

Gold in Urine: A Case History

by R. FUDGE AND R. W. TRUMAN

(*Scientific Adviser's Department, Canynge Hall, Whatley Road, Bristol, 8*)

An account is given of a problem involving the determination of gold in urine of a patient who had been treated with a pharmaceutical gold preparation. The method used, combining dry ashing with colorimetry, is described and discussed together with some medical aspects of the case.

In response to a request from the Royal Cornwall Hospital, Truro, twenty-four-hour samples of a patient's urine were accepted for gold estimation. The patient, a woman aged 56 years, had suffered for 10 years with rheumatoid arthritis. Treatment initially involved the use of steroids; then a pharmaceutical gold preparation was employed. The latter treatment, administered over a period of 12 weeks, amounted to 500 mg of myocrisin—(1:2-Dicarboxyethyl)-thio-gold disodium salt—equivalent to 250 mg of gold.

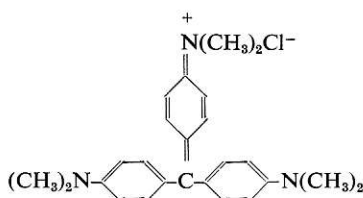
The patient developed aplastic anaemia and an attempt to leach out the gold using British Anti-Lewisite (2:3-Dimercapto-1-propanol) was made over a period of 7 days. Twenty-four-hour urine samples were taken during this period (Table I).

TABLE I
ESTIMATION OF GOLD IN 24-HOUR URINE SAMPLES

Day	Details of Treatment		Twenty-four-hour Urine Samples		
	Myocrisin	British Anti Lewisite 1/M Injections milligrammes	Volume millilitres	Gold microgrammes	Gold parts per million
1	Final dose given				
2		100			
3		100	1400	1820	1.3
4		100	680	840	1.3
5			780	1350	1.7
6			2600	2460	0.9
7		100			
8		100	1480	910	0.6
9					
10			1670	980	0.6

Among the techniques available for the determination of small quantities of gold in solution are fluorimetry using Kojic acid¹; atomic absorption following solvent extraction^{2,3} and polarography⁴. Techniques which have been employed in the analysis of gold in urine include neutron activation⁵, emission spectroscopy⁶ and colorimetry^{7,8,9}. For the latter various reagents have been used, for example, di-2-pyridyl ketoxime⁷, applicable down to 0.05 p.p.m. of gold in urine; *o*-tolidine⁸, applicable down to 0.02 p.p.m. of gold, and methyl green⁹, following extraction with benzene at pH 1.0.

Several methods involving the use of crystal violet—(4-{bis[*p*-dimethyl-amino-phenyl] methylene}-2,5-cyclohexadien-1-ylidene dimethylammonium chloride):



have been published^{10,11} and Kothny¹² describes a procedure employing it for the determination of trace quantities of gold, mercury and thallium in urine. Although in this day and age measurement of heavy metals in urine is usually carried out by atomic absorption techniques, procedures suitable for the determination of gold in urine were lacking (a situation subsequently rectified¹⁴). Lack of such a procedure together with the urgency of the problem led to a chemical method being examined, combining dry ashing¹³ with colorimetry¹⁰. Such a method is both cheap and suitable for such a relatively 'isolated' problem.

Reagents

1. *Concentrated nitric acid*: A.R. quality.
2. *Concentrated hydrochloric acid*: A.R. quality.
3. *Aqua Regia*: Mix 1 volume of concentrated HNO_3 with 3 volumes of concentrated HCl . *Freshly prepared each day*.
4. *HNO_3/HCl mixture*: Mix equal volumes of HNO_3 and HCl (concentrated acids).
5. *Hydrochloric acid solution*: Mix equal volumes of concentrated HCl and distilled water.
6. *Ferric chloride solution*: Dissolve 5 g of hydrated solid in 100 ml of the HCl solution (5).
7. *Hydrogen peroxide*: "20 volume": Of A.R. quality.
8. *Disodium ethylenediaminetetra-acetic acid solution*: Dissolve 5 g of the salt in 100 ml of distilled water.
9. *Crystal Violet solution*: Dissolve 0.2 g of crystal violet in 100 ml of distilled water and filter prior to use.
10. *Toluene*: Of A.R. quality.
11. *Standard Gold solution*. *Solution A*: Dissolve 22.4 mg of auric oxide in the minimum volume of aqua regia. Dilute to 1 litre with distilled water. Store in an amber glass bottle. 1 ml $\equiv$ 20 μg of gold.

Solution B: Dilute 5 ml of solution A to 100 ml with distilled water immediately before use. 1 ml $\equiv$ 1 μg of gold.

Method

Pipette a suitable aliquot (10 to 100 ml) of urine into a round-bottomed porcelain dish. Add 4 ml of concentrated HNO_3 and evaporate to dryness on a water bath. Disperse the residue in 3 ml of aqua regia and again evaporate to dryness. Ash at a temperature not exceeding 400°C for the minimum time necessary to produce a clean ash (approximately 2 hr.). If necessary, add a further 3 ml of aqua regia, evaporate to dryness and re-ash.

Add 10 ml of aqua regia and warm on a hot plate at low heat, whilst swirling to dissolve the residue. Decant into a 50 ml beaker. Re-extract with a further 2×5 ml of aqua regia and combine the extracts. Ensure that all solid matter is transferred, evaporate to dryness and allow to cool. Add 1 ml of HNO_3/HCl mixture, 1 ml of ferric chloride solution and 2 drops of hydrogen peroxide. Evaporate carefully on a hot plate at low heat to approximately half volume. Cool, add 1.25 ml of HCl solution, 4 drops of hydrogen peroxide, and 2 ml of EDTA solution, transfer to a 50-ml glass-stoppered measuring cylinder and dilute to 20 ml with water.

Add 1 ml of crystal violet solution and 12.5 ml of toluene. Invert 10 times and allow the phases to separate. Remove the blue toluene layer, and spin it in a stoppered centrifuge tube for 2 min. at approximately 3000 r.p.m. Measure the optical density of the solution at 605 nm. Compare with a calibration graph prepared from aliquots of the standard gold solution B containing 0 to 20 μg of gold, following the above procedure, and commencing at—

“Add 10 ml of aqua regia and heat on a hot plate . . .”

Correct all optical density readings by subtracting a reagent blank produced by taking 10 ml of aqua regia through the complete procedure.

$$E_{1\text{ cm}}^{\text{1 per cent.}} (\text{for the complex}) = 3.8 \times 10^3$$

Discussion

The destruction of the organic matter in the urine may be effected either by wet oxidation or by dry ashing. Wet oxidation procedures reported include the use of chloric acid⁸, and a perchloric/sulphuric acid mixture¹⁵. Dry ashing has also been described^{12,13}.

Gorsuch¹⁶ anticipates difficulties with both techniques. In the case of wet oxidation heavy charring may lead to the separation of metallic gold, which may be difficult to re-dissolve. Dry ashing may lead to losses due to retention on the ashing vessel. Gorsuch suggests problems are encountered particularly when using silica dishes and other workers recommend the use of porcelain¹⁷.

Our investigations with porcelain and silica dishes indicate that satisfactory recoveries (in excess of 90 per cent.) can be achieved with both, although both are prone to losses, particularly if care is not taken in the initial evaporation stage. We have recommended the use of porcelain dishes since removal of the adhering siliceous matter remaining after ashing is more easily achieved. This is purely a physical phenomenon. Although ashing temperatures as high

as 900°C have been recorded, a maximum temperature of 400°C for the minimum period necessary to achieve ashing (and in any case not exceeding 3 hr.) is recommended. Even at this temperature large losses have been reported¹⁶, but our experience did not confirm this.

The following elements were found not to interfere with the determination of mercury at the levels recorded in these experiments: arsenic, cadmium, chromium, copper, lead, magnesium, selenium, tin and zinc.

Mercury interferes but is lost during the organic destruction. Thallium and antimony, which give a similar reaction with crystal violet, are not often present at significant levels in urine.

Control urines, taken through the entire procedure, did not significantly alter the reagent blank. Ideally, a calibration graph should be obtained for each batch of samples, a reagent blank always being determined.

Taking into account the blank value obtained, 1 µg of gold can be detected with confidence. When 5 µg of gold was added to 500 ml of urine, in excess of 90 per cent. was recovered. Recoveries in excess of 95 per cent. were obtained for the addition of between 5 µg and 15 µg of gold to 50 ml of urine.

Previous work on the excretion of gold in urine has been documented, although gold levels in the urine after treatment with B.A.L. are less well recorded. Work has been carried out showing that the B.A.L. does not leach out the gold from the body, but enters into combination with it, thereby preventing the gold from combining with tissue protein through sulfhydryl linkages, a combination which has been postulated¹⁸ as being responsible for the toxic reactions of the gold in the body. In fact, only a slight increase in the urinary excretion of gold after treatment with B.A.L. has been recorded.¹⁸ Our results support this.

Consideration of recorded urinary levels of gold after the use of myocrisin¹⁸ shows that 5 per cent. of the gold is excreted within 24 hr. of the initial injection while 10 per cent. is excreted during the following week. Hence about 85 per cent. of the gold is retained in the body. In the case which we investigated we would have expected about 210 mg of gold to be retained in the body and the fact that B.A.L. leached out only 8 mg supports the conclusion that the B.A.L. does not remove the gold from the body to any great degree.

The Authors thank Dr. J. D. Hardy, Consultant Physician at the Royal Cornwall Hospital, for providing information regarding the treatment details of the case, and Mr. D. J. Taylor, Deputy Scientific Adviser, for permission to publish this paper.

References

1. Murato, A., and Ujihara, T., *Japan Analyst*, 1961, **10** (5), 497.
2. Greaves, M. C., *Nature*, 1963, **199**, 552.
3. "Analytical Methods for Flame Spectroscopy", Varian Techtron Pty., Ltd., Spring Vale, Australia, 1971.
4. Ezerskaya, N. A., *Izv. Sektora Platiný, I.O.N.Kh., Akad. Nauk, SSSR*, 1955, **32**, 38.
5. Sölvsten, S., *Scand. J. Clin. Lab. Invest.*, 1964, **16** (1), 39.
6. Krejčí, E., and Špačková, A., *Acta Chim. Acad. Sci. Hung.*, 1963, **38** (2), 103.

7. Walton, R. J., Thibert, R. J., Bozic, J., and Holland, W. J., *Canad. J. Biochem.*, 1970, **48** (7), 823.
8. Goodwin, J. F., and Bollet, A. J., *J. Lab. Clin. Med.*, 1960, **55** (6), 965.
9. Tarayan, V. M., Arstamyan, Zh. M., and Mikaelyan, D. A., *Dokl. Akad. Naukarmyan SSR*, 1968, **46** (1), 15.
10. Goryunova, I. F., *Nauch. Trud̄y Irkutsk. Gos. Nauchnoissled. Inst. Redk. Tsvet. Metall.*, 1968, (17), 58.
11. Krasil'nikova, L. N., Efimova, M. C., and Ponomareva, L. S., *Sb. Nauch. Trud. Vses. Nauchno-issled. Gornometallurg. Inst. Tsvet. Metall.*, 1965, (9), 9.
12. Kothny, E. L., *Analyst*, 1969, **94**, 198-203.
13. Doležal, J., Höfer, M., and Zýka, J., *Českosl. Farm.*, 1959, **8** (10), 578.
14. Baláza, N. D. H., Pole, D. J., and Masarei, J. R., *Clin. Chim. Acta*, 1972, **40**, 213.
15. Azevedo, M. D., and Silva Carvelho, J., *Bull. Soc. Chim. Biol.*, 1961, **43** (11), 1247.
16. Gorsuch, T. T., "The Destruction of Organic Matter", Pergamon Press, Oxford, 1970, p. 66.
17. Doležal, J., Höfer, M., and Zýka, J., *Českosl. Farm.*, 1959, **8** (10), 557.
18. Hollander, J. L., "Arthritis and Allied Conditions", Henry Kimpton, London, 1960, p. 284.
19. Dawson, M. H., Boots, R. H., and Tyson, T. L., *Tr. Assoc. Am. Physicians*, 1941, **56**, 330.
20. Nugent, T. E., Nugent, F. W., and O'Neil, F. B., *Canad. M.A.J.*, 1953, **68**, 225.