Cultured human cells with high levels of gold-binding cytosolic metallothionein are not resistant to the growth inhibitory effect of sodium aurothiomalate

A GLENNÅS^{1,2} AND H E RUGSTAD¹

From the ¹Division of Clinical Pharmacology, Institute for Surgical Research, Rikshospitalet, the National Hospital, and ²Oslo Sanitetsforening Rheumatism Hospital, Oslo, Norway

SUMMARY We have previously shown that cells with a high content of the gold-binding protein metallothionein (MT) are resistant to the growth inhibitory effects of gold(III) chloride and auranofin. To investigate whether MT confers resistance to sodium aurothiomalate two cell lines of cultured human epithelial (HE) cells were used; the parental cell line (HE) and a substrain (HE₁₀₀) containing high levels of cytosolic MT. Sodium aurothiomalate and thiomalic acid without gold both caused a dose-dependent growth inhibition of both cell lines when used in the concentration range 25–300 µmol/l culture medium and for four days' exposure. MT, despite binding about one third of the cytosolic gold, did not protect against the antiproliferative effect of sodium aurothiomalate. The gold and the thiomalate moieties were distributed differently within the cells; 30% of the cellular gold and 80% of the thiomalate were recovered in the cytosol. Gold was mainly protein bound in both cell lines, as shown by G75 Sephadex gel filtration of the cytosols. In the HE₁₀₀ cells about 30% of the gold eluted with MT. The thiomalate eluted mainly with substances with molecular weights less than 1000. Cellular synthesis of MT was not observed during sodium aurothiomalate treatment. The results indicate that the sodium aurothiomalate molecule dissociates and support the suggestion that the thiomalate moiety is, in part, responsible for the antiproliferative effect of the drug.

Key phrase: drug resistance.

Metallothionein (MT) is the name given to a group of low molecular weight isoproteins (6000–7000) with a unique amino acid composition, in that 30% of the 61 amino acid residues are cysteine. MT has the capacity to bind certain heavy metals.¹ The protein is widely distributed among different animal species and seems to occur in all tissues but is found mainly in the cytosol of liver and kidney cells.¹ Cadmium (Cd) is the most potent MT inducer metal known at present, and MT is probably important in the detoxification of chronic Cd intoxications.¹

Sharma and McQueen² and Mogilnicka and Webb³ have shown the occurrence of low molecular

weight gold-binding proteins in the liver and kidneys of rats after intramuscular administration of the gold-containing drug, sodium aurothiomalate, to the animals. These proteins have not been fully characterised, but their behaviour on gel filtration columns suggests that they may be of MT nature. The biological functions of the proteins have not yet been studied.

We have previously shown that cultured human cells containing high amounts of cadmium-induced cytosolic MT are resistant to the antiproliferative effects of 350 µmol gold(III) chloride/l culture medium⁴ and 2 µmol/l of the antiarthritic drug, auranofin.⁵ These concentrations when given continuously were lethal to the parent cell line with undetectable levels of cytosolic MT. The demonstration of the binding of gold to the MT within the

Accepted for publication 23 July 1985.

Correspondence to Dr Anne Glennås, Division of Clinical Pharmacology, Institute for Surgical Research, Rikshospitalet, N-0027 Oslo 1, Norway.

resistant cells led to the conclusion that MT may act as one protective factor against the growth inhibitory effect of the two gold-containing compounds.

The purpose of the present study was to investigate the possible protective role of MT against the antiproliferative effect of sodium aurothiomalate. Cultured human cells with and without detectable amounts of cytosolic MT were used. The cellular concentrations and cytosolic distribution of the gold and the thiomalate moieties of the drug were investigated. For comparison, the cells were also exposed to thiomalic acid without gold.

Materials and methods

CELLS AND CELL CULTURING

The cells were human epithelial cells (HE) derived from normal skin (NCTC 2544) and obtained from the American Type Culture Collection, Maryland, USA. They were grown as monolayers in Dulbecco's modification of Eagle's medium (Flow Laboratories, Irvine, Scotland) supplemented with serum, antibiotics, and mycostaticum.⁴ A substrain (HE₁₀₀) had previously been adapted to 100 µmol CdCl₂/l culture medium, an otherwise lethal concentration for the cells.⁶ There is considerable evidence that the Cd resistance of these cells is due to induction of MT synthesis and the binding of Cd to MT. About 2–3% of the cytosolic proteins in these cells are estimated to be MT.⁷ Characterisation of the cells by methods including transmission electron microscopy, surface antigen typing, and isoenzyme analysis⁸ has confirmed their epithelial and human nature. Flow cytometric studies, cellular glutathione determinations,^{7,8} two-dimensional polyacrylamide gel electrophoresis of cellular proteins, and preliminary studies on DNA repair capacity⁹ have shown no major differences between the HE and HE₁₀₀ cells, apart from the Cd-MT content.

DRUGS AND CHEMICALS

Disodium auro [2,3-¹⁴C₂]thiomalate (specific activity 0.31 mCi/mmol) and [2,3-¹⁴C₂]thiomalic acid (specific activity 0.88 mCi/mmol) were kindly supplied by Rhône-Poulenc (May & Baker Ltd, Dagenham, Essex, England). The radiochemical purity of the ¹⁴C-labelled disodium aurothiomalate was determined at the start of the experiments by thin layer chromatography (TLC) with *n*-butanol/glacial acetic acid/water 80:20:20 on silica gel plates (Polygram Sil G/UV 254, Schleicher & Schull GmbH, Dassel, W Germany). Subsequent determination of radioactivity in sections of the plates indicated a radiochemical purity of 90%. The purity of the labelled thiomalic acid was 68% as estimated by TLC with toluene/methanol/glacial acetic acid

45:8:4 on silica gel. Batches of sodium [¹⁹⁹Au] aurothiomalate (specific activity 123.5 mCi/mmol and 163.0 mCi/mmol) were synthesised at the Department of Biophysics, University of Manchester. Sodium aurothiomalate (Myocrisin) was the commercially available drug, and thiomalic acid was purchased from Sigma Chemical Company, Mo, USA. The thiomalic acid was stored under nitrogen until use, and all compounds were protected from light. Fresh solutions of the drugs were prepared for each experiment. Cadmium chloride purum was obtained from Fluka AG, Switzerland.

CELL GROWTH STUDIES

Cells from both cell lines (HE and HE₁₀₀) were subcultured into Costar tissue culture wells (16 mm) (Costar, Maryland, USA) at a density of 1.0 × 10⁴ cells per well. The HE₁₀₀ cells were removed from the Cd-containing medium, and all cells were grown without metal supplement for 24 h. Cultures were then exposed to medium containing 25, 50, 100, 200, or 300 µmol of sodium aurothiomalate or thiomalate derived from thiomalic acid per litre of culture medium. Controls were grown without drug supplementation. Cells from six replicate wells of each cell line, drug, and drug concentration were harvested by trypsinisation and counted in a Coulter counter at the start of treatment and daily until day 4. Media with and without the drug supplements were changed on day 2. On day 4 the viability of the cells was tested by the dye exclusion test (trypan blue).¹⁰ Differences in sensitivity to the drugs between the cell lines were calculated by means of an area method (see Appendix).

CELLULAR CONCENTRATION AND DISTRIBUTION OF ¹⁹⁹Au AND ¹⁴C

After removal of the Cd supplement for 24 h exponentially growing HE and HE₁₀₀ cells were exposed to 300 µmol/l of sodium [¹⁹⁹Au]aurothiomalate, disodium auro [2,3-¹⁴C₂]thiomalate, or [2,3-¹⁴C₂]thiomalic acid for another 24 h. The cell cytosols were then obtained by ultrasound sonication and ultracentrifugation (105 000 g, 1 h, 4°C). Aliquots of sonicates and cytosols were analysed for radioactivity and for protein content (Abbott bichromatic analyser 100) with human albumin (Behringwerke AG, W Germany) as a standard. Differences in concentrations and the distribution of ¹⁹⁹Au and ¹⁴C were tested by Student's *t* test, taking significance as *p* < 0.05. The remaining cytosols were chromatographed on a G75 Sephadex column (1.6 × 90 cm) with 10 mM ammonium formate buffer pH 8 as eluent. The elution rate was 18 ml/h and the fraction volume 3 ml. The concentrations of Cd, Zn, and Cu in the eluted fractions

were determined by atomic absorption spectrophotometry (Instrumentation Laboratories aa/ae spectrophotometer 257). The ^{199}Au concentration was determined with a gamma scintillation spectrometer (Packard 5221). The counting efficiency was 35% with a 120–185 keV window. The ^{14}C activity was determined in a Mark II liquid scintillation system (Tracor Analytic Inc., Ill., USA). Each fraction showed a similar degree of quenching. The counting efficiency determined by an external standard ratio method for ^{14}C was 89%. The coefficients of variation (standard deviation corrected for background as a percentage of net counts) of the counts per minute had a range of 0.2–1.1% (^{199}Au) and 0.5–2.7% (^{14}C) for the gel filtration peaks and 1.0–3.3% (^{199}Au) and 20.8–32.3% (^{14}C) for the regions between the peaks.

IN-VITRO INCUBATION OF Cd-MT AND ISOTOPE-LABELLED SODIUM AUROTHIOMALATE

Cd-induced MT, obtained after G75 Sephadex gel filtration of cytosols from HE₁₀₀ cells as described above, was dissolved in 10 mM ammonium formate buffer pH 7.5. The concentration of MT was approximately 10 $\mu\text{mol/l}$. Sodium [^{199}Au]aurothiomalate or disodium auro[2,3- $^{14}\text{C}_2$]thiomalate was added to a final drug concentration of 20 $\mu\text{mol/l}$ and

incubated for 1 h at 20°C. The drug concentration used was equal to the cytosolic gold concentration after drug exposure of living cells. After a second G75 Sephadex gel filtration run the concentrations of ^{199}Au , ^{14}C , Cd, and Zn in the eluted fractions were determined.

INCUBATION OF ^{14}C -LABELLED SODIUM AUROTHIOMALATE WITH CULTURE MEDIUM
To examine the distribution of Au and ^{14}C the culture medium was supplemented with 300 $\mu\text{mol/l}$ of disodium auro[2,3- $^{14}\text{C}_2$]thiomalate and incubated for 24 h in tissue culture flasks without cells. After G75 Sephadex chromatography of the medium the ^{14}C concentration in the eluted fractions was determined as above. The Au concentration was determined by flame atomic absorption spectrophotometry.

Results

EFFECT OF SODIUM AUROTHIOMALATE AND THIOMALIC ACID ON CELL GROWTH
The growth of HE and HE₁₀₀ cells during exposure to 25–300 $\mu\text{mol/l}$ of sodium aurothiomalate for four days is shown in Fig. 1. Fig. 2 shows the cell growth during exposure to equimolar concentrations of

Fig. 1 Growth of HE and HE₁₀₀ cells on exposure to 25 (○—○), 50 (■—■), 100 (□—□), 200 (▼—▼), or 300 (△...△) μmol sodium aurothiomalate/culture medium for four days. Controls (●—●) were grown without drug supplement. Δ indicates medium change. Each point is the median of six replicates. Bars indicate the 25th and 75th percentiles for the distribution of cell counts.

Fig. 2 Growth of HE and HE₁₀₀ cells on exposure to 25 (○—○), 50 (■—■), 100 (□—□), 200 (▼—▼), or 300 (△...△) μmol thiomalate derived from thiomalic acid/l culture medium for four days. Controls (●—●) were grown without drug supplement. △ indicates medium change. Each point is the median of six replicates. Bars indicate the 25th and 75th percentiles for the distribution of cell counts.

thiomalate derived from thiomalic acid. Both compounds caused a dose-dependent growth inhibition of both cell lines. No significant differences between the HE and HE₁₀₀ cells were detected in the responses when grown with sodium aurothiomalate or thiomalate. On day 4 of treatment with 300 μmol sodium aurothiomalate/l 6.5% (percentage of median values for the cell counts) of the HE cells and 5.4% of the HE₁₀₀ cells remained adherent to the wells compared with the control cells. On treatment with 300 μmol thiomalate/l 0.7% of the HE cells and 0.7% of the HE₁₀₀ cells remained attached. The viability of the remaining cells was more than 85%. At concentrations of sodium aurothiomalate or thiomalate higher than 100 μmol/l all cells detached from the wells and died within about two weeks.

CELLULAR CONCENTRATIONS OF ¹⁹⁹Au AND ¹⁴C

The concentrations of ¹⁹⁹Au and ¹⁴C in sonicates and cytosols after 24 h exposure of the cells to 300 μmol/l of either sodium [¹⁹⁹Au]aurothiomalate,

disodium auro[2,3-¹⁴C₂]thiomalate, or [2,3-¹⁴C₂]thiomalic acid are shown in Table 1. After exposure to the ¹⁹⁹Au- or ¹⁴C-labelled aurothiomalate ¹⁹⁹Au and ¹⁴C were recovered in the cells (sonicates) in equimolar concentrations, 9.5–13.2 nmol/mg sonicate protein. No significant differences were found between the HE and HE₁₀₀ cells in either ¹⁹⁹Au or ¹⁴C content. About 30% of the cellular ¹⁹⁹Au and 80% of the cellular ¹⁴C derived from the thiomalate moiety were recovered in the cytosol. Accordingly, the cytosolic ¹⁹⁹Au concentrations were lower than those of ¹⁴C, 5.0–6.1 nmol ¹⁹⁹Au/mg cytosol protein and 18.0–23.3 nmol ¹⁴C/mg cytosol protein respectively. No significant differences in the cytosolic concentrations of ¹⁹⁹Au and ¹⁴C between the HE and HE₁₀₀ cells were detected. When the cells were exposed to the ¹⁴C-labelled thiomalate derived from thiomalic acid, similar or slightly higher concentrations and a similar distribution of ¹⁴C between the sonicates and cytosols was found compared with that found after exposure of the cells to the ¹⁴C-labelled disodium aurothiomalate.

Table 1 Concentrations of ^{199}Au or ^{14}C in sonicates and cytosols of HE and HE₁₀₀ cells*

Isotope/drug	HE cells			HE ₁₀₀ cells		
	Sonicate	Cytosol	% of cellular ^{199}Au or ^{14}C in cytosol	Sonicate	Cytosol	% of cellular ^{199}Au or ^{14}C in cytosol
^{199}Au from sodium [^{199}Au] aurothiomalate	9.5±6.1	6.1±5.0	35.3±16.5	11.3±8.6	5.0±3.4	30.0±16.4
^{14}C from disodium auro[2,3- $^{14}\text{C}_2$]thiomalate	12.0±1.6	18.0±6.4	77.5±0.7	13.2±2.5	23.3±5.1	80.5±3.5
^{14}C from [2,3- $^{14}\text{C}_2$] thiomalic acid	20.4±3.4	34.8±0.7	81.5±0.7	18.9±4.9	31.1±1.9	82.0±2.8

*Concentrations (nmol/mg protein) are means±SD from three (^{199}Au) or two (^{14}C) experiments. See methods section for details.

Fig. 3 Elution profiles of ^{199}Au (—), Cd(---), Zn(---), and Cu(-.-.) obtained after G75 Sephadex gel filtration of cytosols from HE and HE₁₀₀ cells after exposure to sodium [^{199}Au]aurothiomalate (panel A–B). Elution profiles of ^{14}C (—), Cd(---), Zn(---), and Cu(-.-.) obtained in the same way and after treatment of HE and HE₁₀₀ cells with disodium auro[2,3- $^{14}\text{C}_2$]thiomalate (panel C–D).

Fig. 4 Elution profiles of ^{14}C (—), Cd (---), Zn (- - -), and Cu (· · ·) after G75 Sephadex gel filtration of cytosols from HE and HE₁₀₀ cells after treatment with [2,3- $^{14}\text{C}_2$] thiomalic acid.

DISTRIBUTION OF ^{199}Au AND ^{14}C WITHIN THE CYTOSOLS

The G75 Sephadex elution profiles of ^{199}Au , ^{14}C , Cd, Zn, and Cu after gel filtration of cytosols from HE and HE₁₀₀ cells treated with sodium [^{199}Au]aurothiomalate, disodium auro[2,3- $^{14}\text{C}_2$]thiomalate, or [2,3- $^{14}\text{C}_2$]thiomalic acid are shown in Figs 3 and 4. The fraction of ^{199}Au eluting with the void volume proteins (peak I, $V_e/V_o=1$, mol.wt > 75 000) was 72% in HE and 46% in the HE₁₀₀ cytosols. In the HE₁₀₀ cytosols 36% of the ^{199}Au coeluted with the Cd-induced MT (peak II, $V_e/V_o \approx 2$, mol.wt ≈ 10 000). No such peak was observed in the HE cytosols, as indicated by the profiles of ^{199}Au , Zn, and Cu. 7% of the ^{14}C eluted with the void volume proteins, whereas 88% eluted with substances of molecular weights less than 1000 (peak III, $V_e/V_o \approx 3$). This pattern was the same in both lines and after treatment with both of the ^{14}C -labelled compounds. No ^{14}C activity was detected in the MT-containing fractions of the HE₁₀₀ cytosols.

IN-VITRO BINDING OF ISOTOPE-LABELLED SODIUM AUROTHIOMALATE TO Cd-MT

Fig. 5 shows the coelution of ^{199}Au derived from sodium [^{199}Au]aurothiomalate and Cd derived from MT after in-vitro incubation of the drug with MT and subsequent gel filtration. About 29% of the Cd bound to MT was exchanged with ^{199}Au under the conditions described. When MT was incubated with disodium auro[2,3- $^{14}\text{C}_2$]thiomalate, ^{14}C and Cd derived from MT eluted separately on the gel filtration column.

DISTRIBUTION OF Au AND ^{14}C IN THE CULTURE MEDIUM

After G75 Sephadex gel filtration of medium incubated with disodium auro[2,3- $^{14}\text{C}_2$]thiomalate about 65% of the Au and 35% of the ^{14}C distributed with the medium proteins. The major part of the ^{14}C eluted with fractions containing substances of molecular weights less than 1000 (profiles not shown).

Fig. 5 Elution profiles of ¹⁹⁹Au(—), Cd(---), and Zn(···) (panel A) and ¹⁴C(—), Cd(---), and Zn(···) (panel B) after G75 Sephadex gel filtration of Cd-induced MT incubated *in vitro* with sodium [¹⁹⁹Au]thiomalate or disodium auro[2,3-¹⁴C₂]thiomalate. The Cd-MT was obtained from a previous G75 Sephadex run of cytosols from HE₁₀₀ cells.

Discussion

The present study shows that cellular Cd-induced MT has the capacity to bind gold when human cells are cultured in the presence of sodium aurothiomalate. Cd-MT also binds gold after *in vitro* incubation with sodium aurothiomalate. The degree of Cd exchange by Au was somewhat higher (29%) after *in vitro* incubation than after treatment of living cells (16%) (Fig. 3B). An almost complete exchange of MT-bound Cd by Au was achieved *in vitro* when an even higher concentration of sodium aurothiomalate (9 mmol/l, data not shown) was used. MT does not confer resistance to the antiproliferative effect of the drug, despite binding about 36% of the cytosolic gold in living cells. This contrasts with the results obtained when auranofin was given to the same cells.⁵ The concentration of 2 μmol auranofin/l culture medium caused an almost complete arrest of growth of the HE cells, whereas the HE₁₀₀ cells continued to grow exponentially. About 15% of the cytosolic gold derived from auranofin was bound to the MT in the HE₁₀₀ cells.

The gel filtration profiles (Figs 3 and 4) gave no indication of induced MT synthesis in the HE cells after 24 h of sodium aurothiomalate treatment. This

does not rule out the possible induction of MT, which has been observed after sodium aurothiomalate administration to rats.^{2,3} *In vivo* alterations in tissue metal distribution caused by gold treatment may indirectly induce MT via zinc or copper, as suggested by Mogilnicka and Webb.¹¹

The gold and the thiomalate derived from sodium aurothiomalate have different intracellular distributions. In the present study the thiomalate distributes similarly regardless of whether it originates from sodium aurothiomalate or thiomalic acid without gold. The thiomalate was mainly present in the cytosol, whereas the gold was recovered mainly in the pellet after ultracentrifugation. Furthermore, most gold was protein bound within the cytosol, while the thiomalate obviously appeared as the thiomalate anion, as its own disulphide, or as mixed disulphides with sulphhydryl-rich oligopeptides. This indicates a dissociation of the sodium aurothiomalate molecule in the cells. Evidence was also obtained that this dissociation takes place even in the culture medium, before the drug penetrates the cells. Preliminary data indicate that the major part of the cytosolic thiomalate is free thiomalate and not its corresponding disulphide (unpublished results). The present results are in accordance with the

studies of Jellum *et al.*,^{12 13} who showed that the gold and thiomalate moieties separate in vivo after intramuscular administration of [¹⁹⁵Au-¹⁴C]aurothiomalate to mice and rats, resulting in protein-bound gold and release of thiomalate. Other in vivo studies¹⁴⁻¹⁶ have confirmed the different distribution of the gold and thiomalate both in animals and humans. The present study shows that the gold and the thiomalate moieties behave differently even within cells.

The results discussed above and the closely similar growth inhibitory effects of sodium aurothiomalate and thiomalate derived from thiomalic acid raise the question of which part of the sodium aurothiomalate molecule is responsible for the antiproliferative effect observed. The possibility that the thiomalate moiety in part mediates the effect ascribed to sodium aurothiomalate cannot be excluded. Other studies¹⁷ have shown that thiomalic acid exerts effects similar to those of D-penicillamine, a thiol-containing antiarthritic drug, on various models of immunologically mediated inflammation in vivo, and in vitro on the responsiveness of lymphocytes to concanavalin A in the presence or absence of macrophages. Munthe and Jellum,¹⁸ in a preliminary study on five patients, suggested that thiomalic acid may have an antiarthritic effect when used in the treatment of rheumatoid arthritis.

In our experiments the concentration range of sodium aurothiomalate used was 25–300 µmol/l culture medium. Thus the lower concentrations correspond to the serum gold levels obtained when the drug is used clinically. The peak serum gold concentration after the intramuscular administration of 50 mg sodium aurothiomalate is reported to be 30–45 µmol/l, and the trough values to be 4–25 µmol gold/l at steady state conditions on chronic treatment with 50 mg every fourth week or every week.^{19 20} The maximum concentration used was that which caused complete growth arrest of the HE cells without causing significant cell death within the experimental period. When compared on this basis the concentration of auranofin equally effective to 300 µmol/l of sodium aurothiomalate is about 2 µmol/l.⁵ This probably reflects the differences in cellular affinity²¹ and in toxicity²² between the two drugs. It may also support the suggestion that the antiproliferative effect of gold-containing drugs may be ascribed to drug components other than the gold moiety or in addition to it.

In conclusion, we have shown that human cells containing high amounts of cytosolic MT are not resistant to the antiproliferative effect of sodium aurothiomalate, though MT traps about one third of the cytosolic gold. Sodium aurothiomalate exposure does not induce the synthesis of detectable amounts

of MT. Sodium aurothiomalate and thiomalic acid have similar growth inhibitory effects. The observed differences in the cellular and cytosolic distributions of gold and thiomalate indicate a dissociation of the sodium aurothiomalate molecule and raise questions about the effector moiety of the molecule.

We are grateful to Mrs K R Modalsli, Miss K Moestue, and Miss A K Syversen for excellent technical assistance, to Dr H L Sharma (Department of Medical Biophysics, University of Manchester, England) for synthesising sodium [¹⁹⁹Au]aurothiomalate, and May & Baker Ltd who provided the ¹⁴C-labelled compounds. Dr L Mørkrid provided statistical advice. A Glennås is a research fellow sponsored by the Norwegian Hydro Company.

Appendix

The relative sensitivity of the two cell lines HE and HE₁₀₀ to sodium aurothiomalate or thiomalic acid was calculated as follows: Cell growth was expressed by the formula:

$$N(t) = N_0 \exp \{f(t, c)\}$$

where N is the cell number at time (t) and c is the drug concentration used.

The following expression was obtained for the area under the curves (AUC) (Figs 1 and 2) by integration from t=0 to t=T:

$$AUC_T = \int_0^T \ln N(t) dt = T \ln N_0 + \int_0^T f(t, c) dt$$

$AUC_0 = T \ln N_0$ was the same for all values of c, thus $\Delta AUC = AUC_T - AUC_0$ was independent of N_0 , the cell number on day 0.

The relative sensitivity to various concentrations of the drug Y(c), of one cell line was expressed as:

$$Y(c) = \Delta AUC(c) / \Delta AUC(c=0)$$

$\Delta Y / (Y' + Y) = (Y' - Y) / (Y' + Y)$ was a linear first-order function of log c, Y denoting the values for the HE cells and Y' those for the HE₁₀₀ cells. Differences in sensitivity to the drugs were tested by a significant correlation between the $\Delta Y / (Y' + Y)$ and log c, taking significance as p < 0.05.

References

- 1 Kägi J H R, Nordberg M, eds. Report from the first international meeting on metallothionein and other low molecular weight metal binding proteins, Zurich, 1978. *Experientia* 1979; **35** (suppl 34): 1–378.
- 2 Sharma R-P, McQueen E G. The binding of gold to cytosolic proteins in the rat liver and kidney tissues: metallothioneins. *Biochem Pharmacol* 1980; **29**: 2017–21.
- 3 Mogilnicka E, Webb M. Comparative studies on the distribution of gold, copper and zinc in the livers and kidneys of rats and hamsters after treatment with sodium [¹⁹⁵Au]aurothiomalate. *J Appl Toxicol* 1981; **1**: 287–91.
- 4 Glennås A, Bakka A, Rugstad H E. Cultured human cells with a high content of metallothionein show resistance against gold-chloride. *Scand J Rheumatol* 1983; **12**: 321–4.
- 5 Glennås A, Rugstad H E. Acquired resistance against auranofin in cultured human cells. *Scand J Rheumatol* (in press).
- 6 Rugstad H E, Norseth T. Cadmium resistance and content of cadmium-binding protein in cultured human cells. *Nature* 1975; **257**: 136–7.
- 7 Bakka A, Endresen L, Johnsen A B S, Edminson P D, Rugstad H E. Resistance against cis-dichlorodiammineplatinum in cultured cells with a high content of metallothionein. *Toxicol Appl Pharmacol* 1981; **61**: 215–26.

- 8 Ree K, Johnsen A S, Rugstad H E, Bakka A, Hovig T. Characterisation of a human epithelial cell line with special reference to its ultrastructure. *Acta Pathol Microbiol Immunol Scand [A]* 1981; **89**: 73–80.
- 9 Endresen L, Thorsrud A K, Jellum E, Willard-Gallo K E, Rugstad H E. Protein mapping of two metallothionein-rich cell strains and their parent lines, using high-resolution two-dimensional electrophoresis. *Anal Biochem* 1984; **143**: 170–8.
- 10 Phillips H J. Dye exclusion test for cell viability. In: Kruse P F Jr, Patterson M K Jr, eds. *Tissue culture. Methods and applications*. New York: Academic Press, 1973: 406–8.
- 11 Mogilnicka E, Webb M. The effects of cadmium and copper on the renal uptake and metallothionein binding of gold in the rat and hamster. *Chem Biol Interact* 1982; **40**: 247–56.
- 12 Jellum E, Munthe E, Guldal G, Aaseth J. Gold and thiol compounds in the treatment of rheumatoid arthritis. *Scand J Rheumatol* 1979; **8** (suppl 28): 28–36.
- 13 Jellum E, Munthe E, Guldal G, Aaseth J. Fate of the gold and the thiomalate part after intramuscular administration of aurothiomalate to mice. *Ann Rheum Dis* 1980; **39**: 155–8.
- 14 Turkall R M, Bianchini J R. In vivo fate of the gold-sulfur bond of gold sodium thiomalate [Abstract]. *Fed Proc* 1980; **39**: 2582.
- 15 Jellum E, Munthe E. Fate of the thiomalate part after intramuscular administration of aurothiomalate in rheumatoid arthritis. *Ann Rheum Dis* 1982; **41**: 431–2.
- 16 Rudge S R, Perrett D, Swannell A J. Free thiomalate in plasma and urine of patients receiving sodium aurothiomalate. *Ann Rheum Dis* 1984; **43**: 66–9.
- 17 Arrigoni-Martelli E, Bramm E, Binderup L. D-Penicillamine-like activity of thiols. *Eur J Rheumatol Inflamm* 1978; **1**: 197–203.
- 18 Munthe E, Jellum E. Tolerance and effectiveness of sodium thiomalate without gold in rheumatoid arthritis [Abstract]. *Scand J Rheumatol* 1980; **9** (suppl 33): 21.
- 19 Gottlieb N L. Comparative pharmacokinetics of parenteral and oral gold compounds. *J Rheumatol* 1982; **9** (suppl 8): 99–109.
- 20 Blocka K L N, Landaw E M. The organic gold compounds: comparative pharmacokinetics. In: Capell H A, Cole D S, Manghani K K, Morris R W, eds. *Auranofin*. Amsterdam: Elsevier, 1983: 32–44. (Excerpta Medica, Current Clinical Practice Series No 7).
- 21 Herrlinger J D, Alsen C, Bèress R, Hecker U, Weikert W. Distribution of gold in serum erythrocytes and white blood cells after in vitro incubation and during chrysotherapy with different gold compounds. *J Rheumatol* 1982; **9** (suppl 8): 81–9.
- 22 Payne B J, Walz D T. The toxicity of three gold compounds in laboratory animals. *Vet Pathol* 1978; **15** (suppl 5): 1–3.