Serum gold

I. Estimation by atomic absorption spectroscopy

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The estimation of serum gold in patients with rheumatoid arthritis treated with gold salts was first reported in detail by Freyberg, Block, and Levey (1941) and Freyberg, Block, and Wells (1942) using the colorimetric method of Block and Buchanan (1940). This method is cumbersome in that wet digestion is needed, a relatively large sample size is employed, and the concentrations found tend to be high when compared to procedures presently available.

Neutron activation analysis for estimation of gold is the most sensitive method available, but few papers applying this method to the measurement of serum gold levels have been reported and in these only a small number of patients treated with gold have been studied (Brune, Samsahl, and Wester, 1966; Krusius, Markkanen, and Peltola, 1970; Sølvesten, 1964). This is probably due to the lengthy nature of the analysis, considerable expense, and the relative unavailability of suitable neutron sources and multi-channel analysers.

Atomic absorption spectroscopy is ideal in many respects for the estimation of metals in biological fluids. The measurements are rapid with minimum sample preparation and the available instruments have adequate sensitivity for serum. Lorber, Cohen, Chang, and Anderson (1968) reported a study of serum gold levels by this method although no comparison with any other method was made.

In the present paper, we describe the use of two different atomic absorption spectrophotometers for the estimation of serum gold and compare the results with those obtained by neutron activation analysis.

Methods

SERA

Normal sera were obtained from healthy laboratory personnel. Patients with rheumatoid arthritis receiving gold salts (Myochrysine-gold sodium thiomalate, or Solganal-gold thioglucose) were obtained from the Rheumatology Ward and Rheumatology Clinic at the

Veterans Administration Hospital, Hines, Illinois. Blood was drawn at various times after the injection of the gold salt and, after separation, the serum was stored frozen until analysed.

GOLD STANDARDS

Standards of gold sodium thiomalate were prepared from Myochrysine by diluting 1 ml. of the commercial preparation to 252.5 ml. giving a solution with 100 μ g. Au/ml. Standards of gold chloride also were prepared from a solution purchased from Aztec Instruments, Inc., Westport, Connecticut 06880. Dilutions of both standards gave the same results by neutron activation and atomic absorption. For routine work, control solutions were prepared by diluting the Myochrysine standards with pooled normal serum to give the desired gold concentration in 50 per cent. serum.

NEUTRON ACTIVATION

50 μ l. sera, blanks, and standards were frozen and lyophilized on squares of polyethylene. The dried samples were covered with another polyethylene square and the edges sealed with heat. The samples were irradiated for 18 hrs in the CP-5 reactor at Argonne National Laboratory, Lemont, Illinois, with thermal neutrons having a flux of 2.4×10^{13} /cm.²/sec. The samples were allowed to cool for 10 days and assayed with a 512 channel pulse-height analyser. The area under the curve, including three channels on either side of the peak at 0.41 Mev, was used for estimation of gold concentration as described by Covell (1959).

ATOMIC ABSORPTION SPECTROSCOPY

The wave length of light emitted from a hollow cathode lamp is characteristic of the composition of the cathode. In the case of gold, the most sensitive wave length is 242.8 nm. When serum containing gold is atomized into a flame, the gold is reduced to atomic gold. The energy emitted from the gold cathode lamp on passing through the flame is absorbed by the atoms of gold in proportion to their concentration in the atomized solution. The absorbed energy is measured and compared with the energy absorbed when standard solutions of gold are atomized.

We have employed two different instruments:

(1) *Jarrel-Ash Model 82-546 (J-A)* equipped with a total consumption burner, an air-hydrogen flame with a multiple pass beam, and a recorder. The instrument was adjusted to give maximum sensitivity at 242.8 nm. with the cathode lamp carrying a current of 14 to 15 mamp. The base line was recorded when no solution was atomizing. Gold concentration was calculated from the height of the recorded peaks.

The base line on the recorder with the J-A instrument may be obtained by aspirating air, water, or serum. To study the relative effects of each type of aspiration, we chose to adjust the instrument while aspirating air. Positive readings were obtained (Fig. 1) when testing water or serum blanks. The deflections for the serum blanks and standards were greater than when water solutions were atomized. Extensive studies showed that this effect was due to the Na + K content of the serum. Dialysis of serum against water, 1 per cent. NH_4Cl , or 1 per cent. sucrose gave blank readings equivalent to those found for water. The difference between the blanks and standards in water and serum were not the same for all salt concentrations as shown in Fig. 2. The salt effect was relatively constant at 0.3–0.8 per cent. NaCl; we therefore used serum diluted with an equal volume of water for analysis. As the instrument was adjusted while aspirating air, it is desirable to know the precision of the serum blank. To estimate this, twenty random serums from hospitalized persons who had never received gold were diluted with an equal volume of water and aspirated. The mean value and the standard deviation was equivalent to $43.5 \pm 2.8 \mu\text{g. Au}/100 \text{ ml. serum}$. Thus the error due to the blank is, in 95 per cent. of the analyses, $\pm 5.6 \mu\text{g. Au}/100 \text{ ml. serum}$.

FIG. 1 Scale deflections on J-A instrument for blanks and standards in water and pooled normal serum diluted with an equal volume of water

(2) *Perkin-Elmer Model 303 (P-E)* equipped with a Boling burner and used with an acetylene-air flame. The concentration of gold was the same when calculated from the value observed from the energy meter or read from the automatic readout module which is an optional attachment.

FIG. 2 Scale readings of blank and gold standard on J-A instrument as a function of salt content. Open circles = no gold added; closed circles = $200 \mu\text{g. Au}/100 \text{ ml.}$; open triangles = difference curve

The salt effect found with the J-A instrument does not occur with the P-E instrument below a NaCl concentration of 1.5 per cent. A plot of blanks and standards in water and 50 per cent. serum (not shown) gave an identical line with the P-E instrument.

With both instruments frequent standardization and correction for drifting was required. After initial standardization with blanks and standards in 50 per cent. serum, several standards were run after each five or six unknowns. Lorber and others (1968) used the method of additions for their assay of serum gold. In that method the serum is diluted with a standard gold solution and the slope of the curve resulting from the points obtained with and without gold is used to calculate the concentration. The method requires twice as much sample and each point is subject to a precision factor which partly nullifies the theoretical advantages of the method. We have compared direct aspiration of the samples with the method of additions and found no significant difference; the standard deviation was greater for the method of additions. The results thus suggested that the direct aspiration of 50 per cent. serum gives satisfactory results.

EFFECT OF SERUM AGE

Since gold is converted to the atomic state in the flame, ageing the serum would not be expected to have an effect on the quantitative measurement. To check this, serum samples (containing between 0 and $550 \mu\text{g. gold}/100 \text{ ml.}$) that had been assayed were again analysed 1 to 40 months later. A comparison of 45 such values showed that the results were statistically the same, $t = 0.925$.

EFFECT OF VISCOSITY

Lorber and others (1968) found that serum with high viscosity gave lower analytical results because of delay in the atomization of the sample. We have confirmed this with a serum with roughly twice the normal relative viscosity (Table I). Sera with relative viscosities of this magnitude are unusual in rheumatoid arthritis (Shearn, Epstein, and Engleman, 1963), and performance of all analyses at 50 per cent. serum concentration should virtually eliminate error due to this effect.

Table I *Effect of viscosity on recovery of serum gold—P-E instrument*

Serum	Normal	Dysproteinaemic
Relative viscosity*	1.54	3.05
Scale reading, serum	8.0	6.5
Scale reading, serum + Au	27.6	23.1
$\mu\text{g. Au}/100\text{ ml.}$	417	353

* Ostwald viscosimeter, 37°, not corrected for specific gravity.

PLASMA v. SERUM

Lawrence (1961) found that plasma defibrinated with CaCl_2 lost most of its gold content. Our results do not bear this out. Table II compares oxalated plasma, the same plasma after defibrination with CaCl_2 , and serum. The values for native plasma are lower than those for defibrinated plasma or serum which is probably related to the effect of fibrinogen on viscosity.

Table II *Gold content of plasma, defibrinated plasma, and serum ($\mu\text{g. Au}/100\text{ ml.}$)*

Instrument	Plasma	Defibrinated plasma*	Serum
J-A	101 376 505 833	141 399 561 862	121 381 554 874
P-E	126 156 333 356	137 176 360 426	141 178 333 424

* Oxalated plasma defibrinated by addition of 10 $\mu\text{l.}$ 40 per cent. $\text{CaCl}_2/\text{ml.}$ plasma.

Results

Comparison of the atomic absorption instruments

Fig. 3 gives the comparison of the two atomic absorption instruments for 30 sera. The correlation coefficient is 0.987 and the standard deviation of the values from the correlation line is 11.6 $\mu\text{g.}/100\text{ ml.}$

Comparison with neutron activation

Figs 4a and 4b compare results on the J-A instrument with neutron activation results on multiple samples

FIG. 3 *Comparison of both atomic absorption instruments for estimation of serum gold*

from two patients during the first month of gold therapy.

Fig. 5 compares in a different way the neutron activation results for 21 sera and the results with the two atomic absorption instruments. For 21 results with the J-A instrument, the correlation coefficient is 0.96, and for twelve results with the P-E instrument the correlation coefficient is 0.94.

FIG. 5 *Comparison of atomic absorption and neutron activation results. Closed circles = J-A instrument; open circles = P-E instrument*

FIG. 4a, b *Comparison of atomic absorption and neutron activation results in two patients during first month of gold therapy. Closed circles = atomic absorption; open circles = neutron activation*

Although an occasional serum shows a moderate discrepancy between the neutron activation and the atomic absorption results, most of the comparisons are close. Atomic absorption analysis appears to give results adequate for clinical purposes.

instruments are affected by the salt content of the sample, and viscosity has a small effect. Serum yields higher results than plasma. The method compares well with results by neutron activation analysis and its simplicity, speed, and range make it ideal for clinical work.

Summary

The principle of atomic absorption spectroscopy is presented and a method for determination of serum gold is given for two different instruments. Some

We are indebted to Mr. Eugene Kanabrocki, formerly of the Department of Nuclear Medicine, Veterans Administration Hospital, Hines, Illinois, for conducting the neutron activation analyses.

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