

# Localization of gold in synovial membrane of rheumatoid arthritis treated with sodium aurothiomalate

Studies by electron microscope and electron probe x-ray microanalysis

HIROAKI NAKAMURA AND MITSUO IGARASHI

*From the Laboratory of Electron Microscopy, Tokyo Metropolitan Institute of Gerontology, and Department of Orthopaedic Surgery, Tokyo Metropolitan Geriatric Hospital, 35-2 Sakae-cho, Itabashi-ku Tokyo-173, Japan*

**SUMMARY** The localization of gold in the synovial membrane of rheumatoid arthritis patients treated with sodium aurothiomalate was examined and quantitative analysis of epon-embedded sections was carried out with a wavelength dispersive x-ray microanalyser. Gold was only detected in the lysosomes of synovial lining type A cells and subsynovial mononuclear cells in the form of filamentous deposits and highly electron-dense granules, the latter being few in number.

The concentration of gold within the lysosomes containing the characteristic deposits and granules was equivalent to that in epon-embedded standard specimens of freeze-dried albumin in which 2.0-87.4 mg/ml of gold was included. In addition, sulphur was detected in the lysosomes containing the filamentous deposits, but the S/Au x-ray signal ratio was not equal to that detected in sodium aurothiomalate. The significance of the coexistence of gold with sulphur in lysosomes is discussed.

Although gold salts have been used in the treatment of rheumatoid arthritis (RA) for more than 40 years (Rodnan and Benedek, 1970), the mode of action has not as yet been understood. Several possibilities regarding the mechanism of the therapeutic effect of gold salts have been put forward: (1) inhibition of the activity of lysosomal enzymes in synovial membrane (Persellin and Ziff, 1966), (2) increased stability of collagen (Adam *et al.*, 1965), and (3) increased fibrolytic activity (Andersen and Winther, 1968). These hypotheses have suggested that the site of action of gold salts was in the synovial membrane and cavity.

The localization of gold in the synovial tissues after administration of gold salts has been observed under optical microscope with histochemical staining (Lewis and Ziff, 1966), by photochemical technique (Doré and Vernon-Roberts, 1976), and by autoradiography (Tonna *et al.*, 1963). These data indicated that the granules of gold were seen in the cytoplasm of synovial lining cells and subsynovial phagocytes as a form of intracellular inclusions.

Under the electron microscope the gold intracellular inclusions were found in the form of filamentous deposits (Norton *et al.*, 1968). Stuve and Galle (1970) found that mitochondria in the renal cortex of rats injected with sodium aurothiopropionol sulphonate contained deposits which were morphologically similar to filamentous deposits in synovial membrane, and they detected gold in the deposits with an x-ray microanalyser.

We examined the localization of gold in epon-embedded sections of the synovial membrane of RA patients treated with sodium aurothiomalate. An electron microscope and electron probe microanalyser were used, and the concentration of gold localized in the synovial membrane was quantitatively analysed to infer the nature of gold intracellular inclusions.

## Materials and methods

### SYNOVIAL TISSUES

Synovial tissues were obtained at the time of synovectomies from the knees of 7 patients with definite or classical rheumatoid arthritis. 5 patients had received intramuscular injections of sodium

aurothiomalate (Shiosol, Shionogi). The synovial tissue of 2 RA patients who were not treated with gold salts was used as control. The gold therapy received by the patients is summarized in Table 1.

Table 1 Patients treated with sodium aurothiomalate

Case no.	Age	Sex	Total dose (mg)	Duration of therapy	Time since previous injection
1	65	F	2380	2.6 yr	2 d
2	52	F	1380	8 m	3 d
3	92	F	395	5 m	7 d
4	64	F	375	4 m	2 yr
5	71	F	2580	3 yr	3 yr

#### ELECTRON MICROSCOPY

All tissues were fixed in 2.5% glutaraldehyde and 1% osmium tetroxide (0.1 mol/l phosphate buffer, pH 7.4), dehydrated in graded alcohol solutions and propylene oxide, and embedded in Epon 812. Ultrathin sections cut with a LKB-111 ultramicrotome were mounted on copper grids, stained with uranyl acetate and lead citrate, and examined with a JEM-100B electron microscope. Some thin sections were mounted on coated one-hole grids to observe the distribution of gold inclusions.

#### ELECTRON PROBE X-RAY MICROANALYSIS

For electron probe analysis, thin sections (0.15–0.2  $\mu\text{m}$ ) of synovial tissue were placed on copper grids coated with carbon, while thick sections (2  $\mu\text{m}$ ) were picked up on a polished carbon base, and both were further coated with carbon. These specimens were examined with a wavelength dispersive x-ray microanalyser (XMA) attached to a scanning electron microscope (JSM-U3S) for observation of transmitted and backscattered electron images. During quantitative analysis, the accelerating voltage was 25 kV, the absorbed beam current  $2 \times 10^{-8}$  A (on the specimen holder), and the integrated x-ray count time was 40 s. The diffracting crystal used was pentaerthritol.

#### STANDARD SPECIMEN FOR X-RAY QUANTITATIVE MICROANALYSIS

The standard specimen for quantitative analysis was prepared following the procedure of Ingram *et al.* (1972); varying amounts of gold sodium chloride were added to a solution of 20% bovine serum albumin and 5% glycerin in phosphate buffer (pH 7.4) to yield final concentrations of 1%, 0.5%, 0.2%, and 0.1%. To measure the concentration of added gold, each final solution was analysed with a UHF Plasma Spectra Scan-300 (Hitachi). The final solution of 1% gold sodium chloride contained 4.72 mg/ml gold. Each solution was dropped into liquid

nitrogen. The frozen droplets were sectioned to 20  $\mu\text{m}$  thickness in a cryostat at  $-25^{\circ}\text{C}$ , and lyophilized. After the dried sections were fixed with osmium tetroxide vapour, they were embedded in Epon 812. The embedded sample was further sectioned to 2  $\mu\text{m}$  thickness, fixed to a carbon base with cement (Cemedain Contract, Cemedain K.K.) diluted with isoamyl acetate, and coated with carbon in a vacuum evaporator. The standard specimens were analysed in the same way as the synovial tissue sections.

To obtain the sulphate to gold ratio of x-ray intensity within sodium aurothiomalate, a drop placed on a carbon-coated grid was dried. The precipitate of sodium aurothiomalate was studied in the same manner.

#### Results

In the synovial tissue of all RA patients were found hyperplasia of the synovial lining cells, proliferation of capillaries, infiltration of the tissue by inflammatory cells, and interstitial deposition of fibrin-like materials. The degree of these findings ranged from severe to slight in accordance with the particular section of tissue.

A remarkable morphological difference between the synovial membrane of those patients treated with gold salt and those not treated was found in regard to the dense bodies usually considered as lysosomes in synovial lining cells and subsynovial mononuclear cells. These single membrane-bound dense bodies showed various shapes which were classified into four types based on the materials they contained: (1) homogeneous medium-density matrix, (2)

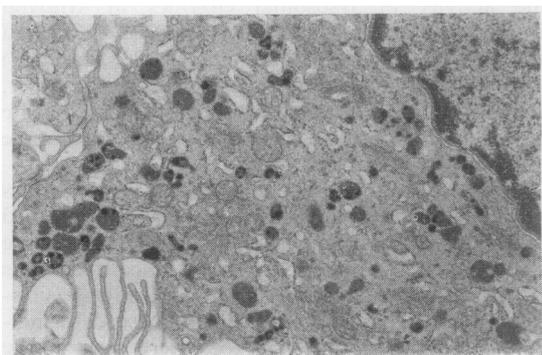


Fig. 1 A synovial lining type A cell of a rheumatoid arthritis patient who received no gold therapy. Homogeneous medium-density matrix lysosomes contain electron-dense granules.  $\times 7400$ .

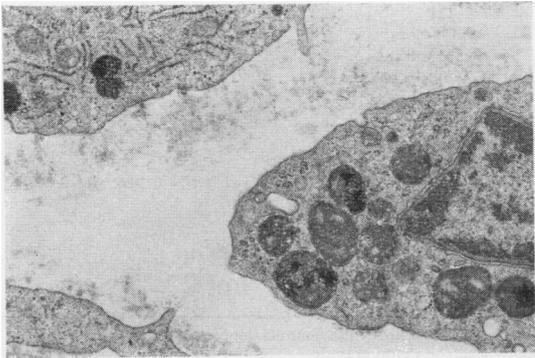
medium density granules (Fig. 1), (3) electron dense filamentous deposits (Figs. 2, 3), and (4) myelin structure, droplets, and filamentous deposits in larger vacuoles (Fig. 5).

Though almost all the dense bodies in the synovial tissue of patients who did not receive gold therapy were homogeneous medium-density matrix, medium-density granules also were found (Fig. 1). Type (3) and (4) dense bodies containing characteristic electron dense deposits were only observed in the synovial tissue of patients treated with sodium aurothiomalate.

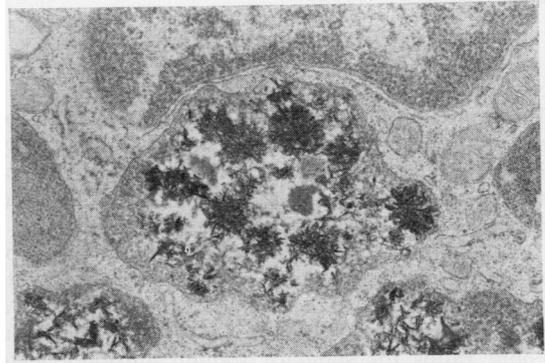
Type (3) dense bodies measuring 0.5–1.5  $\mu\text{m}$  in diameter were found in the synovial lining type A

cells of 2 patients (Cases 1 and 5) (Fig. 2), and the subsynovial mononuclear cells of 5 patients (Cases 1–5) (Fig. 3).

At higher magnification, the filamentous deposits were seen to consist of a radial accumulation of numerous filaments of varying lengths and about 5 nm in diameter (Fig. 4). Also, large single membrane bound type (4) vacuoles 1.5–3.5  $\mu\text{m}$  in diameter were occasionally seen in the subsynovial mononuclear cells around blood vessels in the deeper subsynovial membrane of 3 patients (Cases, 1, 2, 5) (Fig. 5). A few highly electron-dense granules 80–150 nm in diameter were seen in the same area (Fig. 6).



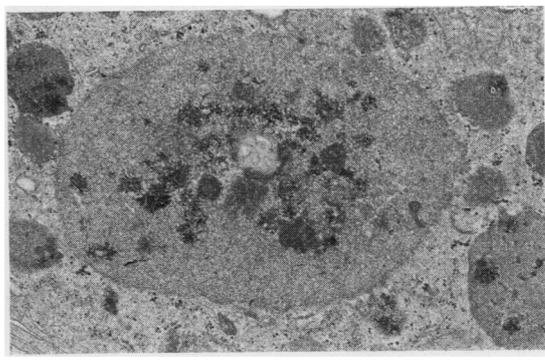
**Fig. 2** Synovial lining type A cell of rheumatoid arthritis patient who underwent gold therapy. Filamentous deposits were observed in single membrane-bound lysosomes.  $\times 13\ 700$ .



**Fig. 4** The filamentous deposits, shown at higher magnification, were composed of fine electron-dense filaments arranged in a radial pattern.  $\times 23\ 100$ .



**Fig. 3** Subsynovial mononuclear cell of rheumatoid arthritis patient who underwent gold therapy. Lysosomes contained deposits morphologically similar to filamentous deposits of synovial lining type A cell.  $\times 7400$ .



**Fig. 5** Residual body of subsynovial mononuclear cell beneath blood vessel. The single membrane-bound large vacuole included numerous filamentous deposits, electron-dense materials, and myelin structure.  $\times 13\ 700$ .

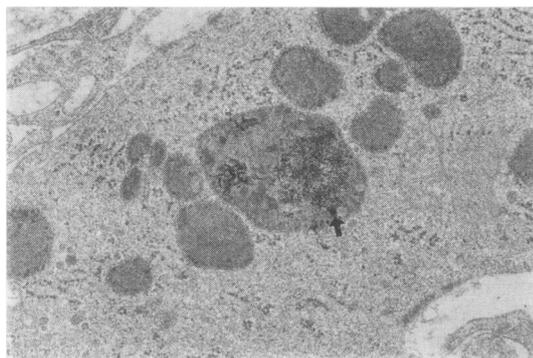


Fig. 6 Lysosomes in synovial mononuclear cell beneath blood vessel contained abundant filamentous deposits and a higher electron-dense granule which is close to the former (arrow).  $\times 16\ 500$ .

#### X-RAY MICROANALYSIS

Electron probe analysis of the thin section of synovial membrane resulted in detection of gold only in dense bodies containing electron-dense filamentous deposits. Furthermore, sulphur was detected in concurrence with gold (Fig. 7a).

The sulphur-to-gold ratios of x-ray intensity of tissue sections and sodium aurothiomalate deposits are shown in Table 2. In one of the 2 patients a significant difference can be seen between the S/Au count ratios for standard sodium aurothiomalate

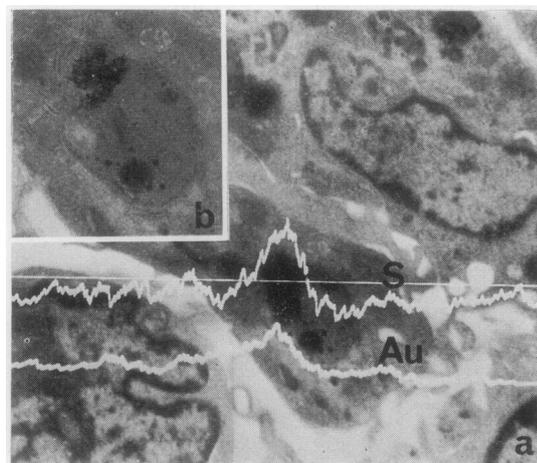


Fig. 7 (a) X-ray line-analysis of gold and sulphate in filamentous deposits. High gold and sulphur peaks were only seen in filamentous deposits.  $\times 1100$ . (b) Transmitted electron micrograph pertaining to (a).

deposits and deposits in tissue sections. No significant difference is noted in any other case. The x-ray intensity of gold detected in highly electron-dense granules was much stronger than that of filamentous deposits, but the intensity of sulphur was very low (Fig. 8a). Gold was not detected in dense bodies having a homogeneous matrix and containing medium-density granules, or other organelles, cytoplasm, collagen fibres, and other extracellular matrix.

Table 2 X-ray analysis for sulphur and gold in thin section and in sodium aurothiomalate

Points*	Sulphur counts†	Gold counts‡	Ratio of S/Au
Case 5			
1	203	146	
2	171	122	
3	91	76	
4	76	57	$1.49 \pm 0.20$ § P < 0.05**
5	10	232	
Case 1			
1	172	129	
2	131	83	
3	76	51	
4	49	28	$1.50 \pm 0.29$ § P > 0.05
5	17	736	
Sodium aurothiomalate‡			
	$146 \pm 56$	$118 \pm 52$	$1.24 \pm 0.13$

\*1-4 points were in the filamentous deposits. 5 point was in the highly electron-dense granule.

†Peak counts minus background counts from the spectrometer offset by 0.1 cm.

‡Means of 10 dried precipitates (3-10  $\mu\text{m}$  diameter) on carbon film.

§Mean  $\pm$  standard error (n = 10).

\*\*Probability of a significant difference between ratios of sulphur to gold in lysosomes and sodium aurothiomalate in standard.

The x-ray intensities of gold obtained by quantitative spot analysis of thick sections are shown in Table 3. In the back-scattered electron image, the filamentous deposits and highly electron-dense granules were seen in synovial lining cells and subsynovial mononuclear cells as extremely bright grains in medium brightness granules 0.5-3.5  $\mu\text{m}$  in diameter.

#### QUANTITATIVE ANALYSIS OF STANDARD SPECIMENS

Spot analysis of standard specimens did not result in uniform x-ray intensity values among the respective spots. However, unit areas were designated and the mean x-ray intensity value of 10 random points within one of these unit areas in a section was very nearly the same as that of the other unit areas. The counting rate for all standard specimens was found

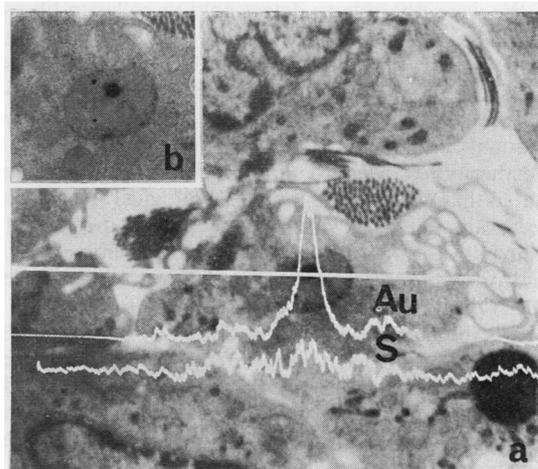


Fig. 8 (a) X-ray line-analysis of highly electron-dense granule. Detection of gold was strong but that of sulphur only slight in dense granule.  $\times 9600$ . (b) Transmitted electron micrograph pertaining to (a). One highly electron-dense granule and two fine electron-dense granules were observed in single membrane-bound vacuole having homogeneous medium-density matrix.

to vary directly and linearly in accordance with the concentration of gold, as illustrated by the typical regression equation:

$$\text{Concentration (mg/ml)} = 0.033 \times \text{counts}/40 \text{ s} - 0.44 \quad (r = 0.9770)$$

where the unit area was  $120 \times 20 \mu\text{m}$  and the mean x-ray count was obtained from 3 blocks (5 sections per block) prepared for each of the four concentrations of gold.

Applying the x-ray intensities of the dense bodies having filamentous deposits and highly electron-dense granules of patients who underwent gold therapy to the above equation, the gold concentrations calculated ranged from 2.0 to 87.4 mg/ml (Table 3).

## Discussion

Although studies of the ultrastructure of RA synovium have been reported in recent years (Barland *et al.*, 1964; Hirohata and Kobayashi, 1964; Norton and Ziff, 1966; Ghadially and Roy, 1967), there has been little description of the ultrastructural changes of synovium after administration of gold salts. Barland *et al.* (1964) found, in their study of the ultrastructure of the synovium of RA patients treated with gold salts, that the synovial lining type A cells contained large residual bodies

Table 3 X-ray intensity and calculated concentration of gold in  $2 \mu\text{m}$  epon-embedded section

Points	Gold x-ray counts			Gold concentration* (mg/ml)
	P	B	P-B	
<b>Case 1</b>				
1	2735	73	2661	87.4
2	1620	34	1586	51.9
3	834	48	786	25.5
4	110	37	73	2.0
Collagen†	35	34	—	—
Epon‡	39	38	—	—
<b>Case 2</b>				
1	136	37	99	2.8
2	302	35	267	8.4
3	496	36	460	14.7
4	666	35	631	21.5
<b>Case 5</b>				
1	664	38	626	21.2
2	493	39	454	14.5
3	333	35	298	9.4
4	262	37	225	7.0
<b>Case 6 (Control)</b>				
37	—	37	—	—
2	39	39	—	—
Collagen†	37	37	—	—
Epon‡	41	39	—	—

\*Calculated mean from regressive equation of standard specimen.

†Counts in collagen fibres.

‡Counts in embedding material.

P = peak counts; B = background counts from the spectrometer offset by 0.1 cm.

characterized by various electron-dense materials and myelin structure. The residual bodies showed high levels of acid phosphatase activity, suggesting that the lysosomes, in the lining cells might contain administered gold ingested by cells.

Norton *et al.* (1968) reported that after intra-articular injection of sodium aurothiomalate filamentous deposits were found in the lysosomes and phagosomes of synovial lining cells and subsynovial mononuclear cells of RA patients and rabbits. Ghadially *et al.* (1976), using an energy dispersive x-ray analyser, confirmed that gold was detectable in lysosomes containing filamentous deposits.

The nature of the gold inclusions is not known, but several possibilities have been suggested: (1) gold binds the SH group of lysosomal enzymes and acts as enzyme inhibitor; (2) metallothioneine, having many SH groups, is biosynthesized in the presence of gold and incorporated in lysosomes; (3) sodium aurothiomalate simply accumulates in lysosomes, etc.

It has been proposed that the mechanism of action of gold and its clinical effectiveness may be mediated by its inhibitory action in lysosomal enzymes *in vitro* (Caygill and Jevons, 1965; Persellin and Ziff, 1966; Paltemaa, 1968; Ennis *et al.*, 1968). The data showed that such an effect was noted at a gold concentration of about  $250 \mu\text{g/ml}$ . But this concentration of gold was much lower than that detected by XMA within

the lysosomes containing filamentous deposits. It is therefore difficult to accept that the gold inclusion observed by electron microscope and XMA was solely due to the inhibition of lysosomal enzymes.

Again, metallothioneine is a special protein in which cadmium and zinc are strongly bound with thioneine (MW about 10 000) which contains many SH groups mostly derived from cysteine (Kägi and Vallee, 1960, 1961). It was said that there was acute biosynthesis of thioneine when excessive quantities of cadmium, zinc, mercury, and copper invaded a living body, and that it combined with the heavy metals to turn into innocuous metallothioneine (Pulido *et al.*, 1966; Evans and Cornatzer, 1970; Wisniewska *et al.*, 1970).

Mizuhira and Kimura (1973) compiled typical x-ray analytical data on Cd-thioneine from electron-dense lysosomes in the kidney and liver cells of mice in which acute cadmium poisoning was induced. However, since it has not been reported that metallothioneine is biosynthesized in the presence of gold, it is not clear whether the sulphate detected together with gold originated from metallothioneine.

After intra-articular injection of sodium thiomaleate alone, synthesized filamentous deposits were found in lysosomes of synovial lining cells and macrophagic cells in synovial membrane (Norton *et al.*, 1968). Our XMA studies showed high concentrations of gold and sulphur in lysosomes containing filamentous deposits. However, the sulphur-to-gold x-ray intensity ratio was higher than their ratio in sodium aurothiomalate. Based on these facts, an assumption of the nature of gold inclusions in synovial membrane produced by aurothiomalate is that gold occurs as sulphur compounds and heavily accumulates in lysosomes.

The structure of the highly electron-dense granules resembled that of colloidal gold particles, but they were larger than the latter. Scarcely any sulphur was detected in these granules by XMA. It may be possible that the filamentous deposits were changed into highly electron-dense granules through some metabolic processes as in the case of ferritin synthesis after administration of iron dextran (Ball *et al.*, 1964).

Gold inclusions were found in the synovial lining cells and subsynovial mononuclear cells of RA patients who had last received an injection 3 years previously. It had previously been reported that gold remained in synovial tissue for quite some time after completion of gold therapy (Grahame *et al.*, 1974), and also maintained its therapeutic effect for 12 months after administration (Empire Rheumatism Council, 1960, 1961). It is possible that this gold is stored mainly in the form of gold inclusions in synovial membrane.

We were not able to detect gold in collagen fibres by XMA. Adam *et al.* (1965), however reported that electron micrographs of tail tendon collagen fibres showed four staining bands per period after administration of gold salts to rats and that gold therapy brought about increased pH and load stability. Furthermore, Mehard and Volcani (1975) have stated that when the contents of biological specimens are examined by XMA, such factors as the loss of elements during fixation, dehydration, embedding, and/or sectioning as well as the detection limit of the XMA must be taken into consideration.

It is therefore possible that this loss of gold or the XMA detection limit may be the reason we were unable to detect gold in collagen fibres of synovial membrane or in lysosomes which did not contain filamentous deposits or highly electron-dense granules.

In our study using an XMA it was not possible to associate any of the therapeutic effects of gold salts with the distribution and localization of gold. However, it did suggest something with regard to the metabolism and preservation of gold in the synovial membrane in that high concentrations of gold and sulphur were detected in lysosomes containing filamentous deposits while the same structure and distribution of gold inclusions were found in synovial membrane 3 years after gold therapy was stopped.

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#### References

- Adam, M., Bartl, P., Deyl, Z., and Romus, J. (1965). Uptake of gold by collagen in gold therapy. *Annals of the Rheumatic Diseases*, **24**, 378-381.
- Andersen, R. B., and Winther, O. (1968). Blood-fibrinolysis during chrysotherapy. *Acta Rheumatologica Scandinavica*, **14**, 161-168.
- Ball, J., Chapman, J. A., and Muirden, K. D. (1964). The uptake of iron in rabbit synovial tissue following intra-articular injection of iron dextran. A light and electron microscope study. *Journal of Cell Biology*, **22**, 351-364.
- Barland, P., Novikoff, A. B., and Hamerman, D. (1964). Fine structure and cytochemistry of the rheumatoid synovial membrane with special reference to lysosomes. *American Journal of Pathology*, **44**, 853-866.
- Caygill, J. C., and Jevons, F. R. (1965). Presence of glucosaminidase activity in human synovial fluid and its inhibition by gold compounds. *Clinica Chimica Acta*, **11**, 233-237.
- Doré, J. L., and Vernon-Roberts, B. (1976). A method for the selective demonstration of gold in tissue sections. *Medical Laboratory Sciences*, **33**, 209-214.
- Empire Rheumatism Council Research Sub-committee (1960). Gold therapy in rheumatoid arthritis. Report of a multi-centre controlled trial. *Annals of the Rheumatic Diseases*, **19**, 95-117.

- Empire Rheumatism Council Research Sub-committee (1961). Gold therapy in rheumatoid arthritis. Final report of a multi-centre controlled trial. *Annals of the Rheumatic Diseases*, **20**, 315-333.
- Ennis, R. S., Granda, J. L., and Posner, A. S. (1968). Effect of gold salts and other drugs on the release and activity of lysosomal hydrolases. *Arthritis and Rheumatism*, **11**, 756-764.
- Evans, G. W., and Cornatzer, W. E. (1970). Copper and zinc metalloproteins in the rat. (Abst.) *Federation Proceedings*, **29**, 695.
- Ghadially, F. N., and Roy, S. (1967). Ultrastructure of synovial membrane in rheumatoid arthritis. *Annals of the Rheumatic Diseases*, **26**, 426-442.
- Ghadially, F. N., Oryschak, A. F., and Mitchell, D. M. (1976). Ultrastructural changes produced in rheumatoid synovial membrane by chrysotherapy. *Annals of the Rheumatic Diseases*, **35**, 67-72.
- Grahame, R., Billings, R., Laurence, M., Marks, V., and Wood, P. J. (1974). Tissue gold levels after chrysotherapy. *Annals of the Rheumatic Diseases*, **33**, 536-539.
- Hirohata, K., and Kobayashi, I. (1964). Fine structures of the synovial tissues in rheumatoid arthritis. *Kobe Journal of the Medical Sciences*, **10**, 195-225.
- Ingram, F. D., Ingram, M. J., and Hogben, C. A. M. (1972). Quantitative electron probe analysis of soft biologic tissue for electrolytes. *Journal of Histochemistry and Cytochemistry*, **20**, 716-722.
- Kägi, J. H. R., and Vallee, B. L. (1960). Metallothionein: a cadmium- and zinc-containing protein from equine renal cortex. *Journal of Biological Chemistry*, **235**, 3460-3465.
- Kägi, J. H. R., and Vallee, B. L. (1961). Metallothionein: a cadmium- and zinc-containing protein from equine renal cortex. *Journal of Biological Chemistry*, **236**, 2435-2442.
- Lewis, D. C., and Ziff, M. (1966). Intra-articular administration of gold salts. *Arthritis and Rheumatism*, **9**, 682-692.
- Mehard, C. W., and Volcani, B. E. (1975). Evaluation of silicon and germanium retention in rat tissues and diatoms during cell and organelle preparation for electron probe microanalysis. *Journal of Histochemistry and Cytochemistry*, **23**, 348-358.
- Mizuhira, V., and Kimura, M. (1973). Localization of cadmium in the mouse liver cells induced by acute cadmium poisoning. *Proceedings of the Electron Microscopy Society of America, 31st Annual Meeting*, p. 402. Ed. by C. J. Arceneaux.
- Norton, W. L., and Ziff, M. (1966). Electron microscopic observations on the rheumatoid synovial membrane. *Arthritis and Rheumatism*, **9**, 589-610.
- Norton, W. L., Lewis, D. C., and Ziff, M. (1968). Electron dense deposits following injection of gold sodium thiomalate and thiomalic acid. *Arthritis and Rheumatism*, **11**, 436-442.
- Paltemaa, S. (1968). The inhibition of lysosomal enzymes by gold salts in human synovial fluid cells. *Acta Rheumatologica Scandinavica*, **14**, 161-168.
- Persellin, R. H., and Ziff, M. (1966). The effect of gold salt on lysosomal enzymes of the peritoneal macrophage. *Arthritis and Rheumatism*, **9**, 57-65.
- Pulido, P., Kägi, J. H. R., and Vallee, B. L. (1966). Isolation and some properties of human metallothionein. *Biochemistry*, **5**, 1768-1777.
- Rodnan, G. P., and Benedek, T. G. (1970). The early history of anti-rheumatic drugs. *Arthritis and Rheumatism*, **13**, 145-165.
- Stuve, J., and Galle, P. (1970). Role of mitochondria in the handling of gold by the kidney. A study by electron microscopy and electron probe microanalysis. *Journal of Cell Biology*, **44**, 667-676.
- Tonna, E. A., Brecher, G., Cronkite, E. P., and Schwartz, I. L. (1963). The autoradiographic localization and distribution of neutron activated gold (Au-198) in skeletal tissue and synovia of mice. *Arthritis and Rheumatism*, **6**, 1-10.
- Wisniewska, J. M., Trojanowska, B. (1970). Binding of mercury in the rat kidney by metallothionein. *Toxicology and Applied Pharmacology*, **16**, 754-763.