Gold Sodium Thiomalate Toxicity in Cadmium-Sensitive and Cadmium-Resistant Chinese Hamster Ovary Cells (42906)

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Abstract. **Gold sodium thiomalate was incubated with one cadmium-sensitive cell line and two cadmium-resistant variants. The resistant lines have been reported to synthesize metallothionein (MT) in response to both cadmium and zinc, whereas the sensitive line does not. All cell lines showed a dose-dependent inhibition of growth as a result of gold sodium thiomalate treatment. However, daily comparisons of cell numbers indicate that the cadmium-resistant lines actually increase in number at the highest gold concentrations, whereas numbers of cells in the nonresistant line decrease. MT biosynthesis was measured by monitoring the incorporation of [^{35}S]cysteine into low molecular weight protein. None of the cells synthesized MT in response to gold. When incubated with both zinc and gold, MT was synthesized by both of the cadmium resistant lines; however, the amount of MT synthesized was reduced in the presence of gold which appears to inhibit the uptake of [^{35}S]cysteine by all the cell lines. Although MT is synthesized in the presence of zinc and gold sodium thiomalate, the MT does not have a significant effect on the ability of these cells to withstand high concentrations of gold.**

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Gold-based drugs are currently used for large numbers of rheumatoid arthritis patients because they are among the few drugs shown to be capable of inducing remission of this disease. (1–3). Although these drugs can induce remission, their toxic side effects prevent more widespread use. Among these side effects, one likely to lead to cessation of treatment is heavy metal nephropathy (4), a syndrome similar to that induced by a variety of heavy metals such as cadmium, lead, and platinum. Protection from this type of damage might allow increased use of gold-based pharmaceuticals in the treatment of arthritis.

For cadmium toxicity, it has been shown (5) that pretreatment with low levels of cadmium can lead to protection of kidney cells from a subsequent high dose of the metal. The basis of this protection is the induction of metallothionein (MT), a low molecular weight protein which binds zinc, cadmium, and other heavy metals (5). Since MT can bind gold (6), it might also be able to protect cells from the toxic effects of gold.

We tested whether gold sodium thiomalate (GST; trade name Myochrysine), one of the commonly used gold drugs, induces MT, and also whether induction of MT provides any protection from gold drug toxicity. Tissue culture of cells provides a useful model system for testing the interaction of gold drugs and MT. We used Chinese hamster ovary (CHO) cells, derived from a parental line originally established by Tijo and Puck (7). Hildebrand and colleagues (8–10) have established a series of lines which show increased resistance to cadmium relative to the parental line. For the experiments reported here, these cadmium-resistant lines were used to test the relationship between MT levels and GST toxicity.

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Materials and Methods

Cell Lines. The parental CHO line (CHOp) and the two cadmium-resistant lines were obtained from C. E. Hildebrand. The cadmium-resistant lines were selected by growth in increasing concentrations of $CdCl_2$. They retain their cadmium resistance in the absence of selective pressure (8). The specific lines used for these experiments were the parental line, CHOp, and two cadmium-resistant lines (Cdr20F4 and Cdr200T1). Although the maximum level of cadmium at which the parental cells grow normally is 2.0×10^{-7} M, the two resistant lines can grow normally at 2.6×10^{-5} M and 1.5×10^{-4} M $CdCl_2$, respectively. A significant factor in the ability of the two variant lines to

survive high concentrations of cadmium is that they both synthesize MT, whereas the parental line does not (9). In the parental line, the MT genes are methylated and do not respond to induction either by metals or glucocorticoids (11). The enhanced MT synthesis of the cadmium-resistant lines is due to gene amplification (12). The rationale for the use of these particular lines for these experiments is that they differ in their ability to synthesize MT in response to cadmium or zinc. The CHOp line synthesizes no MT, the Cdr20F4 line synthesizes significant MT but only after heavy metal induction, and the Cdr200T1 line shows low constitutive levels of MT and high levels in response to heavy metals. Thus, these three lines offer the possibility of testing GST interaction with MT under a variety of conditions.

Cell Culture. The cells were maintained in monolayer cultures in medium consisting of RPMI 1640 (Grand Island Biological Co., Grand Island, NY) supplemented with 10% horse serum (GIBCO), 100 units/ml of penicillin, and 100 μg/ml of streptomycin (Sigma, St. Louis, MO). Cells were incubated at 37°C in a humidified atmosphere of 95% air-5% CO_2. Gold sodium thiomalate (Aldrich, Milwaukee, WI) was dissolved in complete medium at 10^{-2} *M*. This medium was either used directly or diluted with nongold containing medium to yield the lower gold concentrations.

To monitor growth rates, cells were diluted to 10^5 cells/ml and 1.5 ml was added to 35-mm tissue culture dishes (Corning, Corning, NY). Cells were incubated either in complete RPMI medium or this medium supplemented with GST or $ZnCl_2$ at the indicated concentration. (Note. We have recently measured zinc concentrations in control media made as described here. The basal zinc concentration is approximately 1.5×10^{-7} *M*.) The concentrations used for the incubations represent the amount of metal compound added to the medium. The actual free metal may vary based on the degree of interaction of the metal compound with medium components. Medium was replenished at 48-hr intervals. Daily cell counts were taken for each treatment by trypsinizing cells from three plates per group (0.1% trypsin, 0.5% EDTA in balanced salt solution). As soon as cells became detached from the plates, cold, complete medium was added to dilute the trypsin. Cells were pelleted by centrifugation at 200*g* for 5 min, then resuspended in complete medium. Exposure to trypsin was as brief as possible to minimize cell damage. All cell numbers were determined using a hemocytometer.

Measurement of Metallothionein Biosynthesis. Cells were grown to confluency in 100-mm tissue culture dishes, 20 plates per experiment. To test for MT biosynthesis in response to zinc or GST, the cells were incubated with 10^{-4} *M* $ZnCl_2$ or 10^{-4} *M* GST in complete medium for 6.5 hr at 37°C. The zinc concentration of 10^{-4} *M* was used because it is the highest level the cells of all lines are able to tolerate and in previous experiments has been shown to induce MT at maximal levels (12). The incubation time was selected because it has been reported that both zinc and cadmium induce significant levels of MT synthesis in this time period (9). Cells were then pulsed with 0.5 μCi/ml of [^{35}S] cysteine (600 Ci/mmol; New England Nuclear) for an addition hour at 37°C. For some experiments, the [^{35}S] cysteine was added directly to medium containing zinc or GST. For later experiments, the protocol was changed in order to determine whether potential gold binding to [^{35}S]cysteine could be influencing the results. In these cases, the test compound was washed off the cells by rinses with Puck's balanced salt solution (13). Subsequently, fresh medium, prewarmed to 37°C, was added followed by addition of [^{35}S]cysteine. The cells were rinsed with cold Puck's and harvested by scraping. Cells were then centrifuged at 200*g* for 5 min. The pellet was resuspended in 2.0 ml of hypotonic buffer (10 m*M* Tris, pH 8.0) and homogenized with a Dounce homogenizer. The homogenate was centrifuged at 28,000*g* in a Sorvall refrigerated centrifuge for 45 min. The supernatant was placed on a Sephadex G-75 column (1.4 × 54 cm). Fractions of 0.8 ml were collected and ^{35}S activity was determined by liquid scintillation spectrometry. Columns were calibrated using ^{125}I-α-bungarotoxin (M_r 6.6 kDa) as a marker. The bungarotoxin normally eluted at fractions 32–40, slightly later than the putative MT peak at 28–38.

The data are reported as ^{35}S cpm/plate. This normalization procedure provides an indirect measurement of MT biosynthesis per cell as follows. Plates were selected at random from those containing normally growing populations. As shown in Figure 1, incubation with 10^{-4} *M* zinc or GST has no significant effect on cell populations from any of the lines during the first 24 hr. Here the incubation was only 6.5 hr and thus per plate populations within a given cell line should be equal. Although growth rates differ among the cell lines, these experiments compare levels of ^{35}S incorporation after metal treatment of a cell line only to controls of the same line.

To determine the amount of gold bound to metallothionein, Cdr200T1 cells were used. The cells were grown to near confluency, then incubated with 10^{-4} *M* GST for 6.5 hr. The soluble protein fraction was obtained as indicated above, and values for gold concentration were determined by atomic emission spectroscopy. This fraction was then chromatographed on a Sephadex G-75 column. MT fractions (tubes 28 through 38) were pooled and lyophilized. The dried material was resuspended in 0.8 ml of 10 m*M* Tris (pH 8.0) and analyzed for gold by atomic emission spectroscopy (14).

Results

To determine the effect of GST on cell growth, cells of the parental CHOp, Cdr20F4, and Cdr200T1

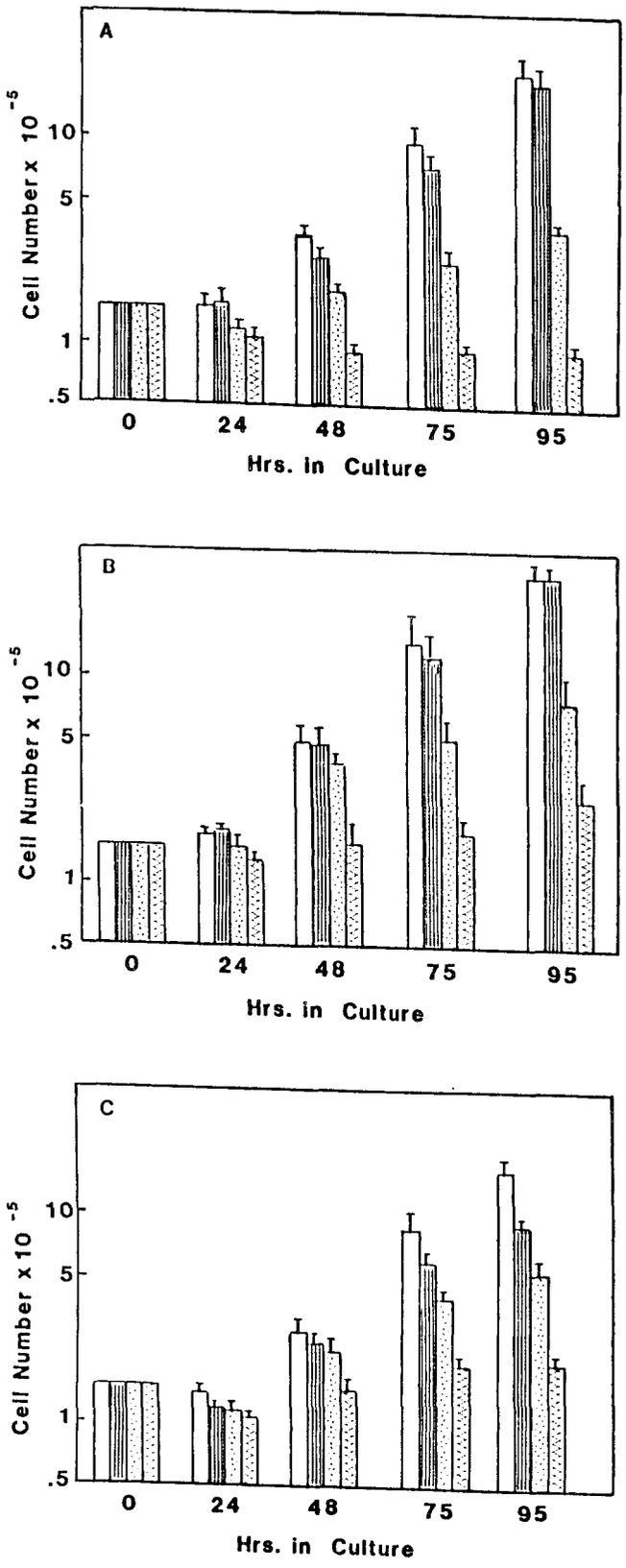

Figure 1. Growth of cells in increasing concentrations of GST. Cells of each line were plated at 1.5×10^5 cells/35-mm plate in complete medium or medium containing GST at 10^{-4}, 10^{-3}, or 10^{-2} *M*. At indicated times, cells were removed from the plate with trypsin-EDTA, centrifuged, resuspended, and counted in a hemocytometer. Data represent the results of three experiments, standard errors are indicated. (a) Parental CHO cells. (b) Cdr20F4 cells. (c) Cdr200T1 cells. The no gold control is indicated by □, the 10^{-4} *M* GST by ▥, 10^{-3} *M* GST by ⊡, and 10^{-2} *M* GST by ⊠.

lines were incubated with increasing concentrations of the drug (Fig. 1). The cells of each line when grown in standard complete medium exhibited similar growth patterns. After an initial lag of 24 hr, the cells began to grow exponentially. The maximum growth rate for each line was different (approximate doubling time for parental line CHOp = 18 hr, Cdr20F4 = 16.5 hr, and Cdr20T1 = 20 hr). In each case GST addition resulted in a dose-dependent inhibition of growth. The inhibitory effect of the drug began to be seen at 48 hr but was increasingly apparent over the next 2 days of culture. Cells of all three lines grew at essentially normal rates in 10^{-4} *M* GST. At higher concentrations, some differences were apparent between the different lines. The most striking point is that the parental CHO cell line grown in 10^{-2} *M* GST showed a decline in cell number over the 4-day period of the experiment, whereas the two cadmium-resistant lines showed a measurable increase in cell number after 4 days in the same levels of gold.

The effects of GST on the different cell lines were further characterized by analysis of variance (ANOVA). The ANOVA analysis verified the observation that the growth rates of the cell lines differed significantly ($F = 87$, $P < 0.001$). Two-way ANOVA analyses were also carried out. The interaction of GST and cell line ($F = 80$, $P < 0.001$) verified that the effects of GST on each cell line became progressively greater with time. The treatment with GST also showed a significant interaction with cell line ($F = 10$, $P < 0.001$), indicating that the cell lines responded differently to the drug.

As indicated earlier, the two Cdr lines differ from the parental CHOp line in their ability to synthesize metallothionein. Experiments were therefore done to monitor MT biosynthesis in order to determine whether any of the cells synthesized MT in response to GST. For these studies, cells were incubated in control medium, 10^{-4} *M* $ZnCl_2$ or GST at 10^{-4} or 10^{-3} *M*. These concentrations were selected because the normal level of GST in patient serum peaks at approximately 10^{-4} *M* (35 μg of Au/ml = 1.75×10^{-4} *M*) (15). [^{35}S]cysteine incorporation was followed as a measure of MT biosynthesis. The parental CHOp line did not synthesize MT under any condition tested (data not shown). All chromatograms of CHOp show two peaks of ^{35}S, one eluted at fractions 20–25 which represents high molecular weight proteins and one at fractions 60–70 which could be either free [^{35}S]cysteine, or some other low molecular weight compound, such as, [^{35}S]cysteine incorporated into glutathione. There is no ^{35}S peak eluting in the region of MT. Results with the Cdr20F4 line (Fig. 2) demonstrate that cells of this variant line do not synthesize detectable amounts of MT when incubated with normal medium. The chromatogram shows only two peaks, each with approximately 50% of the total radioactivity eluting as either high molecular weight or low, and no radioactivity in the MT molecular weight range.

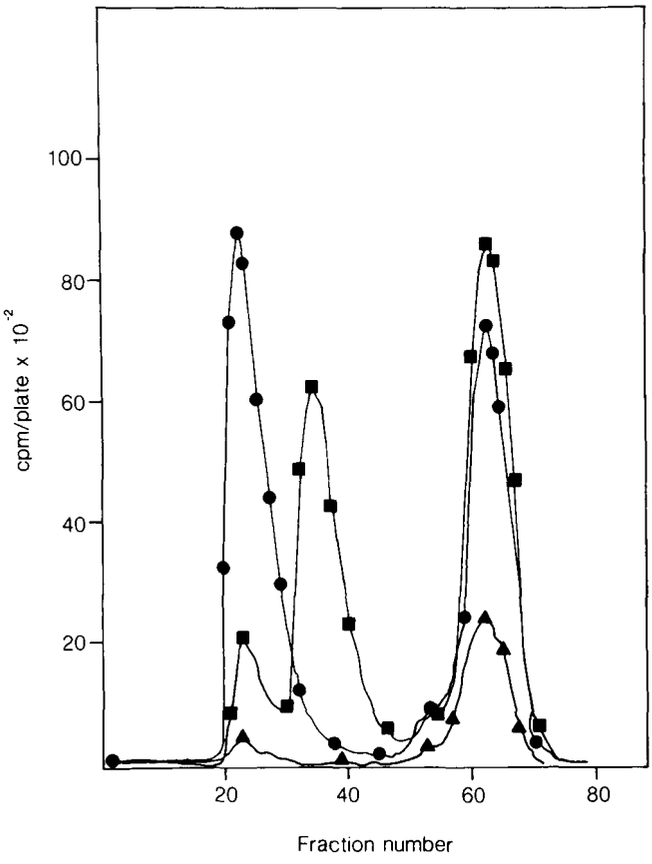

Figure 2. [^{35}S]cysteine incorporation in Cdr20F4 cells. Confluent cultures were incubated in complete medium (●), medium supplemented for 6.5 hr with 10^{-4} *M* $ZnCl_2$ (■) or medium supplemented with 10^{-4} *M* GST for 6.5 hr (▲). Twenty plates were used per experiment. Cells were then incubated with 0.5 mCi/ml [^{35}S]cysteine (5 ml/plate) and removed from the plates by incubation with trypsin-EDTA. Cells were collected by centrifugation at 200*g* for 5 min. The cell pellet was resuspended in 2 ml of 10 mM Tris (pH 8.0) and homogenized, then the homogenate was centrifuged at 28,000*g* for 45 min. The total supernatant was layered onto a Sephadex G-75 Column (1.4 × 54 cm) and 0.8-ml fractions were collected and analyzed by scintillation spectroscopy.

When Cdr20F4 cells were treated with $ZnCl_2$, induction of MT was observed (Fig. 2). Approximately 35% of the radioactivity was located in the fractions 28–38 and only 10% in the high molecular weight fraction. The relative heights of the high molecular weight protein peaks in Figure 2 seem to suggest a shift in synthesis such that [^{35}S]cysteine is preferentially incorporated into MT after zinc induction. The radioactivity found in fractions 28–38 was originally taken to represent MT biosynthesis on the basis of relative migration with the α-bungarotoxin marker. Our results showing induction of a relatively low molecular weight protein in response to $ZnCl_2$ treatment are identical to results of previous published studies using these cell lines (8). Enger *et al.* (9) have shown that this observed increase in [^{35}S] cysteine incorporation is entirely due to induction of MT.

The Cdr20F4 line showed no detectable levels of MT synthesis when incubated with GST at a concentration of 10^{-4} *M* (Fig. 2). There was also no detectable induction of the protein after incubation with either 10^{-3} or 10^{-5} *M* GST (data not shown). Thus, Cdr20F4 cells do not synthesize MT in response to GST treatment during a 6.5-hr exposure. Figures 2 and 3 illustrate another effect of GST on these cells, a decrease in the total amount of [^{35}S]cysteine present after GST incubation. This decrease in [^{35}S]cysteine content on GST incubation occurs with the CHOp, Cdr20F4, and Cdr200T1 lines. Since the GST in the medium could bind the [^{35}S]cysteine, this could influence the amount of cysteine available for uptake by the cells. These experiments were therefore repeated using a second protocol in which the GST was removed before [^{35}S] cysteine addition. The results were essentially the same as those indicated in Figure 2 (data not shown). There was no detectable induction of MT by GST and there was a reduced amount of radioactivity found in the cell extracts.

Tests of the Cdr200T1 line for MT biosynthesis confirmed the fact that these cells synthesize MT at a low level in the absence of inducer (Fig. 3). Furthermore, they can be induced to synthesize MT at a high rate upon treatment with 10^{-4} *M* $ZnCl_2$. In the absence

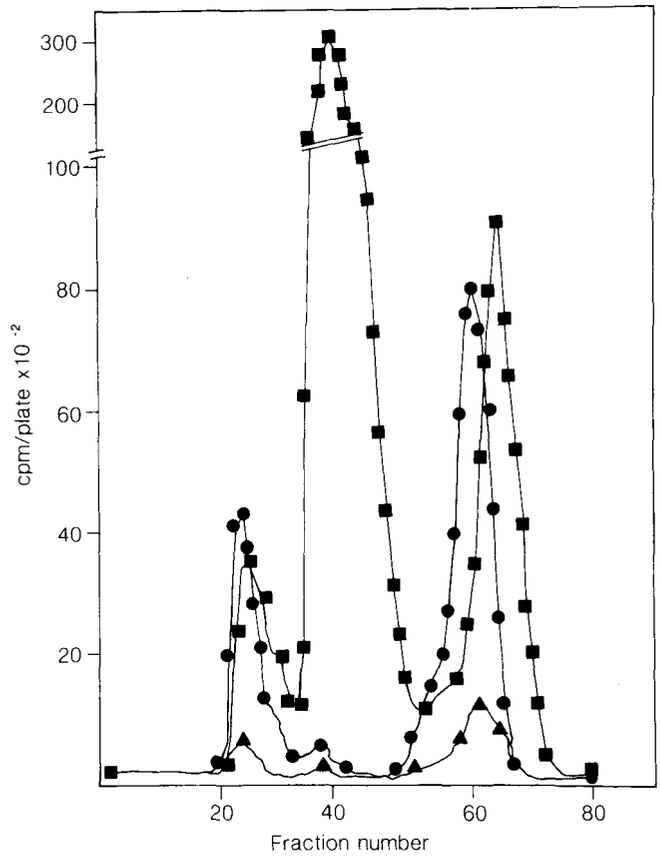

Figure 3. Measurement of [^{35}S]cysteine incorporation in Cdr200T1 cells. Confluent plates of cells were incubated with complete medium (●), medium supplemented with 10^{-4} *M* $ZnCl_2$ for 6.5 hr (■), or medium supplemented with 10^{-4} *M* GST for 6.5 hr (▲). Methods of treatment and chromatography were as described for Figure 2.

of inducer, approximately 8% of the radioactivity was associated with the MT peak, whereas with 10^{-4} *M* $ZnCl_2$ induction approximately 68% of the radioactivity was in the MT peak (Fig. 3). As with the Cdr20F4 line, the Cdr200T1 line was not induced to synthesize MT in response to GST (Fig. 3). There was a reduced amount of radioactivity in these cells when incubated with GST as was observed with the other cell lines. The simplest explanation is that for all three cell lines, GST causes a significant inhibition of cysteine uptake by GST.

The distribution of radioactivity in the different chromatographic fractions provides a measure of the relative incorporation of the [^{35}S]cysteine into different molecular weight materials. For the CHOp cells, chromatograms of cells grown with or without GST show the same distribution of radioactivity in the high and low molecular weight fractions. The Cdr200T1 cells also show no change in the relative distribution of radioactivity with or without GST. The Cdr20F4 cells, however, show a significant difference in this comparison. The cells incubated with GST show a relatively lower amount of radioactivity in the high molecular weight fraction, suggesting an overall inhibition of protein synthesis.

Since there was no apparent induction of MT by GST, we examined the effect of GST on $ZnCl_2$ induction of the protein. Both the Cdr20F4 cells and Cdr200T1 cells were incubated simultaneously with 10^{-4} *M* $ZnCl_2$ and 10^{-4} *M* GST (Fig. 4). In both cases, the total radioactivity in the cells was lower than in the cells incubated with zinc alone but in both bases, induction of MT was observed. With the Cdr200T1 cells incubated with GST and zinc, there was a lower percentage of total [^{35}S]cysteine counts in the MT peak than was observed with zinc alone (40% vs 68%). Thus, GST may depress the zinc-induced [^{35}S]cysteine incorporation into MT to some degree. However, it is clear that significant amounts of MT are synthesized by these cells in response to zinc (compare Figs. 3 and 4).

Since induction of MT by zinc was observed in the presence of GST, cells of all lines were grown in both zinc and GST to determine whether zinc offered any protection from GST toxicity. For these experiments, the zinc concentration was fixed at 10^{-4} *M* and the GST concentration was varied between 10^{-4} and 10^{-2} *M*. Cell numbers at each time point were determined as for Figure 1. Figure 5 shows the data for cells of Cdr200T1 line grown for 95 hr in zinc and GST. There was no increase in cell number in the presence of zinc and GST compared with that for GST alone. This was true for all cell lines and all times examined. The ANOVA three-way analysis testing zinc, cell line and GST also indicated that zinc had no significant effect on cell growth. This suggests that induction of MT by zinc does not result in any protection of these cells from GST toxicity.

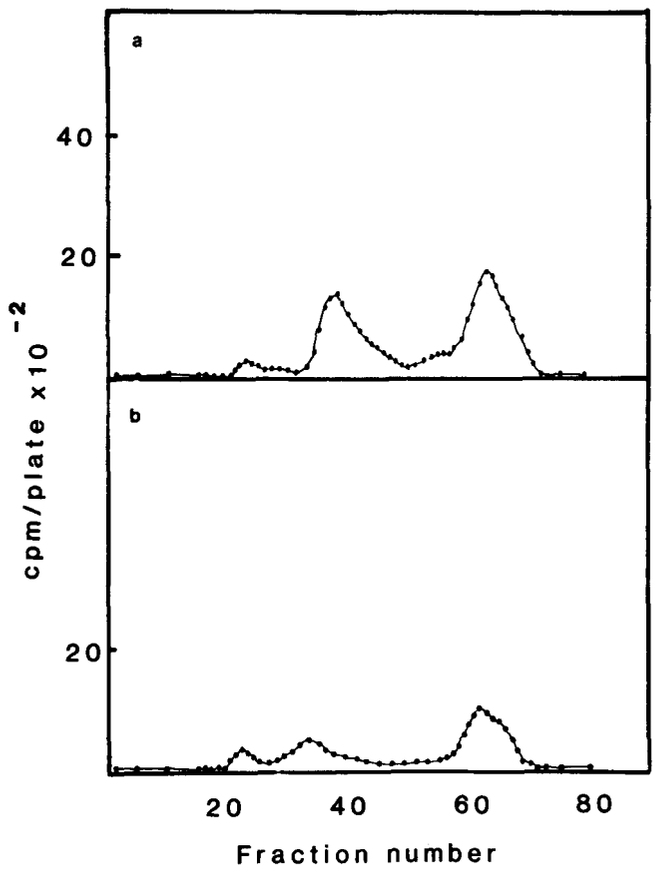

Figure 4. Measurement of $ZnCl_2$ induction of MT in Cdr200T1 and Cdr20F4 cells in the presence of GST. [^{35}S]cysteine incorporation study was carried out as in Figure 2 but both 10^{-4} *M* $ZnCl_2$ and 10^{-4} *M* GST were present for 6.5 hr prior to the addition of [^{35}S]cysteine, Cdr200T1 (a), or Cdr20F4 (b).

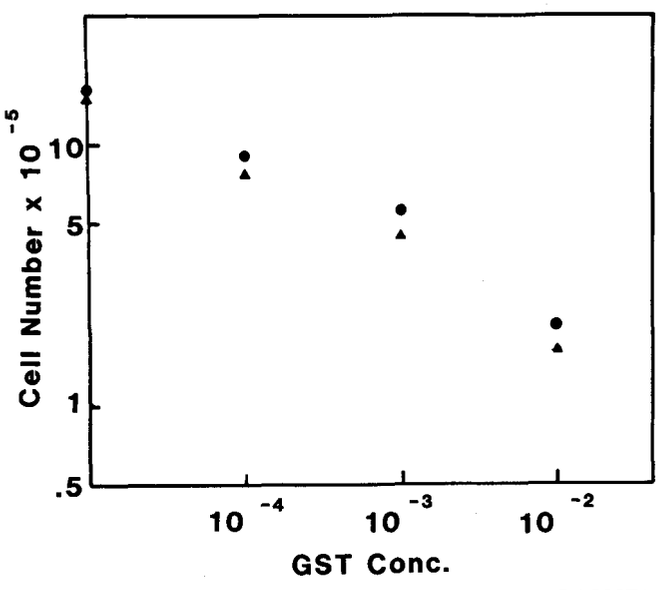

Figure 5. The effect of increasing GST concentration on Cdr200T1 cells incubated in the presence and absence of zinc. Cells of the Cdr200T1 line were incubated in indicated gold concentrations for 95 hr with (▲) or without (●) added zinc. At that point, the number of cells present was counted as indicated in Materials and Methods. The values plotted are the mean values for three separate experiments.

The Cdr200T1 cells were used to determine what proportion of the gold present in the cells was bound to MT. After preinduction for 24 hr with $ZnCl_2$, cells were treated with 10^{-4} *M* GST for 6.5 hr. Measurement of the gold in the MT peak indicated that 31% of the gold in the soluble protein fraction was found in the MT peak.

Discussion

Since toxicity prevents more widespread use of gold compounds in the treatment of arthritis, determination of a mechanism by which cells are better able to withstand the toxic effects of gold could have important implications for chrysotherapy. These studies were carried out to determine whether the presence of metallothionein in cells could provide any protective effect from the toxicity of gold sodium thiomalate.

The use of tissue culture for this type of study has clear advantages. Cells can be treated with known amounts of known compounds, providing a more direct test of the effect of a compound than is possible *in vivo*. Also, variant cell lines can be selected which can survive under specified conditions better than the parental cells. In the case of the cells used here, the variant lines were selected for their ability to withstand higher levels of cadmium than the parental line. The primary basis for this difference is the ability of these lines to synthesize MT (10). These three cell lines were used to ask two specific questions. The first is, can GST induce MT? The second question is, does the presence of MT provide any protective effect from GST toxicity?

Growth rates of the different cell lines were monitored to determine the effect of GST at different concentrations. All cell lines showed a dose-dependent inhibition of growth. The data suggest that both of the variant lines were better able to withstand the toxicity of the drug than the parental CHOp line, although, as shown in Figure 5, the presence of zinc in the incubation medium appeared to have no effect on the ability of the cells to grow in the presence of GST.

Since both the cadmium-resistant lines show reduced sensitivity to GST compared with the cadmium-sensitive line, the question is whether this apparent resistance can be explained by induction of metallothionein. The experiments reported here used [^{35}S]cysteine incorporation to monitor MT biosynthesis. Using this method, we were unable to detect any induction of MT by incubation of the cells with GST. Neither the CHOp parental line nor the Cdr20F4 line showed any detectable incorporation of [^{35}S]cysteine into a fraction of the appropriate molecular weight for metallothionein. The Cdr200T1 line, which makes constitutive levels of MT, did show some [^{35}S]cysteine incorporation in the MT size range, but the level did not appear to be significantly different from the uninduced level. The most straightforward conclusion is that resistance of the two cell lines to gold toxicity cannot be explained by induction of MT.

The other major question addressed by this study was whether the presence of MT enhances the ability of cells to survive GST. Our results indicate that it does not. This can be seen most clearly by examination of the results of experiments using Cdr20F4 cells. These cells show gold resistance compared with the parental CHOp line but show no enhanced resistance in the presence of zinc, where MT induction has been demonstrated (Fig. 4). Since there is no difference in cell number whether or not MT is present, the most obvious conclusion is that MT does not protect the cells against gold toxicity. One possible interpretation of the lack of protection by zinc is that at the concentrations of gold that were present in the cells, Au-MT would not be formed. Clearly, different metals vary in their ability to bind to the thionein apoprotein (16). However, it has been shown that Au has a high affinity for thionein, since Au can readily replace zinc during *in vitro* incubations (6). Furthermore, our data show that in the case in which zinc was used to preinduce MT for 24 hr before addition of the GST, approximately 31% of the cytosolic gold was found in the MT fraction 8 hr after GST administration. This clearly indicates that at the concentrations of GST used in these experiments, there are significant levels of Au bound to MT when it is present. The level of 31% found here is similar to that reported in experiments in which Au was bound to MT *in vivo* after preinduction with other metals (6). If the gold resistance of these cell lines is not due to MT, then other factors must be explored. Possibilities include high molecular weight-binding proteins (17) or glutathione (10), each of which contributes along with MT to the cadmium-resistance of the CHO cells we have studied.

Other investigators have used tissue culture cells to examine the relationship between gold drugs and MT. Butt *et al.* (18) used the Cdr20F4 line to determine whether auranofin, another gold drug, induces MT. Their study involved two phases. The first was to monitor synthesis of MT mRNA in response to auranofin treatment using Northern analysis. The second was to monitor [^{35}S]cysteine incorporation into the two electrophoretically separable isoforms of MT, MT1, and MT2. The Northern analysis compared levels of MT mRNA before or 6 hr after exposure of the Cdr20F4 cells to auranofin. In the absence of auranofin, no MT mRNA was detectable. After 6 hr in auranofin, the MT mRNA level was very high, equal to that seen after 15 hr of cadmium induction. Auranofin, therefore, does induce MT mRNA. Comparing levels of [^{35}S]cysteine incorporation into MT isoforms presents a different picture, however. There is clearly detectable synthesis of MT1 and MT2 by the Cdr20F4 cells after auranofin induction, but the levels are significantly lower than those seen after cadmium induction. The authors did not address this apparent inconsistency at the time. An explanation for the smaller amount of [^{35}S]cysteine incorporation into MT for auranofin induction com-

pared with cadmium induction appeared in a later article from the same group (19). That article indicated that auranofin-induced MT has a faster degradation rate than cadmium-induced MT. This observation could then account for the lower amounts of [^{35}S] cysteine-containing MT after Au induction. However, in the light of our experiments another obvious explanation follows. Since GST appears to inhibit [^{35}S]cysteine uptake, auranofin may have the same effect. Thus, although there are significant levels of MT mRNA produced in response to auranofin treatment, very little MT protein synthesis might be detected because of low levels of [^{35}S]cysteine in the cells.

Two separate groups (19, 20) have suggested that there is a strong correlation between resistance to auranofin in culture and the presence of MT. Work with CHO cells (19) has shown that cells resistant to auranofin make significantly higher levels of MT than controls. The authors do not, however, demonstrate that the MT present binds significant amounts of gold. Using human epithelial cells, Glennas *et al.* (20) have shown a correlation between high levels of MT and auranofin resistance. In these experiments, total MT levels were monitored by radioimmunoassay. Both cadmium-resistant cells and auranofin-resistant cells show higher levels of MT than the parental cells.

Our results on GST effects on cells are clearly at odds with results of experiments using auranofin. Auranofin does seem to induce MT mRNA and auranofin resistance is correlated with MT protein. In contrast, our results show no evidence of induction of MT by GST and no evidence for protection from GST toxicity by simultaneous $ZnCl_2$ induction of MT. After these experiments were completed, reports by Glennas and Rugstad (21, 22) appeared in which they studied GST effects on human epithelial cells. In contrast to their earlier results with auranofin (20), they showed that GST does not induce MT. Furthermore, preinduction of MT by cadmium in cadmium-resistant cells does not offer any protection from GST toxicity. Their result that approximately 36% of cytosolic gold is bound to preinduced MT is in good agreement with our value (31%). The results of Glennas and Rugstad (21, 22), along with those reported here, suggest that auranofin and GST may work at different sites and that resistance to the two drugs may be based on different mechanisms. The lipophilic properties of auranofin yield a drug which can cross cell membranes easily (23). This clearly permits the primary target to be intracellular. In contrast, GST might act initially at the level of the membrane or on a membrane transport system. If effects on transport are the initial results of GST treatment, then the presence of intracellular MT might be expected to offer no protection from GST toxicity. Further experiments on both drugs are necessary to explore these differences.

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