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Determination of Gold in Blood Fractions by Atomic-absorption Spectrometry Using Carbon Rod and Carbon Furnace Atomisation

H. Kamel, D. H. Brown, J. M. Ottaway and W. E. Smith

Department of Pure and Applied Chemistry, University of Strathclyde, Cathedral Street, Glasgow, G1 1XL

A comparison of procedures that involve the use of either carbon rod or carbon furnace atomisation in order to determine, by atomic-absorption spectrometry, the level of gold in whole blood, plasma and serum from patients undergoing gold treatment for rheumatoid arthritis is described. A procedure using carbon furnace atomisation is preferred because of its simplicity and sensitivity. The detection limits for gold, obtained by using the preferred procedure, in serum, plasma and whole blood are 0.002, 0.002 and 0.004 5 μg ml^{-1}, respectively. The relative standard deviations are 1.9% for 0.063 μg ml^{-1} in serum, 2% for 0.061 μg ml^{-1} in plasma and 7.3% for 0.030 μg ml^{-1} in whole blood. The method is used to confirm that most gold is carried in the serum fraction of blood, to determine the gold level in white cells and to demonstrate that the gold level in the ultra-filtrate is low.

Gold complexes have been used in the treatment of rheumatoid arthritis for some time and, although they can give rise to toxic reactions in some patients, they remain among the few drugs that can cause a remission of the disease.[1–3] The original discovery of their effectiveness was accidental and, as yet, no definitive mechanism for their action has been postulated. As part of a programme designed to elucidate their mode of action, a method was required for the determination of the amount and distribution of gold in the blood of patients undergoing chrysotherapy (gold treatment). A simple and sensitive method for this determination using carbon furnace atomic-absorption spectrometry has been developed and is reported in this paper.

A variety of analytical techniques have been applied to the determination of gold in blood but, with the exception of studies involving neutron-activation analysis,[4,5] most recent studies have made use of atomic-absorption spectrometry. Gold has not yet been identified in man as a prerequisite for normal life processes and at present the gold concentration in human blood before gold treatment is below detectable levels. During treatment, the level in blood serum, the blood fraction that has been most extensively studied, is between 1 and 10 μg ml^{-1}.[1,2] Although this level is within the range of most flame atomic-absorption instruments, the dilution procedures that are necessary in order to obtain good nebulisation of serum render the methods low in sensitivity.[6–8] Further, it is not generally appreciated that gold complexes can readily be reduced by reagents such as amino-acids, acetates, etc., to a gold(0) colloid, which adheres at least partially to the walls of vessels. Therefore, techniques that require pre-treatment of the samples or a prolonged analysis time are undesirable, especially as there are considerable variations in the chemical composition of the blood of patients with rheumatoid arthritis. For example, we have recorded albumin concentrations as disparate as 3.43 and 2.47 g per 100 ml of blood serum. Consequently, the reducing tendency of each patient's blood might be an important variable in such methods and would have to be checked.

Carbon rod atomisation techniques have been applied to the analysis of blood serum for gold in three previous studies.[9–11] In this paper, an assessment is made of various possible procedures and a procedure involving direct injection of blood serum into a carbon furnace is selected as being the most suitable. The simplicity and increased sensitivity of this procedure are used with advantage to analyse the various fractions of whole blood and provide an answer, for the first time, to the question of the distribution of gold between cells, serum proteins and the ultra-filtrate.

Experimental

Reagents

De-ionised water and reagents of the highest available purity were used throughout.

Gold(I) stock solution (250 $\mu g\ ml^{-1}$). Dissolve one ampoule of the drug myochrysine (May and Baker), containing 50 mg of sodium aurothiomalate, in 100 ml of de-ionised water. Also prepare a gold(III) stock solution (250 $\mu g\ ml^{-1}$) from sodium chloroaurate. The gold(III) solution is much more easily reduced and reacts in a complex manner with proteins. It is used to standardise the above gold(I) stock solution, which is the preferred working standard in all the procedures referred to in this paper.

Preparation of Calibration Solutions

(*i*) *Aqueous standard solutions.* Dilute 2 ml of gold(I) stock solution (250 $\mu g\ ml^{-1}$) to 100 ml with water. Transfer 0, 1, 2, 3, 4, 5 and 6 ml of the solution to 100-ml calibrated flasks and dilute each to the mark with water. This procedure gives calibration solutions with concentrations of 0, 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 $\mu g\ ml^{-1}$.

(*ii*) *Serum standard solutions.* Dilute 4 ml of gold(I) stock solution (250 $\mu g\ ml^{-1}$) to 100 ml with water. Transfer, by using a microburette, 0, 0.05, 0.1, 0.15, 0.2 and 0.25 ml of this solution into 10-ml calibrated flasks and dilute the solutions to the mark with gold-free serum.

(*iii*) 10- *and* 25-*fold diluted serum standard solutions.* Dilute 4 ml of gold(I) stock solution (250 $\mu g\ ml^{-1}$) to 100 ml with water. Transfer, by use of a microburette, 0, 0.05, 0.1, 0.15, 0.2 and 0.25 ml to a series of 10-ml calibrated flasks, each containing 1 ml of gold-free serum. Dilute the solutions to the mark with water; this procedure yields 10-fold diluted serum standard solutions. To obtain 25-fold diluted serum standard solutions use an analogous procedure, adding 0, 0.15, 0.30, 0.45, 0.60 and 0.75 ml of the gold stock solution, diluted as before, to 1 ml of serum in 25-ml calibrated flasks and dilute each to the mark.

(*iv*) *Plasma and blood standard solutions.* Repeat (*ii*) and (*iii*), using plasma or whole blood instead of serum in order to obtain a similar series of plasma or blood standard solutions.

Solutions of whole blood are particularly unstable but, in general, all diluted standard solutions under (*i*)–(*iv*) must be prepared fresh daily.

Separation of Blood Fractions

Prepare serum, plasma, ultra-filtrate and cell fractions I and II of blood by using standard biochemical methods according to the procedure shown in Fig. 1. Separate the red and white cells by lysis of the red cells, then centrifuge the product and wash the residue frequently with physiological saline solution. This residue consists mainly of white cells with a small concentration of platelets. (The amount of red cells present is determined by difference.)

Apparatus

Carbon filament atomisation

A Shandon Southern Instruments A3470 filament atomiser, mounted in an A3400 atomic-absorption spectrometer, was used for the measurements described under *Procedure A* below. Signals were measured on a Honeywell Electronik 194 recorder. Samples are added to a Type 2 graphite rod manufactured from RWO graphite (Ringsdorf Werk GmbH), which has a hemispherical sample cavity that can accept sample volumes of up to 5 μl. The design of the rod ensures that maximum temperature occurs at the sampling point while the clamped ends of the rod remain relatively cool. An attached power-control unit has variable time and temperature selectors for sequentially drying, charring and atomising the samples and, once set, this sequence of operation proceeds automatically. A Pye Unicam gold hollow-cathode lamp was used as the source and argon was used as the inert gas medium during the heating stages. Samples were transferred to the cavity by means of a micropipette. Operating conditions are given under *Procedure D*.

Carbon furnace atomisation

The instrument used for the measurements described under *Procedure B* was a Perkin-Elmer 306 atomic-absorption spectrometer, equipped with an HGA-72 heated graphite tube atomiser and a deuterium-arc background corrector. Atomisation signals were measured on a Servoscribe strip-chart recorder. The HGA-72 has variable time and temperature

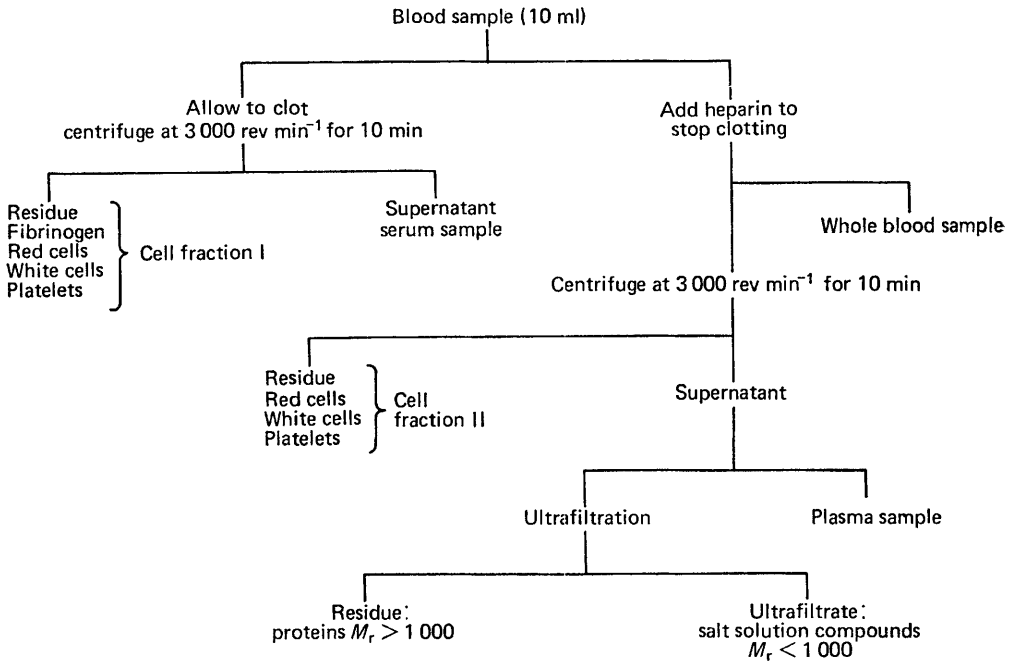

Fig. 1. Procedure for the separation of a blood sample into its components.

selectors for sequentially drying, ashing and atomising the samples and, once set, the sequence of operations proceeds automatically.

Samples are atomised in a graphite tube that is 5.3 cm long and 1 cm in diameter, under an atmosphere of argon. In this atomiser, it is also possible to increase the sensitivity by stopping the flow of purge gas automatically during the atomisation stage, thus retaining the metal atoms in the beam for a longer period of time. Operating conditions are given in *Procedure D*.

Procedures

Procedure A. Determination of gold in serum, plasma and whole blood by an extraction method using 4-methylpentan-2-one[7] and atomic-absorption spectrometry with carbon rod atomisation

To 1 ml of gold-free serum or 1 ml of a serum sample containing between 0.5 and 5 μg of gold, placed in a 20-ml stoppered glass tube, add 2 ml of saturated potassium permanganate solution and mix. Then add 1 ml of 6 M hydrochloric acid and mix again. After leaving it to stand for 20 min, place the tube in a water-bath for 1 h at 75 °C and then boil the contents for 2 min. Cool the resulting suspension to room temperature and add 2 ml of 4-methylpentan-2-one, stopper the tube and shake it vigorously for 2 min. Next, inject 5 μl of the 4-methylpentan-2-one layer into the recess in the carbon rod atomiser and carry out the determination as described under *Procedure D*. A similar procedure is adopted for plasma and blood samples, using 1 ml of sample in each instance. Prepare standards for these procedures as in (*ii*) above and carry out the full procedure.

Procedure B. Determination of gold in serum, plasma and whole blood by direct injection or by dilution and injection into the carbon furnace atomiser

Inject 5 μl of a sample of serum, plasma or whole blood containing between 0.05 and 0.3 μg ml^{-1} of gold directly into the carbon furnace (*Procedure D*). For higher gold concentrations (up to 7.5 μg ml^{-1}), use the dilution procedure described in the preparation of 25-fold diluted serum solutions before injection into the carbon furnace.

Procedure C. Standard additions method for gold in serum, plasma or whole blood using carbon furnace atomisation

Place 1 ml of a sample of serum, plasma or whole blood containing between 0.05 and 0.3 μg of gold in a 10-ml calibrated flask, transfer 0.25 ml of standard gold solution (2 μg ml^{-1}) to the flask and make up to the mark with de-ionised water. Repeat the procedure with the addition of 0.5, 0.75, 1.0 and 1.25 ml of the 2 μg ml^{-1} standard gold solution to further 1-ml portions of the same sample. Carry out the analysis as described under *Procedure B*.

Procedure D. Operation of the instruments

The carbon furnace and carbon rod atomisers were operated under the optimised conditions given in Table I.

TABLE I

OPTIMISED OPERATING CONDITIONS FOR ATOMISERS

Condition	Carbon filament	Carbon furnace
Wavelength/nm	242.8	242.8
Lamp current/mA	10	10
Spectral band width/nm	0.7	0.7
Drying temperature/°C	—	140
Voltage used for drying/V	4	—
Drying time/s	30	30
Ashing temperature/°C	—	475
Voltage used for ashing/V	5	—
Ashing time/s	15	30
Atomisation temperature/°C	—	2 000
Voltage used for atomisation/V	5	—
Atomisation time/s	5	5
Volume of the sample/μl	5	5
Argon flow-rate/l min^{-1}	1.5	1.5

Sequentially inject samples and standards into the carbon furnace or carbon filament atomiser and record the peak height of the atomic-absorption signal during the atomisation step. Interpolate sample concentrations from the calibration graph obtained from determinations on standards or directly from the standard addition calibration graph.

Results and Discussion

The results of analyses of blood samples from six patients undergoing gold therapy, carried out by using Procedures A, B and C, are given in Table II. As the procedures use different atomisation techniques on different instruments and different sample treatments (solvent extraction, dilution and standard addition), the reasonable agreement of the results suggests that both carbon rod and carbon furnace atomisation can be used to determine the amount of gold in serum, plasma and whole blood with acceptable accuracy. The results from Procedure A show a small negative bias compared with those from Procedures B and C and the use of Procedure B is to be preferred. These points are discussed further below.

Use of Procedure A

By using undiluted serum samples in an analogous manner to that described by Matousek and Stevens,[12] it was found that ashing was incomplete and the residue left on the rod often obstructed the light path and led to erroneous results. The residual deposit was difficult to remove satisfactorily from the fragile rod and this operation had to be carried out after every seven samples. Increasing the temperature of ashing to red heat, as suggested by Aggett,[9] resulted in large losses of gold in the sample, either by spluttering or by premature atomisation of the gold.

With 10-fold diluted samples, problems resulting from background absorption by the smoke were considerable, as the instrument used had no background correction facility. The use of the 4-methylpentan-2-one extraction procedure (A) was therefore essential in order to obtain reliable results. To take account of interference from other ions or molecules that are extracted with the gold, a calibration graph of the amount of gold extracted from standard

TABLE II

DETERMINATION OF GOLD IN BLOOD FRACTIONS OF PATIENTS UNDERGOING GOLD THERAPY

Concentration of gold measured in $\mu g\ ml^{-1}$.

Sample	Patient	Procedure A			Procedure B (following 10-fold dilution)			Procedure C
Serum	1	3.60	3.65	3.61	4.13	4.09	4.11	4.06
	2	0.53	0.51	0.54	0.58	0.57	0.57	0.56
	3	0.60	0.65	0.64	0.63	0.62	0.63	0.66
	4	1.10	1.14	1.12	1.19	1.19	1.20	1.20
	5	1.01	1.03	1.05	1.06	1.06	1.07	1.07
	6	4.12	4.10	4.09	4.56	4.53	4.54	4.50
Plasma	1	3.65	3.62	3.69	3.79	3.77	3.78	3.80
	2	0.61	0.60	0.62	0.65	0.66	0.68	0.67
	3	0.70	0.71	0.73	0.75	0.76	0.75	0.75
	4	1.19	1.20	1.16	1.23	1.24	1.23	1.25
	5	0.98	1.00	1.10	1.05	1.06	1.05	1.05
	6	4.21	4.18	4.20	4.21	4.20	4.21	4.18
Whole blood	1	2.17	2.20	2.24	2.44	2.46	2.47	2.44
	2	0.30	0.31	0.30	0.34	0.35	0.35	0.33
	3	0.39	0.38	0.38	0.40	0.41	0.42	0.40
	4	0.69	0.70	0.67	0.73	0.71	0.72	0.72
	5	0.60	0.59	0.58	0.61	0.62	0.65	0.64
	6	2.52	2.53	2.50	2.69	2.68	2.69	2.70

gold(I) stock solutions, prepared as in (*i*) above, was compared with a calibration graph of the amount extracted from standard gold serum solutions prepared as in (*iii*), using the conditions of Procedure A. Extraction from the serum standards was uniformly low at all concentrations; the preparation of standards by extraction of serum solutions was therefore essential. Calibration graphs were linear in the range 0.002–0.5 $\mu g\ ml^{-1}$ of gold.

It was found that a smaller amount of oxidant or a lesser time of oxidation than those specified in Procedure A resulted in larger discrepancies. Reproducible sample application was difficult, particularly as the carbon rod appears to be permeable to 4-methylpentan-2-one. It seems possible that the negative bias on results obtained with Procedure A is caused by the incomplete extraction of gold from a strong gold - protein complex in the samples, which is not formed during the short preparation time of the serum standards. Despite this bias, the results from all three methods can be considered to be in adequate agreement.

Use of Procedure B

In this procedure, optimisation of the time and temperature of ashing is important in order to ensure that all of the matrix is destroyed, that the evolution of smoke and other causes of background absorption are minimised and that none of the gold is lost at this stage. Ashing at 475 °C for 30 s satisfies all three requirements. By using method (*iii*) under Preparation of Calibration Solutions, samples containing between 0.05 and 0.30 $\mu g\ ml^{-1}$ of gold in 0-, 5-, 10- and 25-fold diluted serum solutions were prepared. The results of the analysis of these solutions using Procedure B, under the operating conditions of Procedure D, are shown in Fig. 2. As, for each serum dilution, the results give a straight line graph that passes through the origin, it is clear that the ashing procedure and background corrector are working efficiently in overcoming the effect of smoke for all serum dilutions. However, the sensitivity of the signal for a constant level of gold increases with dilution of the serum, reaching a maximum at the 10- and 25-fold dilutions.

The atomisation signal for 0.1 $\mu g\ ml^{-1}$ was observed as a function of time for 0-, 5- and 10-fold serum dilutions (Fig. 3) and it was clear that peak area was increasing with dilution as well as peak height. Blank serum gave no signal at all concentrations. In concentrated serum solutions, some gold appeared to be held up and released towards the end of the atomisation stage (Fig. 3). Possibly a very stable protein complex, which was not completely destroyed under these conditions during the ashing procedure, prevented rapid atomisation of part of the gold. Another possible explanation is that during the ashing stage some gold

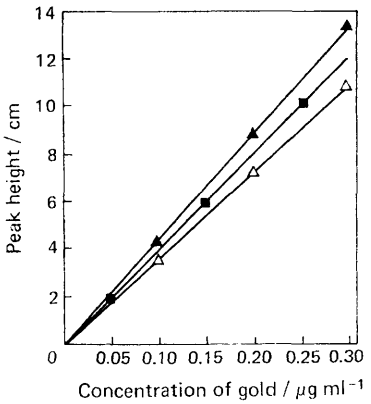

Fig. 2. Results of the analysis of samples containing between 0.05 and 0.3 μg ml^{-1} of gold in 0- (△), 5- (■), 10- (▲) and 25-fold (▲) diluted serum, compared with aqueous gold standards (▲). The points for the 10- and 25-fold diluted sera and the aqueous gold standards are coincident.

was deposited away from the centre of the tube, thus reducing the sensitivity of the signal. This effect would be reduced as the serum level was reduced.

Because there was no problem of sensitivity, we used a 10-fold diluted serum solution in the concentration range 0.05–0.3 μg ml^{-1} of gold and a 25-fold diluted serum solution between 0.3 and 7.5 μg ml^{-1}.

The major reasons for preferring Procedure B to Procedure A are its relative simplicity, its more economic use of time and materials and its higher sensitivity. A comparison of the relative standard deviation and detection limits for serum gold determinations using the two procedures is given in Table III, together with the same information using Procedure B for

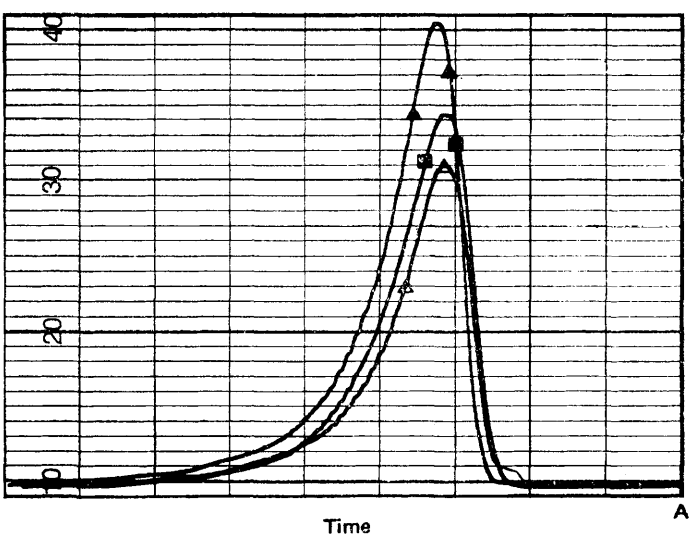

Fig. 3. Atomic-absorption signals as a function of time for samples containing 0.1 μg ml^{-1} of gold at various serum dilutions. △, No dilution; ■, 5-fold dilution; ▲, 10-fold dilution. Atomisation starts at A. Chart speed, 1 cm s^{-1}. Each division is equivalent to 1 s.

plasma and blood samples. The accuracy of Procedure B was established by use of the standard addition method, Procedure C. An example is shown in Fig. 4 and the results are tabulated for each instance in Table II. Both plasma and serum samples gave constant results over a period of 24 h when the samples were kept at room temperature but there was some reduction in the signal for whole blood.

TABLE III

COMPARISON OF PROCEDURES A AND B FOR THE ANALYSIS OF VARIOUS BLOOD FRACTIONS

Sample	Procedure	Mean concentration found/μg ml^{-1}	Number of results	Relative standard deviation, %	Detection limit	
					μg ml^{-1}	g
Serum 1	A	0.361	6	2.5	0.005	2.5×10^{-11}
Serum 2	A	0.061	6	4.1		
Serum 2	B	0.063	10	1.9	0.002	1×10^{-11}
Plasma 2	B	0.061	10	2.0	0.002	1×10^{-11}
Whole blood 2	B	0.031	10	7.4	0.004 5	2.2×10^{-11}

Biochemical Implications of the Results

The results demonstrate a number of possible advantages that can accrue from the use of this technique in a clinical laboratory. It is clear that most of the gold in blood is bound to the serum proteins, confirming that the gold level in serum, which is usually the determination reported in clinical studies, offers a reasonable approximation to the bulk gold level in blood. Two recent studies[1,2] suggest that this measurement is not a useful measure of whether or not gold therapy will be successful but there is a suggestion[1,2] that there could be a link between abnormally high levels of gold and toxic skin reactions; this suggestion requires further investigation. The simplicity of Procedure B would make the routine screening of a large number of patients undergoing gold therapy a relatively simple matter.

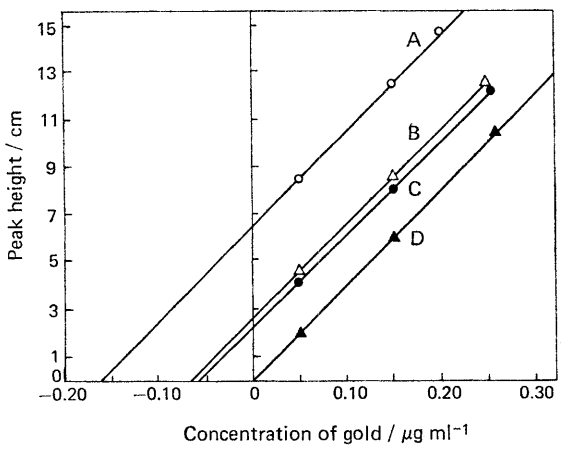

Fig. 4. Example of the standard additions method for the determination of gold in serum; A, sample 1, 4.06 μg ml^{-1}; C, sample 2, 0.56 μg ml^{-1}; B, sample 3, 0.66 μg ml^{-1}; and D, aqueous gold standards.

It is possible that the gold concentration in a fraction of blood other than serum is a more useful indication of the effectiveness of gold therapy. The blood of patient 6 was therefore studied in more detail, taking advantage of the higher sensitivity of the carbon furnace technique in order to determine the gold level in less heavily doped samples without the need for extra pre-concentration stages. A sample of the ultra-filtrate contained no detectable gold (less than 0.004 μg ml^{-1}) and we have confirmed that this was not due to a reaction of the gold with the sinter. We have demonstrated that free myochrysine will pass through

the sinter, so that it can be concluded that there was no free drug in this sample of blood serum, although it may be that some of the gold thiomalate anion is bound unchanged to the serum proteins.

Cell fraction I (Fig. 1) contained 4.1% of the total blood gold, with not less than 90% of it in the white cells. It was difficult to obtain a more exact figure because of problems with the white cell separation procedure. It is possible that the amount of gold within particular types of cell might give a better indication of the nature of the clinical response and the simplicity and sensitivity of the carbon furnace may prove to be particularly useful in this type of study.

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