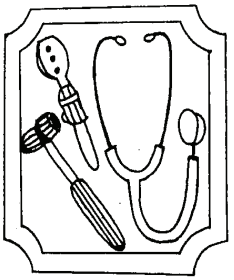


# THE Original Internist

## C • O • N • T • E • N • T • S




*CLINT PUBLICATIONS*

VOL. 15, NO. 3 · ISSN 1529-4722 · SEPTEMBER 2008

# *Clinical Applications of Gold And Silver Nanocolloids*

by Guy E. Abraham, MD

**Introduction**

Three noble metals, also called precious metals, are currently used in medicine: gold, silver, and platinum. None of these metals are considered essential, and there are no daily requirements. Nanocolloids are the metallic form of these metals finely divided with particle sizes below 10 nanometers (nm). One nanometer is one billionth of a meter. As a general rule, the ionic forms of these metals display more toxic reactions than the metallic form of these metals. The metallic form acquires new physical, chemical, and physiological properties when finely divided with particle sizes in the low nanometer range. Only the ionic, most toxic forms of these noble metals are used for medicinal purposes. Platinum is by far the most toxic[1 and vide infra] and is currently used in patients with ovarian and breast cancer.[2 and vide infra] Metallic (non-ionic) colloidal gold and silver have more potential clinical applications and are much safer than platinum.

The purpose of this manuscript is:

- To give a brief history of gold and silver in medicine;
- To review the research performed on colloidal gold and silver by the author — preparation and characterization of gold and silver nanocolloids;
- To discuss the uses of gold nanocolloids in various physiological and pathological conditions;
- To share some preliminary results obtained with colloidal silver.

In the discussion of this manuscript, some mechanisms of action of colloidal gold are presented and a proposed protocol for future research on colloidal gold and silver is outlined. Although some of the data in this manuscript have been previously published, they are reproduced in this review in order to have this information under one cover. The author's experience with colloidal gold started 23 years ago while with colloidal silver only six years ago. Therefore the bulk of this presentation will be on colloidal gold.

Gold belongs to the transition group I in the periodic table and exists in nature in two basic forms — metallic gold and ionic gold salts. Metallic gold is available in various particle sizes, from metallic gold clusters[3] with diameters less than 1 nm, to particle sizes ranging from 1-100 nm[3-7] called colloidal gold and particles larger than 100 nm which are chemically and physiologically inert and will be called coarse gold particles. Colloidal gold absorbed light of different wavelengths, from 510 nm to 560 nm depending on the particle sizes, with the smaller particles absorbing light of shorter wavelength.

When colloidal gold particles are dispersed in an aqueous medium, this metastable system behaves like a hydrophobic (insoluble in water) sol (an aqueous dispersion of solid particles) with a net negative charge at the surface of these particles.[6] Colloidal silver has a net positive charge, although not ionic silver per se. An electrical potential called zeta potential is created around the colloidal gold particles by an ionic double layer of counterions.[7] Aqueous dispersion of nanocolloidal gold possesses unique characteristics, due to the fact that a significant percentage of gold atoms are exposed at the surface of the particles, capable of interacting with the aqueous medium and other molecules. Whereas, coarse gold particles are relatively inert, reacting poorly with other compounds, colloidal gold particles, because of the hydrophobicity of the surface atoms on the colloidal particles, adsorb strongly monolayers of macromolecules which retain their structural properties, biological activities and receptor recognition.[8]

The most common forms of ionic gold are oxidation levels I (monovalent) and III (trivalent). Ionic gold salts that are not soluble in water have been prepared in a colloidal dispersion form, but their properties are similar to soluble ionic gold salts. For the sake of clarity of this presentation, colloidal gold implies metallic, not ionic gold, unless otherwise stated. Ionic gold binds covalently to other molecules to form gold salts (Table 1). Monovalent gold possesses a high affinity for the sulfur moiety, and reacts with it to form aurothiolates. Chemical complexes of monovalent gold readily disproportionate in solution with formation of metallic monoatomic gold and trivalent gold according to the reaction $3AU^{+} \rightarrow 2AU^{o} + AU^{+++}$.[9] Therefore, it would be expected that monovalent gold organocomplexes, such as the aurothiolates, if administered orally or parenterally, would be disproportionate *in vivo* with formation of metallic monoatomic gold and trivalent gold. *In vivo* clustering of metallic gold atoms would eventually form colloidal particles of gold.

Aurothiolate organocomplexes are the only form of gold currently used in medical practice for the treatment of rheumatoid arthritis (RA), and they are associated with a

**Table 1**

## Properties of Different Forms of Gold

| Forms of Gold | Electric Charge | Chemical Reactivity | Mobility in Electrical Field |
|---|---|---|---|
| Ionic gold (monovalent, trivalent) | Positive | Covalent bond | Moves toward cathode |
| Colloidal metallic gold (1-100 nm) | Negative | Hydrophobic adsorption | Moves towards anode |
| Coarse metallic gold | None | Minimal to none | None |

high incidence of adverse reactions.[10] Colloidal gold has been used safely since antiquity, does not display any cytotoxicity *in vitro* and *vivo*, and may offer a better alternative than the aurothiolates in clinical medicine since the active ingredient in aurothiolates is most likely colloidal gold formed by *in vivo* disproportionation and the side effects are mainly due to trivalent $AU^{+++}$ generated from disproportionation. Common sense would favor the active ingredient in its pure state over a precursor that generates both the active form and another form causing side effects.

**Historical Background**

Mahdihassan[11] claimed that the Chinese were the first to prepare and use red colloidal gold as the alchemical drug of longevity, but he gave no reference. According to Mahdihassan, the word alchemy derives from two Chinese words: "Kim" (gold) and "Yeh" (juice). "Kimyeh" (gold juice) entered the Arabic language as "kimiya", and with the definite article, "al," the Arabic word for the red colloidal gold was "alkimiya," which in the western world gave the word "alchemy". So alchemy means preparation and use of colloidal gold. It was believed then that the red color of the gold preparation was essential for its effectiveness. This was confirmed recently and will be discussed in this manuscript.

The procedure for the preparation of red colloidal gold by comminution is still in use today in India, prescribed by ayurvedic physicians for rejuvenation and revitalization in old age under the name "Swarna Bhasma" (red gold) and is reported to be extremely safe. Granules of metallic gold are placed in a granite mortar, mixed with some herbal extracts and rubbed with a granite pestle until the mixture develops a brick red color, a procedure requiring two months. The red-orange color suggests that the particles were very small, less than 20 nm,[4] which is an excellent preparation. However, some ayurvedic physicians wanted the color of the colloidal gold to be blood red, so they added red mercury sulfide (cinnabar) to the gold colloids. This may be the reason colloidal gold lost its healing properties and went into disrepute in recent times.

There is at least one Biblical account going as far back as 3,500 years ago describing an aqueous dispersion of colloidal gold, prepared by comminution and administered to hundreds of thousands of subjects as an anti-stupidity remedy without ill effects.[12] Higby[13] in his review article on gold in medicine quoted Arnald de Villa Nova (1235-1311) using gold to improve vision as a cardio tonic and anti-aging medicine: "It helps vision, and above all cleanses and clears the substance of the heart and the fountain of life." The availability of "Aqua Regia" (Royal Solvent) in the 15th century[13] opened the way to prepare colloidal gold by the chemical method. Aqua Regia is a mixture of highly concentrated preparations of hydrochloric acid and nitric acid. The addition of bulk metallic gold to Aqua Regia dissolves the gold and forms a gold salt, $AuCl_4H$, a trichloride of gold with one molecule of HCl attached to $AuCl_3$. The excess nitric acid and hydrochloric acid are evaporated under heat. The powder form of auric chloride is then dissolved in water containing stabilizers and reduced to colloidal gold by various reducing agents.

In the 16th century, the procedure to prepare gold trichloride from metallic gold was well known. Paracelsus in the early 17th century, described the preparation of a red colloidal gold by reduction of gold trichloride with an alcoholic extract of plants[14] and reported that this liquid colloidal gold preparation was effective in curing diseases, improving strength, prolonging life, and rejuvenating. For melancholy, Paracelsus prescribed liquid colloidal gold because "it makes one's heart happy."[13] Quoting from HB Weiser's book *Inorganic Colloid Chemistry* published in 1933:[14] "Thus around 1600, Parcelsus described the preparation of 'Aurum Potable, Oleum Auri, Quinta Essentia Auri' by the reduction of auric chloride with the alcoholic extract of plants, followed by the addition of sugar or syrup. The mixture, which was red in color, could be

*(Continued on next page)*

concentrated to an oily consistency without coagulating, probably because of the presence of protecting colloids in the alcoholic plant extracts used in its preparation. This aurum potable or potable gold of alchemist was supposed to have fabulous medicinal virtues curing all manner of diseases." Macker, however, in his dictionary of chemistry published in 1774, some two centuries after Paracelsus, wrote that colloidal gold had no medicinal value: "All these gold tinctures are nothing but gold which is made extremely finely divided, floating in an oily fluid."[14]

The first published scientific study of colloidal gold however was by Michael Faraday in 1857.[15] In this classic publication, Faraday described properties of colloidal gold four years before the word "colloid" was coined by Graham in 1861, used the word "molecule" two years before it was modernized by Cannizarro in 1859, and described the Tyndall effect before this optical property of colloid was reported by Tyndall.[7] Prepared by reduction of an aqueous solution of gold trichloride with phosphorus, Faraday observed that the colloidal gold aqueous dispersion was ruby red, but upon addition of various salts, the color changed to blue and the colloids precipitated. He rightly concluded that the color of colloidal gold depended on particle sizes and that the ruby red suspension had the smallest particle sizes. He showed by chemical tests that the ionic form of gold (AU III) used as starting material was no longer present in the aqueous medium but was changed to metallic gold ($AU^o$) dispersed in a very finely divided form. He found that gelatin protected the dispersed gold particles from precipitation by salts. He postulated that this new form of metallic gold possessed new properties due to the interaction of the surface of these gold particles with the surrounding aqueous medium. Faraday made no mention of medicinal application of his colloidal gold. Faraday's brilliant deductions have been confirmed recently. Our current knowledge on colloidal gold and other nanoparticles is an extension of Faraday's findings 150 years ago. There is no evidence, however, that Faraday used colloidal gold for medicinal purposes.

**Preparation of Colloidal Gold**

Since Faraday's original research on the reduction of gold trichloride by phosphorus to generate colloidal gold, several investigators have published various methods for the preparation of colloidal gold at concentrations of 50-100 mg of gold per liter of aqueous medium. This was achieved by reduction of the aqueous solution of gold trichloride with reducing agents. To name just a few reductants: formaldehyde, ethanol, tannic acid, sodium ascorbate, and sodium citrate.[16] In the preparation of colloidal gold for use in molecular biology, the most commonly used reductant is sodium citrate, using the procedure described by Frens.[4] This is due to the versatility of the citrate method which allows, by changing the citrate/gold molar ratios within certain limits, the preparation of a wide range of colloidal gold particle sizes, from 8 nm[4] to 150 nm.[5] Faraday's procedure using phosphorus as the reducing agent yielded particle sizes from 2-12 nm. Of the procedures currently available for the preparation of colloidal gold, the reduction of gold trichloride using the citrate method of Frens[4] is most widely used and the best characterized in terms of sizes and shapes of the colloidal particles

Table 2

**Various Methods for the Preparation of Currently Available Colloidal Gold**

| Methods | Purity | Concentration | Particle sizes | Published Data |
|---|---|---|---|---|
| Mining of colloidal gold dust from abandoned gold mines (Reef Gold) | Very impure. Contains aluminum, silica, and many other elements. | Very low. A few PPM. | Unknown. | Anecdotal. |
| Comminution (Grinding) | Mixed with other ingredients. May contain mercury sulfide (cinnabar). | Unknown. Probably very high. | The red color suggests <20nm. | Anecdotal. |
| Electrolysis with gold anode | Very pure. | Very low. A few PPM. | Unknown. | Anecdotal. |
| Reduction of gold trichloride | Contains electrolytes and stabilizers. May contain antimicrobial preservatives. | Can be prepared at concentration up to 5000 PPM. | 2-150 nm. | Several publications in molecular biology. Two publications in human subjects by the author. |

**Table 3**

**Relationship between Diameter and the Percentage of Gold Atoms Exposed on the Surface of Colloidal Gold**

| Forms of Metallic Gold | Diameter of Particle (nm) | Gold Atoms/Particles | Percentage of Gold Atoms Exposed at the Surface of Particle* |
|---|---|---|---|
| Monoatomic gold | 0.28 | 1 | 100 |
| Gold nanoclusters | 0.8 | 11 | 70 |
| Gold colloids | 1 – 100 | $20 – 10^9$ | 2.0 – 60 |
| Coarse metallic gold | > 100 | $> 10^9$ | < 2.0 |

*Based on the assumption that the gold colloids and the gold atoms are spherical.

(Table 2). Gold preparation by the ayurvedic procedure using comminution may contain mercury. The electrolytic method generates gold colloids in the low nanometer range but in low concentrations. Therefore, large volumes of colloidal gold suspension must be consumed for physiological effects. Metallic gold is found in nature in various particle sizes from large boulders of several kilograms to fine dust of colloidal gold as thin veins of reef gold in quartz rocks. Reef gold used in powder forms contains several other elements. Some of them, such as lead, may be toxic.

There are opposing forces acting on the colloidal gold suspensions, some of which favor stability and others aggregation of the colloid particles to form larger colloids and eventually precipitation of these large particles. I will first discuss the factors predisposing to the aggregation of gold colloids.

When dispersed in an aqueous medium, gold colloids form a negatively charged hydrophobic sol. This hydrophobicity gives these gold particles a tendency to aggregate together to form larger particles. Since gold has a density of 19.3, the effect of gravity on the mass of these particles, combined with hydrophobicity causes these aggregated particles to precipitate rapidly. Brownian motion is temperature-dependant and affects the kinetic energy of the colloid particles. Therefore, the higher the ambient temperature, the more unstable the colloidal gold dispersion becomes. Colloidal gold particles absorb light with a maximum absorption between 510 and 560 nm depending on particle sizes.[5] Some of the absorbed energy dissipates as heat, which increases Brownian motion. So light has the same destabilization effect as heat on the gold colloidal particles. Electrolytes tend to compress the protective ionic double layer surrounding the gold particles, decreasing their stability.

On the other hand, these are factors that favor stability. The electrical charges on the surface of the gold colloids favor aggregative stability due to ionic double layers of counterions surrounding the surface of these negatively charged particles, preventing proximity of these particles and minimizing aggregation. Since the electrical potential of the ionic double layer, which depends on surface atoms of gold, favors stability, the greater the percentage of gold atoms exposed at the surface of the particles, the greater the aggregative stability of the colloidal gold aqueous dispersion. The smaller the particle sizes, the greater the percentage of gold atoms exposed at the surface of the particles (Table 3). Therefore, the smaller particles form a more stable gold sol because they would possess the smallest mass and, the highest electrical potential of the ionic double layer. In theory, monoatomic metallic gold would possess the maximum stability. The smallest colloidal gold available for studies in molecular biology contains 11 atoms of gold and has a diameter of 0.8 nm.[3] The adsorption of hydrophylic colloids like gelatin or surfactants on the gold colloids confers protection against the aggregative effects of electrolytes.[17,18]

**Current Uses of Gold in Biology and Medicine**

At the beginning of this century, colloidal gold was used in diagnostic tests for liver functions and for cerebrospinal fluid analysis,[19] and more recently as a home test kit for pregnancy. The last 30 years however have witnessed an extensive use of colloidal gold in molecular biology, due to the fact that colloidal gold particles have strong reflectivity in light and electron microscopy, and adsorb strongly to macromolecules without affecting their biological activity. First used in 1962 as a tracer in transmission electron microscopy (TEM), colloidal gold was later applied in 1975 to scanning electron microscopy (SEM). Particles of colloidal gold with diameter of 20 nm or less are used in TEM because of its high resolution whereas larger gold

*(Continued on next page)*

particles were used in SEM.[6] For a recent review on this subject, consult the 3-volume set published in 1989 by Academic Press and edited by MA Hayat — *Colloidal Gold: Principles, Methods, and Applications*.

Metallic gold particles with diameters less than 100 nm can be incorporated inside cells by a mechanism known as microendocytosis.[20] Gold particles first adhere to the cell membrane which then invaginates to form cytoplasmic vesicles containing the gold particles which are transported into intracellular granules. Colloidal gold particles are usually concentrated in lysosomes. At body temperature, over 90% of the colloidal gold particles were incorporated inside the cells within one hour of incubation.[21] Culture of various cell types with colloidal gold showed no evidence of cytotoxicity.[22-27] No *in vivo* cytotoxicity was reported with the use of colloidal gold administrated intravenously to ponies and pigs at doses of 400 mg of gold.[24]

Ionic forms of gold, on the other hand, display cytotoxicity in various cell types. Monovalent and trivalent gold oxydize glutathione in intact red blood cells, whereas colloidal gold particles were without effect.[28] Monovalent aurothiolates currently used in medical practice inhibit adenylcyclase activity in lymphocytes;[29] increase oxygen free radical production in T-cells which results in cellular death following depletion of glutathione;[30] induce the formation of stress proteins in mouse macrophages[31] in response to oxidative stress caused by production of oxygen free radicals;[30] inhibit DNA synthesis in lymphocytes;[32] and suppress immunoglobulin synthesis.[33]

Aurothiolates, which are ionic forms of gold covalently bound to a sulfur moiety, have been used in the treatment of RA since their introduction by Forestier in 1929.[34] In a follow-up publication, describing his experience on the use of gold compounds in 550 cases of RA for over six years,[35] Forestier reported that the only forms of gold effective in the management of RA were organic compounds containing monovalent cathonic gold covalently bound to a sulfur moiety (aurothiolates), and given by weekly intramuscular injection to achieve a total cumulative dose of 2.5-3.0 g. He stated that colloidal gold was ineffective. He did not mention the dosage, the form of colloidal gold, whether metallic or cathionic, nor the method of administration.

With the aurothiolates, Forestier observed a 70-80% success rate with improvement of pain, swelling, mobility, and general condition of patients. He reported several side effects of his treatment: fever, pruritus, skin rash, ulcers in the mucus membrane of the mouth, conjunctivitis, keratitis, diarrhea, bronchitis, and reactivation of latent diseases such as herpes zoster and skin boils. Laboratory tests in his patients reveal significant hematologic abnormalities in some patients, including agranulocytosis, thrombocytopenia with purpura resulting in one death. Albuminuria and abnormal liver function tests were reported in some patients. Considered as a whole, he concluded that these side effects should not be taken too seriously. Several reports have been published confirming the efficiency of the parental forms of aurothiolates in RA, but also expounding further on some of the side effects observed by Forestier: pulmonary damage,[36-40] myelotoxicity, leucopenia, thrombocytopenia, and anemia.[41-46] In an

**Table 4**

**Incidence of Adverse Reactions from Oral and Parenteral Aurothiolates Administered to Patients with Rheumatoid Arthritis at an Average Daily Dosage of 3 mg of Gold (Based on 18 Comparative Clinical Trials)***

| Side Effect | Ridaura (445 patients) | Injectable Gold (445 patients) |
|---|---|---|
| Proteinuria | 0.9% | 5.4% |
| Rash | 26% | 39% |
| Diarrhea | 42.5% | 13% |
| Stomatitis | 13% | 18% |
| Anemia | 3.1% | 2.7% |
| Leucopenia | 1.3% | 2.2% |
| Thrombocytopenia | 0.9% | 2.2% |
| Elevated Liver Function Tests | 1.9% | 1.7% |
| Pulmonary | 0.2% | 0.2% |

* From *Physicians' Reference Desk 49th edition*, Medical Economics, Montvale, New Jersey, 1995.

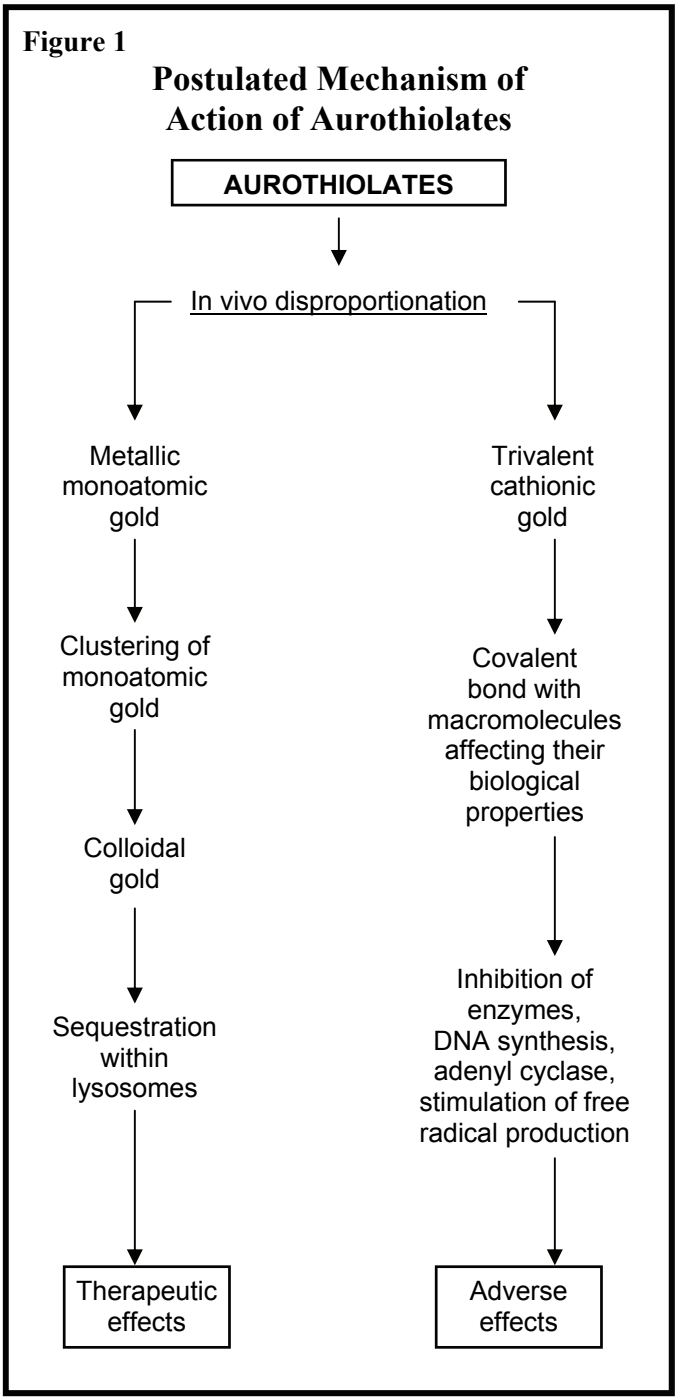
attempt to minimize these side effects, an oral preparation was introduced in 1976.[47] However, this preparation caused diarrhea/loose stools in 50% of the patients, was less effective than the parenteral forms of aurothiolates and produced the same side effects as the injectable forms of gold salts although to a lesser extent (Table 4).

Since all monovalent organogold complexes currently approved for use in medical practice display significant side effects, and colloidal gold is relatively safe without any *in vitro* or *in vivo* toxicity, it is amazing that only one clinical trial has been published by the author on the use of colloidal gold in RA. There is no published data on the use of colloidal gold in medical practice for any medical condition. It is very likely that some of the therapeutic effects of the aurothiolates in RA are due to the colloidal metallic gold generated from disproportionation, and the side effects are caused by the trivalent cathionic gold formed from this disproportionation and the aurothiolates themselves (Figure 1).

It may not be a coincidence that the oral phosphine gold preparation which is more resistant to disproportionation than the injectable aurothiolates,[9,48] is less effective but also has lower incidence of side effects than the injectable preparations. This is exactly what would be expected from Figure 1. A greater degree of disproportionation would result in greater *in vivo* levels of therapeutic colloidal gold, but also a greater levels of side effects causing cathionic trivalent gold. The most prevalent side effects of aurotherapy are skin rash and diarrhea. Trivalent cathionic gold salts cause contact dermatitis and skin rash.[49] The diarrheogenic action of aurothiolates can be explained by their ability to stimulate intestinal secretion *in vitro*, an effect shared by cathionic trivalent gold.[50]

In studies performed *in vitro* and *in vivo*, metallic colloidal gold particles are ultimately sequestered within lysosomes of phagocytes, visible under electron microscopy (EM). Ionic gold salts are not visible under EM. After administration of aurothiolates to RA patients, gold particles visible under EM selectively accumulate in the lysosomes of synovial cells and macrophages.[51] It is believed that stabilization of lysosomes by these gold particles plays a role in their therapeutic actions.[52] Since disproportionation of aurothiolates generate monoatomic metallic gold with a diameter of 0.28 nm, a size below the resolution of EM, the only way the gold particles in the lysosomes could be visible under EM is by clustering of metallic monoatomic gold to form colloidal gold particles visible under EM. Therefore, the argument that colloidal gold formed by disproportionate is the active and beneficial ingredient from aurotherapy with aurothiolates seems very plausible. The gold visualized by EM is metallic gold.

**Figure 1**



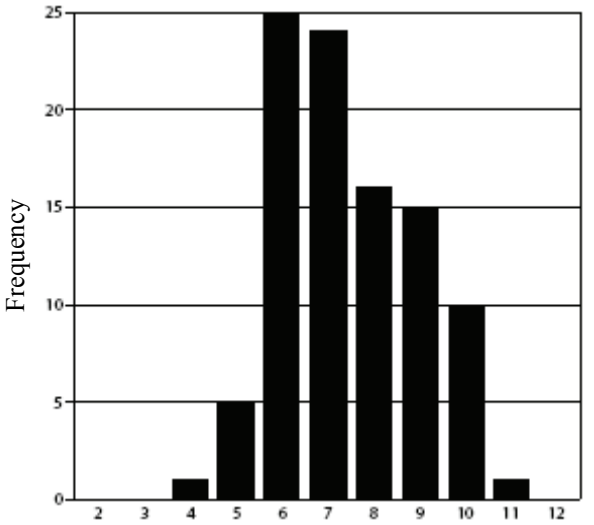

**Recent Clinical Studies with Colloidal Metallic Gold by the Author**

In 1985, I initiated the gold project after I became interested in the confirmation of previous claims that colloidal gold improves vision, memory, mood, cognitive functions, cardiac functions, and longevity. Aurothiolates, which are extremely toxic, were the only form of gold used in medical practice then for rheumatoid arthritis. To my surprise, there was no

*(Continued on next page)*

**Figure 2**

**Histogram of Colloidal Gold Particle Sizes in Aqueous Suspension Prepared by Author**




published data on the use of colloidal gold in patients with rheumatoid arthritis, and for that matter in any other medical condition.

My first project was to study the effect of colloidal metallic gold on patients with severe rheumatoid arthritis not responding to other treatment modalities. This study was performed in collaboration with rheumatologist, Peter Himmel, MD, in 1995, supported by a grant from Optimox Corporation. The results of this study were published in 1997.[53] To this author's knowledge, this was the first published clinical study of colloidal gold. For the convenience of the reader, these data will be reproduced in this manuscript.

***Preparation of Colloidal Gold*:** Aqueous dispersion of colloidal gold (Aurasol®) was prepared by the author at a concentration of 1,000 mg/L (1,000ppm) using the citrate method of Frens,[4] with several proprietary modifications. Maltodextrins (Food Grade) were used at the concentration of 2.5% to prevent autoaggregation of the small colloid particles. It required 10 years of on and off research to optimize the stability of the colloids in concentrated preparations as high as 5,000mg/L (5,000ppm) and to maintain particle sizes of the gold colloids with ruby red color below 20 nm consistently. Many batches with blue and violet colors were discarded in the process. Gold colloids of the blue color could not pass through a 100-nm filter and precipitated out of the solution within days of preparation. But gold colloids of the violet color were more stable and could pass through a 100-nm filter, but not a 20-nm filter. They were used in the study of the normal subjects to be described later. However, they were devoid of noticeable effect in the subjects tested.

Using the optimized procedure, the sizes of the colloid particles of ruby red color were less than 20 nm in several batches, confirmed by quantitative recovery after passing through a 20-nm filter (Whatman Anotop 10. pore size 0.02 nm PN# 68091102). Based on particle sizing by TEM (Courtesy of R.H. Albrecht, Department of Animal Health and Biomedical Science, University of Wisconsin, Madison, WI), 99% of the particles were less than 10 nm (Figure 2).

Gold trichloride absorbs light with a maximum absorption of 290 nm, whereas colloidal gold has a maximum absorption of 510-560 nm depending on the size of the particles. In order to confirm that all the gold trichloride was reduced to metallic colloid gold, UV-VIS spectrum of the gold suspension was performed before and after ultracentrifugation. No peak at 290 nm was observed following ultracentrifugation which causes sedimentation of colloids, leaving only the ionic gold in solution. The absence of a peak at 290 nm post centrifugation suggested that there was no ionic gold in the supernatant and therefore, in the colloidal gold suspension. This was satisfactory evidence that all the gold trichloride was reduced to colloidal metallic gold.

Accelerated shelf-life studies confirmed the stability of the aqueous dispersion for at least two years at ambient temperature. The following metals were measured in the aqueous colloidal gold dispersions and were undetectable at 0.5 ppm (<0.5 mg/L): antimony, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, lead, mercury, molybdenum, nickel, selenium, silver, thallium, vanadium, and zinc. The lead levels were measured again in a more sensitive assay and were undetectable at 50 ppb (<0.05 mg/L). Sterilization was achieved by microfiltration through 100-nm pore size and sodium benzoate was used as an antimicrobial preservative. Particle sizing with the 20-nm micro-cartridges was performed following sterilization through large volume 100-nm cartridges. The aqueous suspension of colloidal gold prepared in this fashion was stable for up to 13 years (as of this writing) at room temperature when kept in sealed dark brown plastic containers.

**Clinical Studies**

***Effects of Colloidal Gold on Patients with Rheumatoid Arthritis*:** The particle sizes of the batches of colloidal gold used in the following studies were all below 10 nm, unless otherwise stated. The author postulated that the active ingredient in aurothiolates therapy for RA is colloidal metallic gold generated by *in vivo* dispro-portionation with subsequent clustering of monoatomic

metallic gold, and the side effects were due to the aurothiolates themselves and the trivalent gold (AU III) generated from disproportionation (Figure 1). If this postulate is valid, one would expect colloidal gold to have therapeutic effects in RA and be devoid of side effects. In order to minimize the placebo effect, the 10 worst cases (9 of 10 seropositive) with long standing (7-40 years duration) erosive RA with minimal to no response to previous treatment, were selected from Dr. Himmel's practice. The clinical data on these patients are displayed in Table 5.

Nine of the 10 patients had previously received aurothiolates therapy without effect and five of the nine experienced side effects of skin rash, stomatitis, and proteinuria. Six of the 10 patients were totally work disabled. After informed consent was obtained, the patients underwent complete clinical and laboratory evaluations and weekly afterwards for four weeks and monthly for an additional 13 months (52 weeks) of oral colloidal gold administration. Clinical evaluation included performance parameters assessed by the method of Pincus, *et al*,[54] severity of tenderness and swelling of joints for 86 joints based on the quantitation of Lansbury[55] and the classification described in the *Dictionary of Rheumatic Diseases*:[56] Class I — complete functional capacity with ability to carry on all usual duties without handicaps; Class II — functional capacity adequate to conduct normal activities despite handicap or discomfort or limited mobility of one or more joints; Class III — functional capacity adequate to perform only a few or none of the duties of usual occupation or self-care; and Class IV — largely or wholly incapacitated with patient bedridden or confined to a wheelchair, permitting little or no self-care.

Laboratory evaluation involved the following blood and urine tests: hemoglobin, hematocrit, white blood cells and subsets, platelets, liver, renal functions and urinalysis. Specialized immune function tests were performed by a commercial laboratory (Immunoscience Laboratory, Beverly Hills, USA): the cytokines tumor necrosis factor α (TNF-α) and interlukins-6 (IL-6); natural killer (NK) cells lytic activity; the immune complexes IgG, IgM, and IgA; rheumatoid factor (RF)

*(Continued on next page)*

**Table 5**

## Clinical Data on the RA Patients

| Patient | Sex/ Race | Age (Years) | Height (Inches) | Weight (Pounds) | ARA Functional Class | Work Status | Response to Previous RX | Previous Aurothiolate RX |
|---|---|---|---|---|---|---|---|---|
| 1 | M/W | 52 | 61 | 195 | III | Disabled | Minimal to none | Myochrysine, Proteinuria |
| 2 | M/W | 58 | 67 | 126 | III | Disabled | Minimal to none | Myochrysine, Proteinuria |
| 3 | F/W | 58 | 66 | 160 | III | Homemaker | Minimal to none | Myochrysine, Proteinuria |
| 4 | F/W | 54 | 65 | 168 | III | Works full time | Minimal to none | Myochrysine, Proteinuria |
| 5 | F/W | 31 | 63 | 128 | II | Homemaker | Minimal to none | Myochrysine, Proteinuria |
| 6 | F/W | 37 | 67 | 145 | IV | Disabled | Minimal to none | Myochrysine, Proteinuria |
| 7 | F/W | 43 | 63 | 108 | II | Works full time | Minimal to none | Myochrysine, Proteinuria |
| 8 | F/W | 58 | 64 | 138 | III | Disabled | Minimal to none | Myochrysine, Proteinuria |
| 9 | M/W | 59 | 74 | 204 | III | Disabled | Minimal to none | Myochrysine, Proteinuria |
| 10 | M/W | 52 | 72 | 280 | III | Disabled | Minimal to none | Myochrysine, Proteinuria |

**Table 6**

## Effects of a Colloidal Gold Preparation (Aurasol®) at 30 mg/day on Some Clinical Parameters of Disease Activity, Fatigue and Satisfaction with Ability to do Work in 10 RA Patients

| Clinical Parameters | Pre-RX | 1 Week | 4 Weeks | 12 Weeks | 16 Weeks | 24 Weeks | 52 Weeks |
|---|---|---|---|---|---|---|---|
| **Tenderness** | | | | | | | |
| Mean | 54.8 | 19.2 | 8.4 | 9.5 | 9.5 | 5.4 | 5.9 |
| SE | 16.2 | 6.3 | 4.5 | 2.6 | 2.6 | 2.0 | 2.5 |
| *p*-value | — | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| **Swelling** | | | | | | | |
| Mean | 42.3 | 15.9 | 13.2 | 8.8 | 4.5 | 3.3 | 3.6 |
| SE | 10.3 | 5.9 | 5.8 | 3.7 | 1.3 | 1.2 | 2.2 |
| *p*-value | — | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| **Stiffness a.m. (hours)** | | | | | | | |
| Mean | 2.8 | 2.3 | 1.8 | 2.0 | 0.54 | 0.51 | 0.67 |
| SE | 0.67 | 0.66 | 0.71 | 0.78 | 0.28 | 0.31 | 0.36 |
| *p*-value | — | NS | NS | NS | <0.01 | <0.01 | <0.01 |
| **Fatigue** | | | | | | | |
| Mean | 5.3 | 4.8 | 3.1 | 3.1 | 2.9 | 4.1 | 2.6 |
| SE | 1.0 | 0.95 | 0.28 | 0.28 | 0.82 | 0.75 | 0.88 |
| *p*-value | — | NS | <0.01 | <0.01 | <0.01 | NS | <0.01 |
| **Satisfaction with ability to do work** | | | | | | | |
| Mean | 3.1 | 2.5 | 2.5 | 2.0 | 1.6 | 2.3 | 2.3 |
| SE | 0.32 | 0.25 | 0.29 | 0.21 | 0.28 | 0.12 | 0.32 |
| *p*-value | — | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |

NS = Not Significant

by Elisa; and erythrocyte sedimentation rate (ESR).

Since the preliminary data by the author suggested that amounts of up to 15 mg/day of colloidal gold were without clinical effect in RA, patients 1-5 received 30 mg/day for the first week and 60 mg/day for the second week, whereas patients 6-10 received 60 mg/day for the first week and 30 mg/day for the second week. Except for one patient, no significant difference was found between these two amounts on the clinical parameters evaluated. The patients were therefore continued on the trial at 30 mg/day for a duration of 52 weeks.

The mean body weight after 52 weeks on colloidal gold was not significantly different from the pre-treatment value. The effects of the colloidal gold (Aurasol®) on tenderness and swelling of joints were rapid and dramatic, with a significant decrease in both parameters after the first week, which persisted during the study period (Table 6). The mean score for tenderness and swelling were respectively, for pre- and post-1 week 58.8 and 18.2 ($p<0.01$) and 42.5 and 15.9 ($p<0.01$). By 24 weeks of gold administration, the mean scores were ten times lower than the pre-treatment levels being respectively 5.4 and 3.3 for tenderness and swelling, and remained lower throughout the study. The duration of a.m. joint stiffness (in hours) showed a decreasing trend that reached statistical significance at 16 weeks with pre- and post-16 week mean score of 2.8 and 0.54, respectively ($p<0.01$). Self-assessed degree of fatigue showed a decreasing trend which became significant at four weeks and remained significantly lower with pre- and post-52 week scores of 5.3±1 (mean ± SE) and 2.6±0.88, respectively.

Satisfaction with ability to do activities, physician's estimate of disease activity, ARA class, and functional assessment of normal activities, all improved significantly after 16 weeks of gold administration (Table 7). However, there was no change in vigorous activities and psychosocial status. Overall, when evaluated individually, nine of the 10 patients improved markedly by 24 weeks of intervention, and three patients (5, 6, and 7) were in clinical remission with improved work status. The most impressive results were obtained in patient 6 who changed from totally disabled to full-time work, and ARA Class IV to Class I.

The results of the immune function tests are displayed in Table 8. The immune complexes IgG and IgM were

significantly suppressed by 16 weeks of intervention and remained low during the study period with pre- and post-52 week values (mean ± SE) for IgG and IgM, respectively, of: 34.6±7.3 and 19.9±3.4 ($p<0.01$); 24.0±4.9 and 19.4±2.9 ($p<0.05$). IgA levels were low and did not suppress further. Both cytokines TNF-x and IL-6 were suppressed significantly by 16 weeks, with values pre- and post-16 weeks of 207±32.7 and 74±24 ($p<0.05$) TNF-x , and 241±66 and 104±24.5 ($p<0.05$) for IL-6. RF levels were elevated prior to gold ingestion and suppressed significantly at 52 weeks with levels of 143±23.7 and 117.9±18.9 ($p<0.05$). ESR remained elevated throughout the study period, without significant change. NK lytic activity increased significantly after 16 weeks of gold administration with pre- and post-gold mean values of 32.2±2.6 and 50.3±3.6 ($p<0.01$).

There was a complete absence of clinical and laboratory evidence of toxicity in the patients. Clinically, there were no reports of skin rashes, stomatitis, gastro-intestinal disturbances, vasomotor reactions, mylagias, arthralgias, puritus, headaches, or metallic taste.

There was no evidence of hematologic, renal, or hepatic cytotoxicity. In fact, there were improvements of some hematologic parameters (Table 9). In six patients, with an elevated platelet count over 400 before intervention, the platelets decreased to normal in all patients at 52 weeks of gold administration. The mean values were: 374±26 (mean ± SE) before and 289±36 after 52 weeks of gold ingestion ($p<0.01$). In four patients with hemo-globin levels below 12 before gold administration, these levels increased above 12 in all patients at 52 weeks. The mean WBC levels were significantly lower at 52 weeks with pre- and post-gold levels of 9.8±0.71 and 7.8±0.71 ($p<0.05$). The significant drop in the mean WBC values was due mainly to patients 2, 3, 5, and 6 with pre-gold values above 10 and post-gold levels below 10. The post-gold WBC levels were within the normal range in all the patients. Colloidal gold had a normalizing effect on these hematologic parameters.

***Effect of Colloidal Gold on Mental, Physical, and Performance Parameters in Normal Adults*:** Following a pilot study in six subjects, the liquid colloidal gold suspersions were tested in 21 adult subjects of both sexes after informed consent was obtained. Observed changes from the baseline reported by the subjects were classified into three groups of parameters: Group I = Mental; Group II = Physical; Group III = Performance (Table 10). A scoring system allowed a maximum score of 12 for Groups I and II and 8 for Group III with a total

*(Continued on next page)*

**Table 7**

**Effects of Aurasol® at 30 mg/day on Physician's Estimate of Disease Activity, ARA Class and Performance Parameters in 10 RA Patients**

| Clinical Parameters | Pre-RX | 16 Weeks | 24 Weeks | 52 Weeks |
|---|---|---|---|---|
| **Physician's estimate disease activity** | | | | |
| Mean | 3.1 | 1.5 | 1.5 | 1.4 |
| SE | 0.22 | 0.26 | 0.38 | 0.21 |
| *p*-value | — | <0.01 | <0.01 | <0.01 |
| **ARA Class** | | | | |
| Mean | 2.9 | 2.3 | 2.1 | 1.7 |
| SE | 0.17 | 0.25 | 0.27 | 0.20 |
| *p*-value | — | <0.05 | <0.05 | <0.05 |
| **Normal activity** | | | | |
| Mean | 14.7 | 11.1 | 12.1 | 12.0 |
| SE | 0.92 | 0.91 | 0.88 | 1.2 |
| *p*-value | — | <0.05 | <0.05 | <0.05 |
| **Vigorous activity** | | | | |
| Mean | 15.1 | 14.2 | 14.8 | 14.1 |
| SE | 0.86 | 0.8 | 1 | 1.1 |
| *p*-value | — | NS | NS | NS |
| **Psychosocial status** | | | | |
| Mean | 6.7 | 6.5 | 6.3 | 6.8 |
| SE | 1.1 | 0.7 | 0.7 | 0.7 |
| *p*-value | — | NS | NS | NS |

NS = Not Significant

**Table 8**

## Effects of a Colloidal Gold Preparation (Aurasol®) at 30 mg/day on Some Laboratory Parameters of Immune Functions in 10 RA Patients

| Clinical Parameters | Pre-RX | 16 Weeks | 24 Weeks | 52 Weeks |
|---|---|---|---|---|
| **IgG** | | | | |
| Mean | 34.6 | 21.4 | 18.8 | 19.9 |
| SE | 7.3 | 4.4 | 3.0 | 3.4 |
| *p*-value | — | <0.01 | <0.01 | <0.01 |
| **IgM** | | | | |
| Mean | 24.0 | 15.6 | 16.0 | 19.4 |
| SE | 4.9 | 3.1 | 3.5 | 0.91 |
| *p*-value | — | <0.01 | <0.01 | <0.05 |
| **IgA** | | | | |
| Mean | 5.9 | 4.5 | 5.6 | 4.7 |
| SE | 0.81 | 0.79 | 1 | 0.91 |
| *p*-value | — | NS | NS | NS |
| **Cytokines TNF-*x*** | | | | |
| Mean | 207 | 105 | 74 | |
| SE | 33 | 30 | 25 | |
| *p*-value | — | <0.05 | <0.05 | |
| **IL-6** | | | | |
| Mean | 241 | 107 | 104 | |
| SE | 66 | 20 | 25 | |
| *p*-value | — | <0.05 | <0.05 | |
| **NK (lytic activity)** | | | | |
| Mean | 33.2 | 50.3 | | |
| SE | 2.6 | 3.6 | | |
| *p*-value | — | <0.01 | | |
| **RF (Elisa)** | | | | |
| Mean | 143.6 | | 145.9 | 117.9 |
| SE | 23.7 | | 22.1 | 18.9 |
| *p*-value | — | | NS | <0.05 |
| **ESR (mm $h^{-1}$)** | | | | |
| Mean | 42.1 | 32.9 | 35.2 | 36.5 |
| SE | 10.3 | 9.99 | 7.9 | 8.4 |
| *p*-value | — | NS | NS | NS |

NS = Not Significant

score of 32. Daily amounts of 3-30 mg of colloidal gold were tested in six subjects who progressively increased the intake of gold weekly from 3 mg/day to 30 mg/day, using a graduated one-ounce plastic cup. The subjects were asked to assess the effects of the colloidal gold on the parameters displayed in Table 10 and to report any side effects. No diarrhea or the other side effects associated with the aurothiolates (Table 4) were observed in this pilot study with up to 30 mg/day. CBC, urinalysis, liver panel, BUN, and serum creatinine were all within normal limits in all six subjects. According to the six subjects tested, a daily amount of 15 mg gave the best response without further improvement when higher quantities of gold were ingested. Therefore, the amount tested in the 21 subjects was 15 mg/day.

After up to nine months at 15 mg gold/day, none of the

subjects reported a worsening of any of the parameters tested. Fifteen subjects consumed it daily on a regular basis for a period of 3-9 months, and six subjects ingested the colloidal gold intermittently for 3-7 months. The regular users scored significantly higher than the intermittent users (Table 11) in the mental, physical, and performance parameters; and the mean of the total scores for the regular users was twice as high as that of the intermittent users. No adverse reaction was reported by the subjects up to nine months on colloidal gold at 15 mg/day. Some subjects were given colloidal gold with a violet color alternating with gold suspension with ruby red color. The violet color suspension passed through the 100-nm filter but not the 20-nm filter. The subjects tested with the oral preparations of ruby red color responded consistently with the beneficial effects in mental and physical parameters. However, the subjects noticed a significant difference when switched from the red preparation to the violet colloid gold which they considered to be devoid of beneficial effects.

The following are unsolicited responses conveyed by the regular users of the ruby red preparations:

- Six subjects experienced improved eyesight with greater ability to see details at a distance and less need to use eyeglasses for reading.
- Three subjects who played tennis and golf reported improved coordination with better performance noted by themselves and by other players familiar with the subjects.
- Two subjects with constipation experienced regular bowel movements while on the colloidal gold, but constipation recurred after stopping the gold colloids.
- Six subjects with morning stiffness experienced increased flexibility upon awakening.
- Two subjects quit their jobs to start their own company. They attributed this change to colloidal gold which increased their self-confidence, organizational skills, and creativity.
- A rejuvenating effect was observed after six and eight months respectively by two female users in their 50s, and they were asked by friends if they had a facelift.
- Three female subjects reported a normalizing effect of colloidal gold on their body weight (Table 12).

***Effect of Colloidal Gold on the IQ Test*:** In the previous study, a significant subjective improvement of mental performance was reported in 21 adult subjects after ingestion of a preparation of colloidal metallic gold for

*(Continued on next page)*

**Table 9**

## Effects of a Colloidal Gold Preparation (Aurasol®) at 30 mg/day for 52 Weeks on Hemoglobin, Hematocrit, White Blood Cells and Platelets in 10 RA Patients

| | White Blood Cells ( X 1000) | | Hemoglobin (g %) | | Hematocrit (%) | | Platelets ( X 1000) | |
|---|---|---|---|---|---|---|---|---|
| **Patients** | **Pre-Rx** | **52 weeks** | **Pre-Rx** | **52 weeks** | **Pre-Rx** | **52 weeks** | **Pre-Rx** | **52 weeks** |
| **1** | 6.8 | 6.4 | 14.3 | 14.0 | 42.3 | 42.0 | 276 | 305 |
| **2** | 11.2 | 9.0 | 14.2 | 14.1 | 41.9 | 42 | 294 | 390 |
| **3** | 10.5 | 7.6 | 13.3 | 13.1 | 41.1 | 40 | 447 | 275 |
| **4** | 7.0 | 5.2 | 11.9 | 13.4 | 35.9 | 40 | 419 | 332 |
| **5** | 10.6 | 7.4 | 13.1 | 12.9 | 39.1 | 38 | 248 | 211 |
| **6** | 15.4 | 8.0 | 10.4 | 13.5 | 32.3 | 41 | 453 | 359 |
| **7** | 11.5 | 12.4 | 11.7 | 13.8 | 36.7 | 43 | 423 | 359 |
| **8** | 10.8 | 10.8 | 14.1 | 13.8 | 42.8 | 41 | 317 | 226 |
| **9** | 6.7 | 5.0 | 11.8 | 12.1 | 35.5 | 36 | 418 | 381 |
| **10** | 7 | 6.6 | 12.5 | 12.3 | 38.3 | 38 | 446 | 348 |
| $\bar{X}$ | 9.8 | 7.8 | 12.7 | 13.3 | 38.6 | 40.1 | 374 | 289 |
| **SE** | 0.85 | 0.71 | 0.44 | 0.21 | 1.0 | 0.66 | 26 | 36 |
| ***p*-value** | — | <0.05 | — | NS | — | NS | — | <0.01 |

NS = Not Significant

**Table 10**

## Parameters Evaluated Following Oral Administration of Colloidal Gold (15 mg/day) in 21 Normal Subjects

| Effects Observed | | | | |
|---|---|---|---|---|
| **Score** | **0** | **-1** | **+1** | **+2** |
| **Group I** | | | | |
| Mental alertness | Same [ ] | Worse [ ] | Better [ ] | Much Better [ ] |
| Motivation | Same [ ] | Worse [ ] | Better [ ] | Much Better [ ] |
| Mental outlook | Same [ ] | Worse [ ] | Better [ ] | Much Better [ ] |
| Memory | Same [ ] | Worse [ ] | Better [ ] | Much Better [ ] |
| Creativity | Same [ ] | Worse [ ] | Better [ ] | Much Better [ ] |
| Organization | Same [ ] | Worse [ ] | Better [ ] | Much Better [ ] |
| **Group II** | | | | |
| Energy level | Same [ ] | Worse [ ] | Better [ ] | Much Better [ ] |
| Coordination | Same [ ] | Worse [ ] | Better [ ] | Much Better [ ] |
| Quality of sleep | Same [ ] | Worse [ ] | Better [ ] | Much Better [ ] |
| Resistance to cold/flu | Same [ ] | Worse [ ] | Better [ ] | Much Better [ ] |
| Skin texture | Same [ ] | Worse [ ] | Better [ ] | Much Better [ ] |
| General appearance | Same [ ] | Worse [ ] | Better [ ] | Much Better [ ] |
| **Group III** | | | | |
| Overall attitude | Same [ ] | Worse [ ] | Better [ ] | Much Better [ ] |
| Relationship with others | Same [ ] | Worse [ ] | Better [ ] | Much Better [ ] |
| Overall wellbeing | Same [ ] | Worse [ ] | Better [ ] | Much Better [ ] |
| Job performance | Same [ ] | Worse [ ] | Better [ ] | Much Better [ ] |

3-9 months at a daily dosage of 15 mg of gold. In order to use an objective and more standardized approach in evaluating the effect of colloidal gold on mental performance, the WAIS-R battery of tests[57-59] was administered to five subjects (four females, one male) age 15-45 years, before treatment, after one month on colloidal gold at 30 mg/day, and 1-3 months after stopping the gold. The subjects were supplied with colloidal gold in 16-ounce dark brown plastic bottles and told to ingest two tablespoons of the liquid daily for one month. These data are displayed in Table 13. Subjects #3 and #5 were tested one month after stopping the gold; subject #4 was tested two months after; and subject #1 and #2, three months after the end of the study. The results, published in 1998, suggest that colloidal gold at 30 mg/day improved significantly the IQ scores after only one month of administration.[57]

The verbal tests are non-learning and therefore not influenced significantly by repetition.[60] The performance tests can be learned with repetition, and this should be taken into consideration when evaluating the results displayed in Table 13. The mean scores ± standard error (SE) were respectively for pre- and post-gold administration: verbal 61.4±2.4 and 75.4±4.5 ($p<0.005$); performance 51.4±0.83 and 61.6±1.9 ($p<0.01$); total scores (IQ) 112.8±2.3 and 137±3.8 ($p<0.005$). Since both the verbal (non-learning) and performance (learning) scores contributed equally to the increased values observed in the total IQ scores following colloidal gold, the positive effect of colloidal gold cannot be attributed solely to learning the correct responses on the second test due to repetition.

**Table 11**

## Effect of Colloidal Gold at a Daily Amount of 15mg on Mental, Physical, and Performance Parameters in 21 Normal Adults

| | | | | | | | Scores | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Subjects** | **Sex** | **Age (years) X±S.E (Range)** | **Height (inches) X±S.E (Range)** | **Weight Before AU X±S.E (Range)** | **Weight After AU X±S.E (Range)** | **Duration (months) X±S.E (Range)** | **Mental X±S.E (Range)** | **Physical X±S.E (Range)** | **Performance X±S.E (Range)** | **Total X±S.E (Range)** |
| Intermittent users (6) | 4 Males 2 Females | 52.8±4.4 (34-77) | 66.8±1.7 (63-71") | 145.2±8.5 (125-180) | 145.1±8.5 (125-180) | 4.7±0.60 (3-7) | 6.3±0.85 (3-9) | 4.5±1.26 (1-10) | 4.0±0.41 (2-5) | 13.2±2.0 (8-21) |
| Regular users (15) | 6 Males 9 Females | 49.41±3.72 (32-77) | 65±1.0 (60-74) | 146±7.2 (110-178) | 133±10.6 (108-170) | 5.9±0.54 (3-9) | 9.7±0.75 (8-12) | 9.3±0.54 (5-12) | 6.3±0.36 (4-8) | 26.5±1.2 (20-31) |
| **P value** | | NS | NS | NS | NS | NS | < 0.01 | < 0.01 | < 0.05 | < 0.01 |

It is of interest to note that in two subjects (#1 and #2) who repeated the battery three months after discontinuing the colloidal gold treatment, the total IQ scores were close to baseline pre-treatment levels, whereas in two subjects who performed the test one month after stopping the gold (#3 and #5) and in one subject (#4) who did so after two months off colloidal gold, the total IQ scores were still elevated above baseline, suggesting that the effect of the gold on mental performance has a carry-over of 1-2 months after stopping the use of this preparation.

It is generally accepted that intelligence or cognitive functioning is the sum of many mental capacities. For this reason, tests that were developed to measure intelligence quotient (IQ) comprised a series of subtests evaluating the several dimensions of intelligence. Of the several IQ tests available, educators have found that the Full Scale IQ score of the Wechsler intelligence scales (WIS) battery, which is calculated from the sum of the individual scores of 11 tests, (six verbal and five performance tests) is an excellent predictor of academic achievement.[58] The revised version of this IQ test (WIAS-R) has been used extensively to assess the effect of deficiencies and supplementation of specific nutrients and the effects of sex, race, age, and education on mental performance.[57]

According to Lezak,[59] the average scores on a WIS battery provide just about as much information as do average scores on a school report card. We have observed a significant increase (20%) of the mean IQ score in five subjects aged 15-45 years after only one month on oral colloidal metallic gold at 30 mg/day. This effect persisted for up to two months following discontinuation of the gold preparation. To our knowledge, this is the first published study evaluating the effect of colloidal metallic gold on mental performance. Possible mechanisms of action of the colloidal gold preparation are only speculative at this time. However, the potential applications of a non-toxic colloidal metal with marked and rapid positive effect on mental performance are of

*(Continued on next page)*

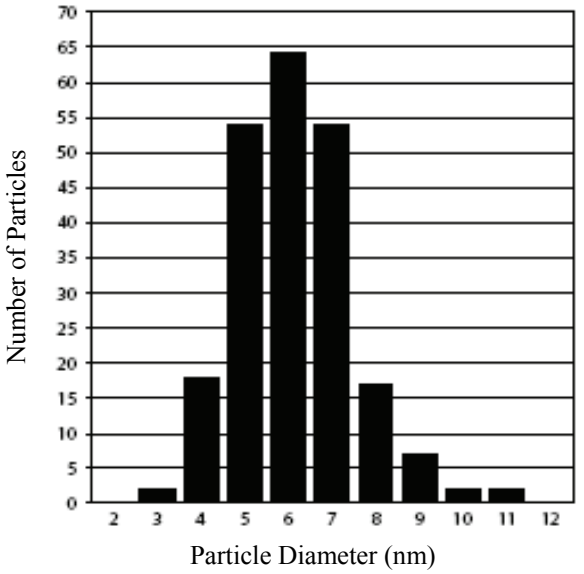
**Table 12**

## Data on 3 Regular users With Significant Change of Weight

| | | | | | | | Score | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Subjects** | **Sex** | **Age (years)** | **Height. (inches)** | **Weight Before AU** | **Weight After AU** | **Duration (months)** | **Mental** | **Physical** | **Performance** | **Total** |
| 1 | F | 61 | 62 | 100 | 108 | 3 | 11 | 9 | 6 | 26 |
| 2 | F | 56 | 65 | 158 | 140 | 6 | 12 | 12 | 7 | 31 |
| 3 | F | 51 | 61 | 155 | 135 | 7 | 8 | 8 | 7 | 23 |

**Figure 3**

**Effects of Spray Drying Colloidal Gold Aqueous Suspension on the Histogram of Particle Sizes**




great practical value, not only in scholastic settings, but also in the workplace and for improving the quality of life.

***Effects of Colloidal Gold on the Elderly*:** In collaboration with Peter Himmel, MD, and his assistant Christine Fagen, BS, the effect of colloidal gold in tablet form was studied in eight elderly patients compared to six patients receiving placebo, using a longitudinal non-crossover double-blind study. This study was funded through a grant from Optimox Corporation. Because the liquid form of colloidal gold stains clothing and would be more difficult to handle by the elderly than a tablet form, the colloidal gold suspension was dried to less than 3% moisture and compounded into tablets of 10 mg gold, using a silica based excipient (Micosolle®) as binder. TEM scanning of the gold powder following resuspension in water reveals that the particle sizes were not adversely affected by the drying process and remained below 10 nm (Figure 3).

Grape juice extract was used in the placebo tablets to match the texture and color of the colloidal gold tablets. The following parameters were assessed at baseline, and after four weeks, and eight weeks on a daily intake of 20 mg of colloidal gold (2 tablets of 10 mg), or placebo: overall well-being, short term memory, pain, fatigue, brain fog, cognitive and physical impacts, the Romberg test, and tandem walking. Measures of overall well-being, pain, and fatigue were assessed by the method of Pincus, *et al*,[54] measures of brain fog was abstracted from the scale of Zung;[60] measures of physical and cognitive performances were taken from Multi-dimensional Assessments of Fatigue Scale and developed by Belza.[61] The neurological tests, tandem walking, and the Romberg test were used as described by DeJong.[62] The four-word short-term memory test of Benedict and Horton[63] was performed in all the elderlies before and after intervention. Informed consent was obtained from all participants.

**Table 13**

**Effects of Colloidal Gold Preparation at 30 mg/day for Four Weeks on IQ in Five Subjects**

| Subject # | WAIS-R SCORES | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | PRE-GOLD | | | AFTER 4 WEEKS ON GOLD | | | 1-3 MONTHS AFTER STOPPING GOLD | | |
| | Non-Learning (verbal) | Learning (performance) | Total | Non-Learning | Learning | Total | Non-Learning | Learning | Total |
| **1** | 54 | 54 | 108 | 60 | 69 | 129 | 53 | 63 | 116 |
| **2** | 63 | 51 | 114 | 73 | 56 | 129 | 64 | 51 | 115 |
| **3** | 67 | 49 | 116 | 89 | 62 | 151 | 82 | 56 | 138 |
| **4** | 56 | 50 | 106 | 72 | 62 | 134 | 65 | 62 | 127 |
| **5** | 67 | 53 | 120 | 83 | 59 | 142 | 74 | 63 | 137 |
| | | | | | | | | | |
| **Mean** | 61.4 | 51.4 | 112.8 | 75.4 | 61.6 | 137 | 67.6 | 59 | 126.6 |
| **SD** | 5.5 | 1.8 | 5.2 | 10 | 4.3 | 8.5 | 9.8 | 4.8 | 9.9 |
| **SE** | 2.4 | 0.83 | 2.3 | 4.5 | 1.9 | 3.8 | 4.4 | 2.1 | 4.4 |
| ***p Value*** | | | | <0.005 | <0.001 | <0.005 | NS | <0.025 | <0.025 |

**Table 14**

## Clinical Data in 14 Elderly Patients

| Placebo | | | | | |
|---|---|---|---|---|---|
| **Patients** | **Sex** | **Age** | **Height (inches)** | **Weight (pounds)** | **Years/III** |
| **1** | F | 65 | 66 | 192 | 3 |
| **2** | F | 74 | 60 | 153 | 14 |
| **3** | F | 73 | 63 | 151 | 2 |
| **4** | F | 77 | 58 | 131 | 3 |
| **5** | M | 84 | 68 | 166 | 11 |
| **6** | M | 83 | 72 | 212 | 4 |
| | Mean<br>SD | 76<br>7.0 | 64<br>5.2 | 167.5<br>29.7 | 6.2<br>5.0 |
| **Aurasol®** | | | | | |
| **Patients** | **Sex** | **Age** | **Height (inches)** | **Weight (pounds)** | **Years/III** |
| 1 | F | 68 | 60 | 210 | 9 |
| 2 | M | 68 | 72 | 264 | 10 |
| 3 | F | 75 | 62 | 167 | 7 |
| 4 | M | 70 | 69 | 244 | 2 |
| 5 | F | 75 | 68 | 139 | 12 |
| 6 | F | 84 | 67 | 200 | 7 |
| 7 | F | 72 | 65 | 165 | 5 |
| 8 | F | 74 | 63 | 135 | 10 |
| | Mean<br>SD | 73.3<br>5.1 | 65.8<br>4.0 | 190.5<br>47.3 | 7.8<br>3.2 |

The anthropometric data on these 14 elderly subjects are displayed in Table 14. There was no significant difference between the placebo group and the intervention group regarding age, height, weight, and duration of illness. Both groups comprised elderly of both sexes with mean age (±SD) of: 76±7 years for the placebo group and 73±5 years for the group receiving colloidal gold.

The results of the test administered are displayed in Table 15. In the patients on placebo for up to eight weeks, there was no significant change from baseline for overall well-being, pain, fatigue, brain fog, physical impact, and cognitive impact. There was a significant (P=0.012) worsening of short term memory after eight weeks on placebo. In the group on colloidal gold, there was a significant improvement of overall well-being, fatigue, brain fog, physical impact, and cognitive impact at eight weeks post gold. The beneficial effect of colloidal gold on short-term memory and pain was evident as early as four weeks post intervention.

There was a significant improvement of equilibrium and coordination as assessed by the Romberg test and tandem walking, in the subjects on colloidal gold but no amelioration of these parameters in the group on placebo. Compared to baseline mean values, the Romberg test improved after four weeks on gold with p value of 0.017; and after eight weeks on gold to a p value of 0.004. Compared to baseline values, improvement of coordination in tandem walking showed a significance of p=0.005 at four weeks and eight weeks on gold.

***Recent Experience with Colloidal Metallic Silver*:** Colloidal silver and gold are used widely in molecular biology. Ionic silver and gold are not visible under light and EM. However, metallic colloidal silver and gold

*(Continued on next page)*

absorb and reflect electromagnetic radiation within certain bands very effectively and are visible after magnification. Silver staining of proteins is commonly used for identification after chromatography[64] and for diagnosis in neuropathology.[65] This property of metallic silver is used efficiently in pathology laboratories. A silver salt is added to the histologic preparation and reacts with proteins to form an ionic silver protein complex. The ionic silver is then reduced to metallic silver using several reducing agents. The metallic silver particles become visible under light and electron microscopy.

Besides displaying the unique properties of noble metals, such as chemical stability, excellent electrical conductivity, and catalytic activity, ionic and colloidal metallic silver were also used widely as an antibacterial, anti-viral and antifungal agent. The combined catalytic and antimicrobial properties of silver ions are currently under investigation in the preparation of biocompatible polymers with silver ions for use as a wound-healing matrix.[66] This silver-biopolymer matrix was nontoxic to the skin cells while possessing antimicrobial activity against yeast, as well as aerobic and anaerobic bacteria. Various mechanism of action of silver ions have been proposed to explain their germicidal effect including inactivation of the bacterial respiratory enzymes by binding to the sulfur moiety of proteins[67,68] and de-stabilization of intermolecular adhesion.[69]

There is a recent resurgence of interest in the antimicrobial properties of metallic (non-ionic) silver nanocolloids, which are colloids with diameters below 10 nm. In 2004, Sondi and Sondi[70] wrote: "To our knowledge, the antibacterial activity of silver ions is well-known and has been studied to detail, while the antibacterial activity of nontoxic elementary silver in the form of nanoparticles has not been reported in the literature." Obviously the Sondis were not aware that colloidal metallic silver has been used for over 100 years as an anti-germicidal agent.

A publication in 1912, by French physician BG DuHamel,[71] gives evidence of extensive applications of colloidal silver in infectious diseases worldwide: "The introduction of the metallic colloids into medicine constitutes a new departure in therapeutics, the significance of which does not appear as yet to be generally recognized. Under these circumstances, a brief review of the physical qualities, physiological properties, and therapeutical effects of these remarkable bodies presents a certain practical interest." After giving a list of infectious diseases treated successfully and safely with colloidal silver, DuHamel wrote: "A glance at the bibliographical notes will show that these remarkable results have not been obtained by any one observer or group of observers, nor in any one country. They are daily being placed on record in medical literature all over the world. In view of their daily increasing field of

**Table 15**

**Effect of Placebo and Colloidal Gold at 20 mg/day on Some Parameters of Physical Well-Being and Cognitive Functions**

| | Placebo (6) | | | Colloidal Gold (8) | | |
|---|---|---|---|---|---|---|
| **Parameters** | **Baseline** | **4 Weeks** | **8 Weeks** | **Baseline** | **4 Weeks** | **8 Weeks** |
| **Overall Wellbeing** | 7.0 ± 34 | 6.8 ± .71<br>p=.68 | 6.6 ± .41<br>p=.23 | 5.0 ± .89 | 5.9 ± 36<br>p=0.21 | 7.1 ± .56<br>p=0.011 |
| **Pain** | 6.6 ± 1.0 | 5.9 ± 1.2<br>p=.72 | 6.4 ± 1.3<br>p=.92 | 3 ± .74 | 4.5 ± .74<br>p=0.02 | 5.0 ± .8<br>p=0.009 |
| **Fatigue** | 4.9 ± 1.2 | 3.4 ± .77<br>p=.19 | 4.8 ± 1.2<br>p=.97 | 3.8 ± .66 | 4.7 ± .42<br>p=.33 | 6.6 ± .69<br>p=0.019 |
| **Brain Fog** | 7.0 ± .58 | 5.7 ± 1.0<br>p=0.066 | 7.2 ± .61<br>P=0.46 | 5.6 ± .63 | 5.8 ± .8<br>p=.78 | 7.6 ± 0.42<br>p=.014 |
| **Physical Impact** | 17 ± 2.9 | 18 ± 2.2<br>p=.36 | 17.7 ± 2.3<br>p=.77 | 21.8 ± 1.9 | 22.4 ± 2.2<br>P=.75 | 18.1 ± 2.7<br>p=0.042 |
| **Cognitive Impact** | 13 ± 2.8 | 11.8 ± 2.7<br>p=.51 | 11.2 ± 2.9<br>p=.32 | 17.6 ± 2.9 | 16.6 ± 2.2<br>p=.32 | 10.7 ± 3.2<br>p=0.022 |
| **Short Term Memory** | 14.8 ± 16 | 13.5 ± 1.4<br>p=0.102 | 13.2 ± 1.2<br>p=-0.012 | 11.9 ± 1.1 | 14.3 ± 1.2<br>p=0.012 | 16.1 ± 3.2<br>p=0.003 |

usefulness, it can hardly be doubted that a new and interesting chapter has been opened up in contemporary therapeutics by the introduction of colloid metals. One point stands out prominently, and that is the absolute innocuousness of these bodies, whether injected into the veins or muscles or into the spinal canal."

Improved technology recently has allowed a more in-depth study of the anti-germicidal properties of colloidal silver and of the mechanism of action involved. The Sondis prepared colloidal metallic silver by reduction of silver nitrate with ascorbic acid using a surfactant as stabilizer.[70] The particle sizes average 12 nm, with a range of 4-32 nm. At concentration of 10 µg silver/ml (10ppm), *in vitro* tests showed a 70% inhibition of bacterial growth of *E. coli* on agar plates.

In 2005, Morones, *et al*,[72] reported the results of their experiments with the use of colloidal metallic silver. These investigators studied the effect of silver colloids in the range of 1-100 nm on gram-negative bacteria inoculated on agar plates. Only silver nanocolloids less than 10 nm were able to bind to the bacterial membrane and penetrate inside the bacteria. They reported that these silver nanocrystals were spherical in shape. The overall effect of metallic silver nanoparticles was different from the effect of silver ions which acted mainly at the surface of the membrane and triggered a protective mechanism in the bacteria. The metallic silver nanocrystals did not trigger this protective mechanism and were able to penetrate inside the bacteria. To summarize their findings, spherical silver nanoparticles in the range of 1-10 nm attach to the surface of the bacterial cell membrane and drastically disturb its proper function, like permeability and respiration. These silver nanoparticles are also able to penetrate inside the bacteria and cause further damage by possibly interacting with sulfur and phosphorus containing compounds.

Yamanaka, *et al*, reported in 2005, that silver ions could penetrate inside bacteria,[73] contrary to Morones' findings. However, the procedure Yamanaka, *et al*, used to prepare the silver suspension is well known to generate colloidal metallic silver with a net positive charge although not ionic silver per se. Because colloidal metallic silver has a net positive charge, it is sometimes called ionic silver, confusing it with ionic silver salts. Quoting Yamanaka, *et al*: "An aqueous solution with a silver ion concentration of 900 ppb was electrolytically prepared by applying a current of 12.5 mA for 28 s between two silver plate electrodes installed in water." This electrolytic process is widely used to generate metallic silver colloids. Ionic silver is not visible under TEM. The fact that they visualized silver particles inside the bacteria by TEM, with the identification of silver by EDX, is proof that they used metallic colloidal silver. One hundred years ago, DuHamel[71] described the electrolytic preparation of colloidal silver having a brownish-red color, characteristic of colloidal metallic silver *(not ionic silver salt which has a milky white appearance)*. Quoting DuHamel: "The physical method consists in passing the electric arc between electrodes of the metal to be converted into colloid, plunged into distilled water. Under these conditions, the electrodes throw off an extremely fine powder which remains suspended in the liquid. A solution of electric colloid silver (small-grained) is of a brownish-red color."

It is likely that the antibacterial effect of metallic silver nanoparticles reported by Sondi[70] was due to particle sizes below 10 nm, as reported by Morones, *et al*.[72] A recent study of the effect of silver nanoparticles on HIV-1 confirms the 10 nm size limit for effectiveness. Elechiguerra, *et al*,[74] used silver nanoparticles with a mean diameter of 21 nm but with a wide range of sizes. They demonstrated that silver nanoparticles undergo a size-dependent interaction with HIV-1, with nanoparticles exclusively in the range of 1-10 nm binding to the HIV-1 at the exclusion of larger colloidal particles.

In a 2008 publication, Choi and Hu[75] reported that colloidal silver particles below 5 nm displayed toxicity to friendly nitrifying bacteria used in water treatment plants. This was the first report of adverse effects of colloidal silver properly characterized. These findings would need confirmation by other investigators. If Choi and Hu's findings can be confirmed for other friendly bacterias, such as the saprophytes of the intestinal tract, then the ideal size of colloidal silver for medicinal uses would be 5-10 nm. However, there is no report of adverse effects due to colloidal silver on the flora of the intestinal tract so far.

Colloidal metallic silver was prepared by the author using reduction of an aqueous solution of silver nitrate (USP grade) in the presence of maltodextrins (food grade) as stabilizers, just like the procedure described previously for gold. Citrate was not required since silver nitrate is easily reduced by proteins and other macro-molecules. Using a proprietary procedure, stable colloidal silver at high concentration (5g/L; 5,000ppm) could be achieved and with particle sizes below 10 nm. This was important in order to prepare a dried powder form with high colloidal silver content and with very small particle sizes consistently. Particle size analysis by TEM of the silver suspension is displayed in Figure 4. Over 99% of the particles were less than 10 nm.

The colloidal silver suspension was evaporated to less than 3% moisture, and the powder was compounded

*(Continued on next page)*

with a silica-based excipient (Micosolle®) into tablets containing 12.5 mg metallic silver per tablet. From the elemental analysis of the silver colloid powder by ICP-MS, all the toxic metals were below the sensitivity of the assay: As <0.001ppm; Se <0.001ppm; Cd <0.001ppm; Sb <0.001ppm; Hg <0.0001ppm; and Pb <0.001ppm. Sodium benzoate used as bacteriostatic agent in the colloidal gold suspensions (Aurasol®) was not required in the colloidal silver preparation since colloidal silver has itself germicidal properties. This preparation was called NanoSilver.

Over the past two years, the NanoSilver tablets containing 12.5mg silver, were tested in normal subjects at daily amounts of 1-6 tablets for up to two years. No side effect was reported, and there was no evidence of toxicity based on hematology, blood chemistry, and urine analysis in the subjects tested. While on the colloidal silver, no subject experienced upper respiratory tract infections, and those with sinus problems experienced runny nose for 1-2 weeks, with clearing of sinuses afterward. However, within two weeks off the preparation, they reported experiencing the usual symptoms of recurrent colds and sinus infections. Unexpected were the observations reported by some subjects on 3-6 tablets/day — increased mental alertness, more energy, and improved eyesight for close and distant vision. These preliminary results are currently under investigation and will be the subject of a more detailed report.

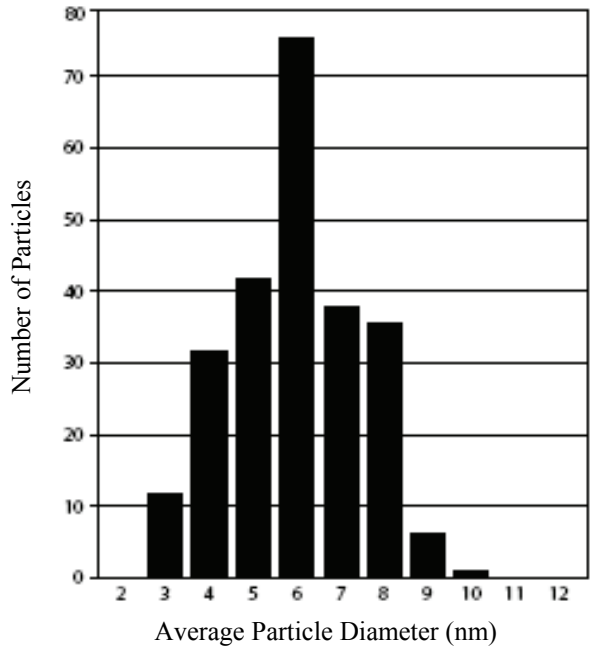
**Discussion**

Data available in the published literature give compelling evidence that colloidal metallic gold is nontoxic both *in vivo* and *in vitro*.[22-27] We found no evidence of toxicity and no side effects in 10 RA patients receiving 30mg/day of gold nanocolloids (Aurasol®) for up to one year.[53] The RA patients who received ionic gold previously in the forms of aurothiolates did not respond satisfactorily to this form of gold and experienced serious side effects (Table 5). All 10 RA patients responded to colloidal gold with significant improvement of all major signs and symptoms as a group. None reported side effects of significance. Although our study was published more than 10 years ago, there has been no published study confirming our findings and no interest from the drug industry to introduce colloidal gold as a safe and effective alternative to the toxic aurothiolates in RA patients.

Colloidal metallic gold (Aurasol®) at 30 mg/day for nine months in a 54-year-old female with class II RA, not responding to previous treatment modalities, resulted in complete remission of RA symptomatology after a total of 7 g of gold. After 10 years off the gold preparation, she was still symptom-free and off all other medications.

**Figure 4**

**Histogram of Particle Distribution of Colloidal Silver prepared by Author TEM Performed by RH Albrecht**




A year ago, she resumed ingestion of one tablet of 10 mg gold (Aurasol®) for the other beneficial effects of gold but not for RA.

The purpose of the gold project, which started 23 years ago, was to confirm previous reports from several hundred years ago, discussing the beneficial effects of colloidal gold — improved eyesight, euphoric anti-depressant, cardiac tonic, and anti-aging to name a few. In some of the normal subjects, gold nanocolloids at 15-30 mg/day improved eyesight for both close and distant vision. Colloidal silver seems to share that effect of colloidal gold on visual acuity when used in amounts within the same range as the amounts of colloidal gold, and with particle sizes below 10 nm.

Paracelsus prescribed colloidal gold in cases of "melancholy because it makes one's heart happy." It is of interest that several subjects on colloidal gold at 15-30 mg/day observed the same mood elevation reported previously. Some even used the same expression, "It makes me happy." Colloidal gold improved mental alertness and cognitive functions, raising IQ scores by a mean of 20% in five subjects tested following only one month on 30 mg gold/day.[57] Gold nanocolloids had a normalizing effect on body weight in the normal subjects. In RA patients, colloidal gold normalized red blood cells, white blood cells, and platelets.

In the elderly, colloidal gold in tablet form at a daily

intake of 20 mg for eight weeks was evaluated using various parameters. These parameters were assessed by procedures previously validated. The design was double-blind longitudinal non-crossover, using grape juice extract in the placebo tablets to give the same appearance as the colloidal gold tablets. The results are displayed in Table 15. There was no beneficial effect on these parameters in the elderly receiving placebo tablets. In the subjects on colloidal gold, there was significant improvement of overall well-being, coordination, equilibrium, pain, energy level, cognition, physical well-being, and short-term memory. Whether an essential element or not, gold nanocolloids may be useful on a long-term basis in the elderly because of their beneficial effect on mental and physical well-being.

***Possible Mechanisms of Actions of Colloidal Gold*:** Metallic colloidal gold possesses properties that could explain its physiological effects. Gold is an excellent reflector of near infrared radiations (NIR).[76-78] Cells transmit, receive, and act upon signals of NIR in predictable manners. Properly located in the cells near transmitters (still unknown) and receivers (centrosomes) of NIR, colloidal gold with small particle sizes (less than 10 nm) could act as wide angle diffusers of NIR signals. Since spatial coherence of electromagnetic signals is required for cellular recognition,[79,80] wide angle diffusers would favor spatial coherence of NIR signals by reaching the whole circumference of neighboring cells at the same time. Intercellular exchange of information would be increased.

Very low radiant exposure of NIR has profound effects on cellular functions: improvement of wound healing;[81,82] increase of collagen synthesis in human skin fibroblast;[83] enhancement of oxidative metabolism in phagocytes;[84] and proliferation of macrophages.[85] The stimulating effect of colloidal gold via NIR on collagen synthesis could explain its rejuvenating effect. Small gold colloids (less than 10 nm) could serve as carriers of signal molecules between cells and between the cytoplasm and the nucleus, increasing intercellular and nuclear cyptoplasm exchange of information, stimulating M-RNA synthesis, even in non-proliferating cells since the nuclear pore sizes in confluent cells are large enough to allow passage of these nanocolloids.[86,87]

Colloidal gold elicited a protective immune response in mice inoculated with plamids encoding Japanese encephalitis virus.[88] Small gold colloids, less than 10 nm, adsorb on the Fc portion of IgG antibodies,[89] leaving the FAB active sites more available for binding to antigens. This stabilizing effect of gold colloids on Ig could improve immune functions and increase resistance to infections.

Since metallic gold is an excellent conductor of electricity on the macroscale, one other possible mechanism of action of colloidal metallic gold in the cells is to serve as a reservoir of monoatomic metallic gold which is slowly released to act as a subcellular superconductor by increasing the speed of intra- and intercellular communications. In this way, intracellular colloidal gold would increase not only the amount of information exchanged between cells within subcellular organelles but also would increase markedly the speed of this transfer of information. Such mechanisms could explain the improved cognitive functions following colloidal gold ingestion in the normal subjects and the elderly.

Electron transfer is strongly catalyzed by colloidal gold in oxidation-reduction reactions.[90] The smaller the particle size, the greater the catalytic effect. Electron transfer is involved in the quenching of free radicals by antioxidants. Since free radical damage is believed to be involved in RA[91] and in the aging process, colloidal gold may exert its anti-aging and anti-RA effects by potentiating the quenching effect of antioxidants on free radicals. Stabilization of lysosomes could result from blockage of free radical damage to the lysosomal membrane by catalyzing electron transfer reactions and from inhibition of lysozymes through electromagnetic modulation. Inhibition of lysozymes can be achieved by certain frequencies of electromagnetic radiation.[92] Since inhibition of lysozymes stabilizes lysosomes,[93] stabilization of lysosomes by colloidal gold could therefore be due to amplification and diffusion of NIR or other electromagnetic signals from other cells which would have an inhibitory influence on lysozymes and a stabilizing influence on lysosomes.

The cardiotonic effect of gold nanocolloids could be due to the smaller particles (less than 6 nm) which are able to penetrate inside the mitochondria and nucleus of the cardiac myocytes. Salnikov, *et al*,[94] reported in 2007, that in isolated rat ventricular myocytes, only gold nanocolloids of 3-nm diameter could penetrate inside the nucleus and the mitochondria whereas particles of 6 nm could cross the cell membrane and concentrate in the cytosol but not in the mitochondria and nucleus of ventricular myocytes. The effect of small gold particles on mitochondrial synthesis of ATP could explain their cardiotonic effect.

In several early publications, an anti-carcinogenic effect of gold salts has been reported. Lewis[95] postulated in 1913, that the antitumor effects of gold salts were due to *in vivo* formation of colloidal gold, which was the active ingredient. His postulate was based on the following experimental results in laboratory animals:

1) Gold compounds that could not be transformed

*(Continued on next page)*

into colloidal gold *in vivo* were ineffective as anti-tumor agents.

2) Gold salts that could disproportionate *in vivo* to form colloidal gold caused necrosis, softening and reduction of tumor sizes, but some animals died from hemorrhage, which Lewis attributed to vascular damage by the salts themselves.

Colloidal gold administration resulted in tumor softening and reduction in size, without hemorrhage. Parenteral colloidal gold administration was reported to have a suppressive effect in mouse leukemia and to potentiate the effect of methotrexates.[96] B-chronic lymphocytic leukemia (BCLL) is an incurable disease characterized by apoptosis resistance. Colloidal gold with particle sizes less than 4 nm enhanced apoptosis in BCLL cells *in vitro*.[97] Angiogenesis, the formation of new blood vessels, is essential for the growth and propagation of tumors. Gold nanocolloids of 5 nm average size decreased angiogenesis by binding to heparin-binding growth factors.[98,99]

The analgesic effect of colloidal gold in RA patients[53] could be due to its effect on opioidergic receptors. In mice and rats exposed to various noxious physical and chemical algesics, colloidal gold prepared by ayurvedic procedures resulted in an analgesic effect.[100] Since naloxone blocked the analgesic effect of gold colloids, Bajaj and Vahora concluded that the analgesic effect of gold colloids was due to an opioidergic mechanism. This opioidergic effect was not observed with the aurothiolate form of gold, which is the ionic, non-metallic form. Bajaj and Vahora commented that colloidal gold was still popular in India (as of 1998) and is "highly valued for its tonic and rejuvenating properties." According to these investigators, ayurvedic physicians recommend colloidal gold as general tonic, hepatotonic, cardio tonic, nervine tonic, aphrodisiac, detoxicant, anti-infective, and anti-aging.

***Proposed Protocol for the Evaluation of Colloidal Gold and Silver in Clinical Medicine*:** Although colloidal gold is the main subject in this section, the recommendations proposed for colloidal gold are applicable also to colloidal silver. Colloidal metallic gold and silver are used extensively in molecular biology, and their properties have been well studied and published in peer review journals. In Table 2 are displayed the different methods of preparation of colloidal gold currently available to the public, and they are compared with the present method of reduction of the trichloride salt. Although there are various methods of preparation of colloidal gold, the reduction of gold trichloride lends itself better to the preparation of various particle sizes of gold colloids with predictable properties. Silver nitrate is the preferred starting material for the preparation of silver nanocolloids in high concentrations.

Only colloidal gold prepared by reduction of the trichloride salt has been well-characterized and its properties published in peer review journals. No evidence of cytotoxicity of metallic colloidal gold has been reported in several *in vitro* and *in vivo* studies. A system of classification of colloidal gold based on particle sizes has been defined. Au5, for example, describes an aqueous suspension of colloid gold with an average particle size of 5 nm. The stabilizer used is identified by a prefix. BSA-Au5 signifies that bovine serum albumin was used as a stabilizer against flocculation. This system could be applied to the characterization of colloidal gold dispersions prepared for medicinal use. In liquid preparation, an antimicrobial preservative could be used to prevent bacterial and mold growth. Colloidal silver would be ideal for such a purpose.

Prudence would dictate that the same basic laboratory work-up prior to and during implementation of aurothiolates be implemented with colloidal gold therapy as a precautionary measure. The adverse reactions described with the use of aurothiolates (Table 4) should be carefully monitored in patients receiving colloidal gold. These precautionary measures implemented for evaluating the potential adverse reactions to colloidal gold could be progressively scaled down and eventually eliminated as a general consensus becomes available on the safety of metallic colloidal gold, prepared by well-defined procedures, characterized by relevant laboratory tests, and used in amounts within a well-defined range for a fixed period of time. It is possible that administration of colloidal gold would be required only for a fixed period of time to achieve complete remission of RA. The author described one such case in this manuscript. Gold nanoparticles could also be used on a long-term basis for their beneficial effect on mental and physical well-being.

A center for colloidal gold research would be desirable for retrieval and dissemination of information on published results of clinical studies. The first project for this center would be to publish an extensive review of available data on the preparation, characterization, and current use of colloidal gold. The second project would involve standardization of the preparation of the aqueous dispersion of colloidal gold for medicinal purposes. A third project would involve the study of bioavailability of colloidal gold, of different particle sizes, their metabolism, distribution, biological half life, and physiological effects. A fourth project would test the safety and efficacy of colloidal gold in RA and other pathologies.

An ongoing project for the research center would be to monitor clinical responses and potential adverse

reactions in medicinal preparations of colloidal gold and silver. Patients may develop skin rashes, itching and diarrhea if the trichloride salt is not completely reduced. If the starting form of the gold used in the preparation of the colloidal gold is the trichloride salt, a cathionic form with oxidant properties and with adverse reactions on the skin and gastrointestinal tract, a complete reduction of the cathionic gold, confirmed by the absence of a UV absorption peak at 290 nm in the supernatant after ultracentrifugation, would be a *sine qua non* requirement for use of this preparation in clinical medicine.

To confirm that all the ionic silver has been reduced to metallic colloidal silver, the silver ion-selective electrode procedure could be used to measure the concentration of ionic silver in the preparation. Ionic silver should be undetectable by the ion-selective electrode measurement in order to use silver nanocolloids suspension for medicinal purposes. The duration of colloidal gold and silver ingestion should be monitored and their effects on the whole blood, serum, and urine levels assessed periodically.

There is no published study on the bioavailability and the metabolism of gold colloids in any animal species. The only balance study in animal and human subjects involved the oral aurothiolates to be discussed later. Hillyer and Albrecht[101] in a 2001 publication reported the first study of the effect of different particle sizes of colloidal gold at 4 nm, 10 nm, 28 nm, and 58 nm on the concentration of gold in various organs of mice drinking water with 200 ppm of colloidal gold. Part of the data is compiled in Table 16. In all the organs studied, the smaller particle sizes of gold resulted in the highest concentrations in the organs analyzed. Since these authors did not perform balance studies in these mice, the amount of gold absorbed and the bioavailability of orally administered colloidal gold could not be computed.

As previously mentioned, the only balance study on gold in human subjects[102,103] involved the orally active aurothiolate Auranofin (Ridaura) from Smith, Kline, and French. At 6 mg/day, an average of 25% is absorbed and 75% is excreted in the feces. The elimination of the 25% retained is equally divided between renal excretion and fecal excretion. At steady state following several weeks on this compound at 6 mg/day, the blood gold concentration averages 0.5 mg/L. The half life of this aurothiolate varies from 2-3 weeks after discontinuation. The major depots of gold from aurothiolates are in decreasing order: bone marrow, liver, skin, and bone. These four depot sites represent 85% to the total body gold content.

In subjects not receiving gold and silver preparation for medicinal purpose, the estimated total body content of gold is 9.8 mg and for silver 0.8 mg.[104] Whole blood levels reported in the literature ranged from 0.37 to 120 ppb for silver and a mean value 0.21 ppb for gold. According to Perrelli and Piolatto,[104] metallic gold is excreted mainly via the kidneys whereas 90% of ingested metallic silver is eliminated via feces.

Plasma gold levels were measured by ICP-MS (BioTrace Lab, Salt Lake City, Utah) in six normal subjects screened by the author and without any evidence of gold ingestion aside from food and no dental prosthesis with gold. The levels were all below 1 ppb (1 μg/L). Using neutron activation analysis, the gold level was measurable in all six subjects, ranging from 0.10-0.72 ppb, after subtracting the mean value of 5 water blanks with mean ± SD of 0.28±0.066 ppb. The mean ± SD of serum gold corrected for blanks was 0.53±0.31 ppb.

Gold levels were measured in serum and whole blood in one male subject after ingestion of 30 mg colloidal gold (Aurasol®) in a liquid suspension. The values obtained by ICP-MS in serial samples are displayed in Table 17. Peak serum gold level was achieved in 5 minutes post-ingestion and became undetectable at 30 minutes. On the other hand, whole blood levels peaked at 30 minutes and became undetectable by eight hours post-ingestion. This suggests a rapid clearance of colloidal gold from the peripheral circulation, whereas aurothiolates have a very long half life with over 90% of blood gold bound to serum albumin.[103] The fraction of gold thiolate associated to red blood cells is not inside the cells but is bound to the erythrocyte membrane.[105]

Research performed on the glomerular filtration of small solutes, water, and macromolecules predicts a two-pore system: a large number of small pores of average diameter of 10 nm; and a limited number of large pores having a diameter of 50 nm.[106] The small pores possess a negative charge which theoretically should repel negatively charged molecules. However, research performed on negatively charged small molecules revealed a minimal effect of charged molecules on their glomerular filtration rate.[107] Therefore, renal clearance of colloidal gold particles possessing a net negative charge of diameters 10 nm or less should be very efficient. This could explain the rapid clearance of gold nanocolloids in the male subject studied. Following this line of reasoning, urinary concentration of gold could be useful in assessing the bioavailability of ingested gold nanocolloids. The intestinal absorption of colloidal gold favors small particle sizes.[101] If gold colloids are administered parenterally instead of by ingestion, the renal clearance of large particles would be very low, resulting in a longer blood half life.

The toxicity of the gold aurothiolates was described previously in this manuscript. The clinical side effects

*(Continued on next page)*

**Table 16**

**Effects of Particle Sizes of Colloidal Gold Ingested by Mice* on Their Concentrations in Various Organs (compiled from reference 101)**

| | Concentrations in PPB (mean ± SD) | | | | | |
|---|---|---|---|---|---|---|
| **Particle Size** | **Blood** | **Brain** | **Heart** | **Lung** | **Liver** | **Kidney** |
| **4nm** | 6.8 ± 0.7 | 4.7 ± 0.26 | 15 ± 2 | 32.4 ± 9.4 | 21.3 ± 3.6 | 75.4 ± 10.7 |
| **10nm** | 0.77 ± 0.07 | 2.1 ± 0.8 | 7.3 ± 3.3 | 8.6 ± 5 | 2.8 ± 0.3 | 17.4 ± 1.5 |
| **28nm** | 0.47 ± 0.2 | 0.7 ± 0.2 | 1.5 ± 0.8 | 0.8 ± 0.35 | 1.2 ± 0.03 | 6.2 ± 1.5 |

* Daily intake approximately 200 micrograms gold for 7 days. Gold levels measured at 12 hours after last dose.

are displayed in Table 4. We previously discussed several publications on the toxicity of aurothiolates at the cellular and molecular levels,[53] most of which are probably due to the gold trichloride formed *in vivo* by disproportionation. On the other hand, there is no evidence of toxicity of colloidal metallic gold at the clinical,[53] histological cellular and molecular levels.[22-27] It is obvious that colloidal metallic gold in the low nanometer range is a safe and effective alternative to the toxic aurothiolates in the management of RA.

If the starting material for the preparation of gold nanocolloids is auric chloride, care must be taken to ensure that all the ionic gold has been reduced to metallic colloidal gold in order to avoid the side effects of the ionic gold in the preparation. Ionic silver is associated with a grayish-blue discoloration of the outer tegument mainly of the exposed areas. It is called argiria,[108] and it has never been documented in subjects ingesting silver nanocolloids well characterized in term of particle sizes and absence of ionic silver in the ingested preparation. The same skin discoloration was described with the use of ionic gold in the treatment of tuberculosis and RA. This side effect is called chrysiasis,[109] and has never been reported with the use of colloidal metallic gold.

In a review on the toxicological aspects of topical silver pharmaceuticals, by Hollinger,[110] all the ionic silver preparations displayed toxicity. The only metallic colloidal silver tested was without any vitro toxic effect although possessing bacteriostatic property. In susceptible mice, ionic silver induced antinuclear autoantibody production.[111]

In studies performed *in vivo* on pregnant rats, gold colloids of particle sizes 4 nm, 5 nm, 30 nm, and 200 nm did not cross the placenta to any significant extent in either direction, that is from mother to fetus and from fetus to mother.[112,113] If these findings can be extrapolated to human subjects, colloidal gold ingestion during pregnancy would not be expected to result in the beneficial effects of gold nanocolloids in the offspring.

In summary, it is important to make a clear distinction between the ionic and metallic forms of gold and silver. The ionic forms of gold and silver display toxicity both *in vivo* and *in vitro*. On the other hand, the metallic colloidal forms of these metals are extremely safe. There is convincing evidence presented here to suggest that an upper limit of 10-nm diameter of colloidal gold and silver is required for physiological and clinical effects. Further research with these gold and silver nanocolloids seems very promising.


### Acknowledgement

The author wishes to express his gratitude to Peter B. Himmel, MD, and Christine Fagen, BS, for collaborating on the colloidal gold studies in RA patients and the elderly; to Ralph M. Albrecht for performing the TEM particle sizing, UV-VIS spectrum pre- and post-ultracentrifugation, and the neutron activation analysis of the serum samples; to Ameen Lalani and Regina Casillas for typing the manuscript.


### About the Author


*Guy E. Abraham, MD, is a former Professor of Obstetrics, Gynecology, and Endocrinology at the UCLA School of Medicine. Some 35 years ago, he pioneered the development of assays to measure minute quantities of steroid hormones in biological fluids. He has been honored as follows: General Diagnostic Award from the*

**Table 17**

**Serum and Whole Blood Levels of Gold* in a Male Subject Following Ingestion of 30 mg of Colloidal Gold (Aurasol®)**

| Time After Ingestion | Serum Level PPB (µg/L) | Whole Blood Level PPB (µg/L) |
|---|---|---|
| **0** | <1.0 | <1.0 |
| **5 Minutes** | 12.6 | 7.6 |
| **15 Minutes** | 5.3 | 8.1 |
| **30 Minutes** | <1.0 | 8.5 |
| **60 Minutes** | <1.0 | 7.7 |
| **2 Hours** | <1.0 | 3.7 |
| **4 Hours** | <1.0 | 3.0 |
| **8 Hours** | <1.0 | <1.0 |

* Measured by ICP-MS at BioTrace Lab, Salt Lake City

*Canadian Association of Clinical Chemists, 1974; the Medaille d'Honneur from the University of Liege, Belgium, 1976; the Senior Investigator Award of Pharmacia, Sweden, 1980. The applications of Dr. Abraham's techniques to a variety of female disorders have brought a notable improvement to the understanding and management of these disorders.*

*Twenty-five years ago, Dr. Abraham developed nutritional programs for women with premenstrual tension syndrome and post-menopausal osteoporosis. They are now the most commonly used dietary programs by American obstetricians and gynecologists. Dr. Abraham's current research interests include the development of assays for the measurement of iodide and the other halides in biological fluids and their applications to the implementation of orthoiodo-supplementation in medical practice.*

## REFERENCES


1) Petrucci F, *et al*. "Biomonitoring of a worker population exposed to platinum dust in a catalyst production plant." *Occupational and Environmental Medicine*, 2005; 62:27-33.
2) Melichar B, *et al*. "Gastrointestinal permeability in ovarian cancer and breast cancer patients treated with Paclitaxel and platinum." *BMC Cancer*, 2007; 7:155.
3) Hainfeld JF. "A small gold-conjugated antibody lable: Improved resolution for electron microscopy." *Science*, 1987; 236:450.
4) Frens G. "Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions." *Nature Phy Sci*, 1973; 241:20-22.
5) Horisberger M and Rosset J. "Colloidal gold: A useful marker for transmission and scanning electron microscopy." *J Histochem & Cytochem*, 1977; 25:295-305.
6) Goodman SL, Hodges GM, *et al*. "A review of the colloidal gold marker system." *Scan Electron Microscopy*, 1980; 11:133-146.
7) Everett DH. *Basic Principles of Colloid Science*. The Royal Society of Chemistry, London, 1988; 5-36.
8) De Roe C, Courtoy PJ, *et al*. "A model of protein-colloidal gold interactions." *J Histochem & Cytochem*, 1987; 35:1191-1198.
9) Sutton BM and Dimartino M. "Gold." In: *Handbook on Toxicity of Inorganic Compounds*. Seiler HG and Sigel H, editors. Marcel Dekker, Inc., New York, 1988; 307-314.
10) *Goodman & Gilman's: The Pharmacological Basis of Therapeutics*. McGraw-Hill, New York, 1996; 644-646.
11) Mahdihassan S. "Cinnabar-gold as the best alchemical drug of longevity, called makaradhwaja in India." *Am J Chinese Med*, 1985; 13:93-108.
12) "Exodus (32:19-20)." *The Oxford Study Bible.* Oxford University Press, New York, 1992.
13) Higby GJ. "Gold in medicine, A review of its use in the West before 1900." *Gold Bulletin*, 1982; 15:130-140.
14) Weiser HB. *Inorganic Colloidal Gold Chemistry*. Wiley, New York, 1933; 1:21-57.
15) Faraday MX. "The Bakerian Lecture — Experimental relations of gold (and other metals) to light." *Phil Trans R Soc Lond*, 1857; 147:145-181.
16) Horisberger M. "The gold method as applied to lectin cytochemistry in transmission and scanning electron microscopy." *Technique in Immunocytochemistry*, 1985; 3:155-178.
17) Glazman YM. "Effect of surface-active agents on stability of hydrophobic sols." *Faraday Discuss*, 1966; 42:255-266.
18) Hawkins HK, Rehm LZ, *et al*. "Colloidal gold labeling of sections and cell surfaces." *Ultrastructural Pathol*, 1992; 16:61-70.
19) Maclagan NF. "The preparation and use of colloidal gold sols as diagnostic agents." *J Exp Path*, 1947; 27: 369-377.
20) Komiyama A and Spicer SS. "Microendocytosis in eosinophillic leukocytes." *J Cell Biol*, 1975; 64:622-635.
21) Aonuma K. "Colloidal gold uptake as a marker for monocyte differentiation and maturation in normal and leukemic cells." *Int'l Hematology*, 1992; 55:265-274.
22) Feldherr C and Akin D. "The permeability of nuclear envelope in dividing and nondividing cell culture." *J Cell Biol*, 1990; 111:1-8.
23) Feldherr C and Akin D. "Signal-mediated nuclear transport in proliferating and growth-arrested BALA/c 3T3 cells." *J Cell Biol*, 1991; 115:933-939.
24) Danien BJ, Sims PA, *et al*. "Use of colloidal gold and neutron activation in correlative microscopic labeling and label quantitation." *Scanning Microscopy*, 1995; 9:773-780.
25) Ackerman GAM and Wolken KW. "Histochemical evidence for the differential surface labeling. Uptake, and intracellular transport of a colloidal gold-labeled insulin complex by normal human blood cells." *J Histochem & Cytochem*, 1981; 29:1137-1149.
26) De Roe C, Courtoy PJ, *et al*. "Molecular aspects of the interactions between protein, colloidal gold and cultured cells: Applications to galactosylated serum albumin and rat hepatocytes." *Arch Intern Physiol Biochem*, 1982; 90:186.
27) Juurlink BHJ and Devon RM. "Colloidal gold as a permanent marker of cells." *Experientia*, 1991; 47:75-77.
28) Garner M, Rglinski J, *et al*. "The interaction of colloidal metal


*(Continued on next page)*

with erythrocytes." *J Inorg Biochem*, 1994; 56:283-290.
29) Lazareic MB, Yan K, *et al*. "Effect of gold compounds on the activity of adenylyl cyclase in human lymphocyte membranes." *Arthritis & Rheumatism*, 1992; 35:857-864.
30) Vint IAM, Foreman JC, *et al*. "The gold anti-rheumatic drug Auranofin governs T-cell activation by enhancing oxygen free radical production." *Eur J Immunol*, 1994; 24:1961-1965.
31) Sato H, Yamaguvhi M, *et al*. "Induction of stress proteins in mouse peritoneal macrophages by the anti-rheumatic agents gold sodium thiomalate and Auranofin." *Biochem Pharmacol*, 1995; 49:1453-1457.
32) Cahill RNP. "Effect of sodium aurothiomalate myocrisin on DNA synthesis in phytohaemagglutin-stimulated cultures of sheep lymphocytes." *Experientia*, 1971; 27:913-914.
33) Lorbat A, Simon T, *et al*. "Chrysotherapy, suppression of immunoglobulin synthesis." *Arthritis Rheum*, 1978; 21:785-791.
34) Forestier J. "La chrysotherapie dans les rhumatismes chroniques." *Bull et Mem Soc Med des Hop de Paris*, 1929; 44:323-329.
35) Forestier J. "Rheumatoid arthritis and its treatment by gold salts." *J Lab Clin Med*, 1935; 20:827-840.
36) Empire Rheumatism Council. "Gold therapy in rheumatoid arthritis. Final report of a multicenter controlled trial." *Ann Rheum Dis*, 1961; 20:315-324.
37) Geddes DM and Brostoff J. "Pulmonary fibrosis associated with hypersensitivity to gold salts." *BMJ*, 1976; 1:1444.
38) Gould PW, McCormack PL, *et al*. "Pulmonary damage associated with sodium aurothiomalate therapy." *J Rheumatol*, 1977; 3:181-182.
39) Scott DL, Bradby GV, *et al*. "Relationship of gold and penicillamine therapy to diffuse intestinal lung disease." *Ann Rheum Dis*, 1981; 40:136-141.
40) Belleli A, Boiardi L, *et al*. "Diffuse intestinal lung disease associated with hypersensitivity to gold salt." *Clin Exp Rheumatol*, 1985; 3:181-182.
41) Kay AGL. "Myelotoxicity of gold." *BMJ*, 1976; I:1266-1268.
42) Coblyn JS, Weinblatt M, *et al*. "Gold-induced thrombocytopaenia; A clinical and immunogenetic study of twenty-three patients." *Ann Intern Med*, 1981; 95:178-181.
43) Adachi JD, Benson WG, *et al*. "Gold-induced thrombocytopaenia; 12 Cases and a review of the literature." *Semin Arthritis Rheum*, 1987; 16:287-293.
44) Amos RS and Bax DE. "Leucopaenia in rheumatoid arthritis: Relationship to gold or sulphasalazine therapy." *Br J Rheum*, 1988; 27:465-468.
45) Madhok R, Pullar T, *et al*. "Chrysotherapy and thrombocytopenia." *Ann Rheum Dis*, 1985; 44:589-591.
46) Epstein WV, Henke CJ, *et al*. "Effect of parenterally administered gold therapy on the course of adult rheumatoid arthritis." *Ann Int Med*, 1991; 114:437-444.
47) Finkelstein AE, Walz DT, *et al*. "Auranofin: New oral gold compound for treatment of rheumatoid arthritis." *Ann Rheum Dis*, 1976; 35:251-257.
48) Pearson RG. "Hard and soft acids and bases." *J of Amer Chemical Society*, 1963; 85:3533-3539.
49) Kligman AM. "The identification of contact allergens by human assay. III. The Maximization Test: A procedure for screening and rating contact sensitizers." *J Invest Derm*, 1966; 47:393-409.
50) Hardcastle J, Hardcastle PT, *et al*. "Effect of Auranofin on ion transport by rat small intestine." *J Pharm Pharmacol*, 1989; 41:817-823.
51) Lockie LM and Smith DM. "Forty-seven years experience with gold therapy in 1019 rheumatoid arthritis patients." *Semin Arthritis Rheum*, 1985; 14:238-246.
52) Panayi GS. "New ideas on the pathogenesis of rheumatoid arthritis." *Ann Ital Med Int*, 1990; 5:1-4.
53) Abraham GE and Himmel PB. "Management of rheumatoid arthritis: Rationale for the use of colloidal metallic gold." *Journal of Nut and Env Med*, 1997; 7:295-305.
54) Pincus T, Summey JA, *et al*. "Assessment of patient satisfaction in activities of daily living using a modified Stanford Health Assessment Questionnaire." *Arthritis Rheum*, 1983; 26:1346-1353.
55) Lansbury J. "Quantitation of the activity of rheumatoid arthritis." *Am J Med Sci*, 1956; 232:300-310.
56) American Rheumatology Association. *Dictionary of Rheumatic Diseases, Vol. I. Signs and Symptoms.* Contact Associates International Ltd, New York, 1992.
57) Abraham GE, McReynolds SA, *et al*. "Effects of colloidal metallic gold on cognitive functions: A pilot study." *Frontier Perspective*, 1998; 7:39-41.
58) Lezak MD. *Neuropsychological Assessment*. Oxford University Press, New York, 1995; 690.
59) Lezak MD. *Neuropsychological Assessment*. Oxford University Press, New York, 1995; 691.
60) McDowell I and Newell C. *Measuring Health — A Guide to Rating Scales and Questionnaires*. Oxford University Press, Inc, New York, 1987; 249-252.
61) Belza BL. "Comparison of self-reported fatigue in rheumatoid arthritis and controls." *Journal of Rheumatology*, 1995; 22:639-643.
62) DeJong RN. *The Neurologic Examination*. Harper and Row, New York, 1967; 524-559.
63) Benedict R and Horton AM. "The construct validity of the four-word short-term memory test: A preliminary study." *International Journal of Neuroscience*, 1990; 2:199-202.
64) Chevallet M, Luche S, *et al*. "Silver staining of proteins in polyacrylamide gels." *National Protoc*, 2006; 1:1852-1858.
65) Uchihara T. "Silver diagnosis in neuropathology: Principles, practice, and revised interpretation." *Acta Neuropathoi (Berl)*, 2007; 113:483-499.
66) Babu R, Zhang J, *et al*. "Antimicrobial activities of silver used as a polymerization catalyst for wound-healing matrix." *Biomaterials*, 2006; 27:4304-4313.
67) Feng OL, Wu J, *et al*. "A Mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*." *J Biomed Mater Res*, 2000; 52:662-668.
68) Bragg PD and Rainnie DJ. "The effect of silver ions on the respiratory chain of *Escherichia coli*." *Journal of Microbiology*, 1974; 20:883-889.
69) Chaw KC, Manimaran M, *et al*. "Role of silver ions in destabilization of intermolecular adhesion forces measured by atomic force microscopy in *Staphylococcus epidermidis* biofilms." *Antimicrobial Agents and Chemotherapy*, 2005; 49:4853-4859.
70) Sondi I and Salopek-Sondi B. "Silver nanoparticles as antimicrobial agent: A case study on *E. coli* as a model for gram-negative bacteria." *Journal of Colloid and Interface Science*, 2004; 275:177-182.
71) DuHamel BG. "Electric metallic colloids and their therapeutical applications." *Lancet*, 1912; 40:89-90.
72) Morones JR, Elechiguerra JL, *et al*. "The bactericidal effect of silver nanoparticles." *Nanotechnology*, 2006; 16:2346-2353.
73) Yamanaka M, Hara K, *et al*. "Bactericidal actions of a silver ion solution on *Escherichia coli*, studied by energy-filtering transmission electron microscopy and proteomic analysis." *Applied and Environmental Microbiology*, 2005; 71:7589-7593.
74) Elechiguerra JL, Burt JL, *et al*. "Interaction of silver nanoparticles with HIV-1." *Journal of Nanotechnology*, 2005; 3:3-13.

75) Choi O and Hu Z. "Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria." *Environmental Science and Technology*, 2008; 42:4583-4588.
76) Albrecht-Buehler G. "Rudimentary form of cellular 'vision.'" *Proc Natl Acad Sci*, 1992; 89:8288-8292.
77) Albrecht-Buehler G. "Cellular infrared detector appears to be contained in the centrosome." *Cell Motility and Cytoskeleton*, 1994; 27:262-271.
78) Albrecht-Buehler G. "Changes of cell behavior by near-infrared signals." *Cell Motility and the Cytoskeleton*, 1995; 32:299-304.
79) Litovitz TA, Mullins JM, *et al*. "Effect of coherence time of the applied magnetic field on ornithine decarboxylase activity." *Biochem Biophys Res Comm*, 1991; 178:862-865.
80) Litovitz TA, Krause D, *et al*. "Simultaneous applications of a spatially coherent noise field blocks the response of cell cultures to a 60 Hz electromagnetic field." In: *Blank M Electricity and Magnesium in Biology and Medicine*. San Francisco Press Inc, 1993.
81) El Sayed SO and Dyson M. "Comparison of the effect of multiwavelength light produced by a cluster of simiconductor diodes and of each individual diode on mast cell number and degranulation in intact and injured skin." *Lasers in Surgery*, 1990; 10:559.
82) Longo L, Evangelista S, *et al*. "Effect of diode laser silver arsenide-aluminum (GA-AL-AS) 904 NM on healing of experimental wounds." *Laser Surgery Medicine*, 1987; 7:444.
83) LAM TS, Abergel CA, *et al*. "Laser stimulation of collagen synthesis in human skin fibroblast cultures." *Laser Life Science*, 1986; 1:61.
84) Karu T, Andrelchuk T, *et al*. "Changes in oxidative metabolism of murine spleen following laser and superluminous diode (550-950 nm) irradiation: Effects of cellular composition and radiation parameters." *Laser in Surgery and Medicine*, 1993; 13:453.
85) Young S, Bolton P, *et al*. "Macrophage responsiveness to light therapy." *Laser in Surgery and Medicine*, 1989; 9:497.
86) Feldherr C and Akin D. "The permeability of nuclear envelope in dividing and nondividing cell cultures, *J Cell Biol*, 1990; 111:1-8.
87) Feldherr C and Akin D. Signal-mediated nuclear transport in proliferating and growth-arrested BABB/c 3T3 cells." *J Cell Biol*, 1991; 115:933-939.
88) Zhao Z, Wakita T, *et al*. "Inoculation of plasmids encoding Japanese encephalitis virus PrM-E proteins with colloidal gold elicits a protective immune response in BALB/c mice." *Journal Virol*, 2003; 77:4248-4260.
89) Simmons SR and Albrecht RM. "Probe size and bound label conformation in colloidal gold-ligand labels and gold-immunolables." *Scan El Micr*, 1989; 3:27-34.
90) Freund PL and Spiro M. "Colloidal catalyst: The effect of sol size and concentration." *J Phys Chem*, 1985; 89:1074-1077.
91) Skosey JL. "Gold compounds and D-penicillamine." In: *Arthritis and Allied Conditions*. McCarty DJ and Koopman WJ, editors. Lea & Febiger, Publishing, 1993; 603-614.
92) Shaya SY and Smith CW. "The effects of magnetic and radiofrequency field on the activity of lysozyme." *Collective Phenomena*, 1977; 2:215.
93) Ghadially FN, Oryschak AF, *et al*. "Ultrastructural changes produced in rheumatoid synovial membrane by chrysotherapy." *Ann Rheum Dis*, 1976; 35:67-72.
94) Salnikov V, Lukyánenko YO, *et al*. "Probing the outer mitochondrial membrane in cardiac mitochondria with nanoparticles." *Biophysical Journal*, 2007; 92:1058-1071.
95) Lewis C. "Die wirking von schwermetallen auf die bosartigen tiergeschwulste." *Berl Klin Wochschr*, 1913; 50:541-542.
96) Alekhina RP, Bukhman VM, *et al*. "Ratio between proliferating and quiescent spleen cell populations during development of Rauscher leukemia and after loading of mononuclear phagocytes with colloidal gold." *Biull Eksp Biol Med*, 1984; 97:790-793.
97) Mukherjee P. "Potential therapeutic application of gold nanoparticles in B-chronic lymphocytic leukemia (BCLL); Enhancing apoptosis." *J Nanobiotechnology*, 2007; 10.1186:1477-3155.
98) Bhattacharya R. "Gold nanoparticles inhibit VEGF165-induced proliferation of HUVEC cells." *Nanolett*, 2004; 4:2479-2481.
99) Mukherjee P, *et al*. "Antiangiogenic properties of gold nanoparticles." *Clin Cancer Res*, 2005; 11(9).
100) Bajaj S and Vohora SB. "Analgesic activity of gold preparations used in Ayurveda & Unani-Tibb." *Indian J Med Res*, 1998; 108:104-111.
101) Hillyer JF and Albrecht RM. "Gastrointestinal persorption and tissue distribution of differently sized colloidal gold nanoparticles." *Journal of Pharmaceutical Sciences*, 2001; 90:1927-1936.
102) Gottlieb NL. "Metabolism and distribution of gold compounds." *J Rheumatol*, 1979; 6:2-6.
103) Blocka KLN, Paulus HE, *et al*. "Clinical pharmacokinetics of oral and injectable gold compounds." *Clinical Pharmacokinetics*, 1986; 11:133-143.
104) Perrelli G and Piolatto G. "Tentative reference values for gold, silver, and platinum: Literature data analysis." *The Science of the Total Enviroment*, 1992; 120:93-96.
105) Campbell JM, Reglinski J, *et al*. "Action of sodium aurothiomalate on erythrocyte membrane." *Annals of the Rheumatic Diseases*, 1992; 51:969-971.
106) Tencer J, *et al*. "Size-selectivity of the glomerular barrier to high molecular weight proteins: Upper size limitations of shunt pathways." *Kidney Int*, 1998; 53:709-715.
107) Tay M, *et al*. "Charge selectivity in kidney ultrafiltration is associated with glomerular uptake of transport probes." *Am J Physiol Renal Physiol*, 1991; 260: F549-F554.
108) Rosenman KD, Moss A, *et al*. "Clinical implications of exposure to silver nitrate and silver oxide." *Journal of Occupational Medicine*, 1979; 21:430-435.
109) Cox AJ and Marich KW. "Gold in the dermis following gold therapy for rheumatoid arthritis." *Arch Dermatol*, 1973; 108:655-657.
110) Hollinger MA. "Toxicological aspects of topical silver pharmaceuticals." *Critical Reviews in Toxicology*, 1996; 26:255-260.
111) Hanson M and Abedi-Valugerdi M. "Mercury and silver differentially induce antinuclear autoantibody production in susceptible H-2S, H-2q and H-2f Mice." *Clin Exp Immunol*, 2003; 10.1046:1365-2249.
112) Challier JC, Panigel M, *et al*. "Uptake of colloidal 198Au by fetal liver in rat, after direct intrafetal administration." *Int J Nucl Med Biol*, 1973; 1:103-106.
113) Takahashi S and Matsuoka O. "Cross placental transfer of 198Au-colloid in near term rats: *J Radiat Res*, 1981; 2:242-249.

◆