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EFFECT OF GOLD SODIUM THIOMALATE ON PROLIFERATION OF HUMAN RHEUMATOID SYNOVIAL CELLS AND ON COLLAGEN SYNTHESIS IN TISSUE CULTURE*

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Abstract—Synovial tissue obtained from patients with rheumatoid arthritis who were undergoing reconstructive joint surgery was used to obtain explant cultures of synovial cells. The experiments described were performed on growing monolayer cultures during the second to fifth passages. Synovial cells were exposed to gold sodium thiomalate (GST) in concentrations equivalent to levels attained in synovial tissues during chrysotherapy (3–50 μg/ml). After 5 days of exposure of cells to 10 or 50 μg/ml of GST, [^{3}H]thymidine incorporation into DNA was inhibited 94 or 99 per cent, respectively. After 10 days of exposure to 10 μg/ml of GST, cell number was decreased 50 per cent, although no change in cell number was found after a 5-day exposure to 100 μg/ml of GST. The total collagen content of the media was decreased in flasks of cells exposed to 50 μg/ml of GST for 15 days, which reflects the decrease in cell numbers observed at this concentration. However, after 15 days of exposure of synovial cells to 12 μg/ml of GST, incorporation of [^{14}C]proline into total collagen per cell increased 4-fold. This increase in [^{14}C]proline incorporation occurred predominantly in type I collagen. In these experiments, the percentage of type III collagen containing [^{14}C]proline and the amount found in media were suppressed 50 per cent by a fifteen day exposure to 3 μg/ml of GST. The ability of GST to increase the relative commitment of these cultures to make type I collagen is dose dependent in the range from 3 to 12 μg/ml. These data indicate that changes in cell proliferation and in the nature (genetic composition) of the extracellular matrix produced are direct effects of GST on the synovial cell in tissue culture and may represent one important mechanism of action of chrysotherapy in the treatment of patients with rheumatoid arthritis.

Gold has been used for approximately 50 years to treat rheumatoid arthritis (RA). The introduction of gold into therapeutics was based on its bacteriocidal (i.e. anti-mycobacterial) property [1]. Following its introduction, a number of hypotheses were proposed to explain the effectiveness of gold; these included increasing collagen crosslinking [2–4] and inhibition of lysosomal enzymes [5–7]. More recently, inactivation of complement [8], inhibition of macrophage and neutrophil phagocytosis [9,10], inhibition of neutrophil chemotaxis [11] and inhibition of lymphocyte proliferation [12,13] have been suggested as possible mechanisms of actions. Steroidal and non-steroidal anti-inflammatory agents also decrease pain and inflammation associated with RA, but they do not prevent the hyperplasia of the synovium, or the erosive destruction of joint tissues by the pannus [1]. In several randomized double blind studies, gold sodium thiomalate (GST) administered to RA patients was effective in preventing progressive joint erosion [14,15].

Collagen is the fibrous structural protein of the extracellular matrix. Since the discovery of different genetic types of collagen [16], there have been numerous reports of abnormalities in the synthesis and accumulation of the various collagen genetic types in a number of connective tissue disorders [17,18]. Type I and type III collagen are the predominant types that have been extracted from synovial tissue [19,20]; both types are synthesized by synovial cells in primary culture and after four passages of cells growing in monolayer.† In the study reported here, we have investigated the dose-related effect of GST on the proliferation of human rheumatoid synovial cells and on the synthesis of type I and type III collagen by these synovial cells in culture.

MATERIALS AND METHODS

Culture methods. Synovial tissues were obtained from patients with RA who were undergoing recon-

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structive joint surgery at the Roger Williams General Hospital, Providence, RI. The fat was removed, and 1 mm^3 fragments of synovial tissue were placed in 100 × 20 mm dishes (Corning Glass Works, Corning, NY). Dulbecco's Modified Eagle's (DME) medium containing 10% (v/v) heat-denatured fetal calf serum (FCS), penicillin–streptomycin solution (100 units penicillin and 100 μg streptomycin per ml medium), and Fungizone (2.5μg per ml medium) (all obtained from the Grand Island Biological Co., Grand Island, NY) was added to the dishes. The cultures were incubated at 37° in the presence of 95% air and 5% carbon dioxide. To subculture the cells, the cell layer was detached by incubation in isotonic phosphate-buffered saline (PBS), pH 7.2, containing 0.1% trypsin (Grand Island Biological Co., Grand Island, NY), 0.02% EDTA and 0.02% glucose. Experiments were performed on cells at the second to fifth passage and the medium was routinely changed every 4–6 days. All cultures were tested for and found to be free of mycoplasma contamination using a fluorescent assay (Bioassay Systems, Cambridge, MA). Gold sodium thiomalate (GST) (Merck, Sharp & Dohme, West Point, PA) was diluted in sterile distilled water. A 0.01 vol. of drug solution was added to culture medium.

Cell proliferation. Cell number was determined by using either a hemocytometer or a Coulter Counter model T_A. The incorporation of [^{3}H]thymidine ([^{3}H]TdR) 542 mCi/mmole (New England Nuclear, Boston, MA) into DNA was measured by adding 0.5 μCi [^{3}H]TdR/ml medium 4 hr before harvesting the cell layer. Cells were released from the dishes using the trypsin solution described above and precipitated twice in a 10% (v/v) trichloroacetic acid (TCA) solution. The TCA pellet was dissolved in 1 ml of 0.05 N NaOH, and radioactivity was determined in a Packard Tricarb liquid scintillation counter.

Collagen extraction and quantitation. Cells in 75 cm^2 flasks (Corning, NY) were incubated with 10 ml of fresh medium containing β-aminopropionitrile (BAPN) (10^{-4} g/ml) and ascorbate (10^{-4}M). Ascorbate was added to flasks every day during the 5-day incubation period. [^{14}C]Proline (sp. act. 260 mCi/mmole) (New England Nuclear) (1 μCi/ml medium) was added 48 hr before the medium was harvested. Labeled proline incorporation in this system was linear over a 72 hr period. In the presence of BAPN to inhibit collagen cross-linking and ascorbate to ensure hydroxylation, the amount of radioactive collagen present in the cell layer was below the limit of detection of the collagenase digestion assay. In view of this, these studies were conducted on collagen harvested from the medium; the cell layer was used for the cell proliferation studies described above. Medium protein was precipitated by adding ammonium sulfate crystals to 45% saturation. After overnight incubation at 4°, the precipitate and solution were centrifuged at 3000 *g* for 30 min. The pellet (which contained more than 95% of the non-dialyzable hydroxyproline) was dissolved in 1 ml of 0.5 M acetic acid and then dialyzed in the cold at 4° against 0.5 M acetic acid. Pepsin (100 μg/ml) was added to the dialysis bags to digest non-collagen protein and convert procollagen to collagen. Dialysis was continued for another 2 days and the samples were then lyophilized.

Samples were prepared for electrophoresis by dissolving them in 0.05 M sodium phosphate (pH 7.2) containing 0.2% sodium dodecylsulfate (SDS) and 10% sucrose. The pH was adjusted with sodium hydroxide and the proteins were made fluorescent by the addition (with mixing) of 0.2 vol. of a 4 mg MDPF*/ml acetone solution. Disulfide bonds of type III collagen were reduced (R) by heating the alkaline samples (pH = 10–11) at 56° for 15 min. Non-reduced (NR) samples were prepared by returning the fluorescent samples to neutrality (pH = 7–8) with hydrochloric acid before heating.

Samples were electrophoresed according to the procedure of Neville [21]. The quantitation of fluorescence in the gels was determined as described by Goldberg and Fuller [22], using the Gilford fluorescent gel accessory model 2515 and the Gilford model 250 spectrophotometer. Areas beneath the peaks were measured with a Numonics electronic planimeter model 210-217 and compared to the fluorescence of known standards. Using a standard curve from purified α1(I) calf skin collagen, the fluorescent area (RFI X mm) of synovial cell medium MDPF-labeled collagen was converted to amount of collagen α chains.

The radioactivity in the bands corresponding to α chains was determined by dissolving 5 mm slices in 0.2 ml hydrogen peroxide and then heating at 60° for 4 hr. Ten milliliters Hydromix (Yorktown Research, Hackensack, NJ) were added to the vials, and the radioactivity was determined in a Packard liquid scintillation counter.

The percentages of type I and type III collagen in SDS polyacrylamide gels were calculated from the following formulas:

$$\% \text{ Type I} = \left[\frac{3}{\frac{\alpha 1(R)}{\alpha 2(R)} + 1}\right] \times 100$$

$$\% \text{ Type III} = \left[\frac{\frac{\alpha 1(R)}{\alpha 2(R)} - \frac{\alpha 1(NR)}{\alpha 2(NR)}}{\frac{\alpha 1(R)}{\alpha 2(R)} + 1}\right] \times 100,$$

where α1 (NR) and α2(NR) are c.p.m. found in the gel slices or fluorescence found in the peaks at the α1 and α2 chain positions, respectively, after electrophoresis of a non-reduced sample; and where α1 (R) and α2 (R) are the c.p.m. found in the gel slices or fluorescence found in the peaks at the α1 and α2 chain positions, respectively, after electrophoresis of a reduced sample.

Collagen [^{14}C]labeled samples were analyzed by carboxymethyl cellulose chromatography, using the procedure described by Miller [23]. Briefly, a 1.8 × 10 cm column (Pharmacia, Uppsala, Sweden) was filled with carboxymethyl cellulose (Whatman, Kent,

* MDPF = 2 - methoxy - 2,4 - diphenyl - 3(2H) - furanone. MDPF is available from the Diagnostic Division, Hoffman–LaRoche, Nutley, NJ.

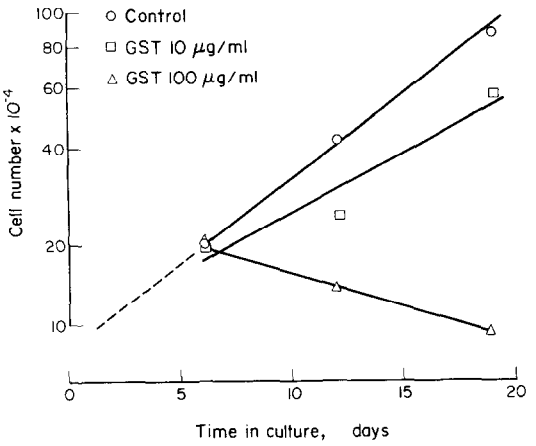

Fig. 1. Effect of GST on cell growth curve. Cells (10^5) were plated in 25 cm^2 flasks on day zero. The medium was changed on day 1 with the addition of GST to the gold-treated flasks. GST was added after medium changes on days 6 and 11. Each point represents the mean of three flasks. Cells were counted using a hemocytometer. Key: (○) control, (□) 10 μg GST/ml medium; and (△) 100 μg GST/ml medium.

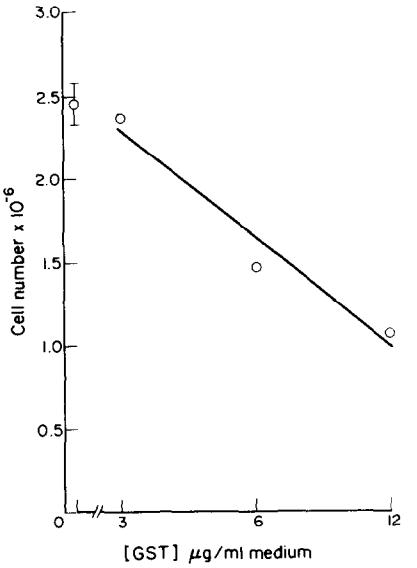

Fig. 2. Dose–response effect of GST on growth curve, following exposure of synovial cells to GST for 15 days. Flasks (75 cm^2) were plated with 500,000 cells. Cells were counted using a Coulter counter. The control is the mean ± S.E. from three flasks.

England), equilibrated in 0.06 M sodium acetate buffer (pH 4.8) and maintained at 42°. The ^{14}C-labeled collagen samples, containing 2–4 mg of type I and type III calf skin collagen (prepared as described previously [22]) as carrier, were denatured by heating at 56° for 30 min in the presence of 10 M urea. The samples were reduced by the addition of 1% (v/v) β-mercaptoethanol and applied to the column. The collagen α chain components were eluted from the column using a linear salt gradient; the starting buffer consisted of 200 ml of 0.06 M sodium acetate buffer (pH 4.8), and the final buffer consisted of 200 ml of starting buffer containing 0.1 M sodium chloride. The eluant was collected in 12-ml fractions; 1 ml of each fraction was mixed with 10 ml Hydromix, and the radioactivity was counted. The percentages of type I and type III ^{14}C-labeled collagen were calculated from the radioactivity eluted in the peaks that correspond to α1 (I), α1 (III) and α2 chains. The α1 (I), α1 (III) and α2 peaks were confirmed by their electrophoretic migration on SDS–polyacrylamide gels.

Table 1. Effects of gold sodium thiomalate on proliferation of human synovial cells in culture

GST (μg/ml)	Cells × 10^{-3}	DNA [^{3}H]TdR* (c.p.m. × 10^{-3})
	Two-day incubation	
0	122 (119, 125)†	41 (35, 46)
50	134 (124, 144)	9 (8,10)
	Five-day incubation	
0	178 ± 12‡	160 ± 25
10	177 ± 6	10 ± 2§
50	185 ± 16	2 ± 0.5§

* Four-hr incorporation of [^{3}H]TdR into TCA-precipitated cell layer.
† Mean of two flasks with individual values in brackets.
‡ Mean ± S.E. for three flasks.
§ $P < 0.01$.

RESULTS

Effect of GST on proliferation of cells cultured from synovial tissue. The effect of GST on synovial cell proliferation and on the growth curve was determined by measuring incorporation of [^{3}H]TdR into DNA and by counting total cell number. The incorporation of [^{3}H]TdR into DNA was inhibited 78 per cent after a 2 day exposure to 50 μg/ml of GST (Table 1). After 5 days of exposure, [^{3}H]TdR incorporation was almost completely inhibited—94 per cent by 10 μg/ml of GST and 99 per cent by 50 μg/ml of GST. The total number of cells per flask did not change significantly after a 5-day exposure to concentrations as high as 100 μg/ml of GST. However, as shown in Fig. 1, 10 μg/ml of GST depressed the cell growth curve between 5 and 20 days, and exposure of cells to 100 μg/ml of GST caused a continuous decrease in the accumulation of cells in the flasks during the same period. When cells were exposed to GST for 15 days, the depression of the growth curve was dose dependent through a range of concentrations from 3 to 12 μg/ml (Fig. 2). The viability of the cells exposed to GST in these experiments was unchanged, as confirmed by trypan blue exclusion. This is consistent with observations reported by Finkelstein *et al.* [13], when the organic gold compound auranofin was investigated in a culture system consisting of human lymphocytes.

Effect of GST on collagen synthesis. The rates of synthesis of type I and type III collagens were quan-

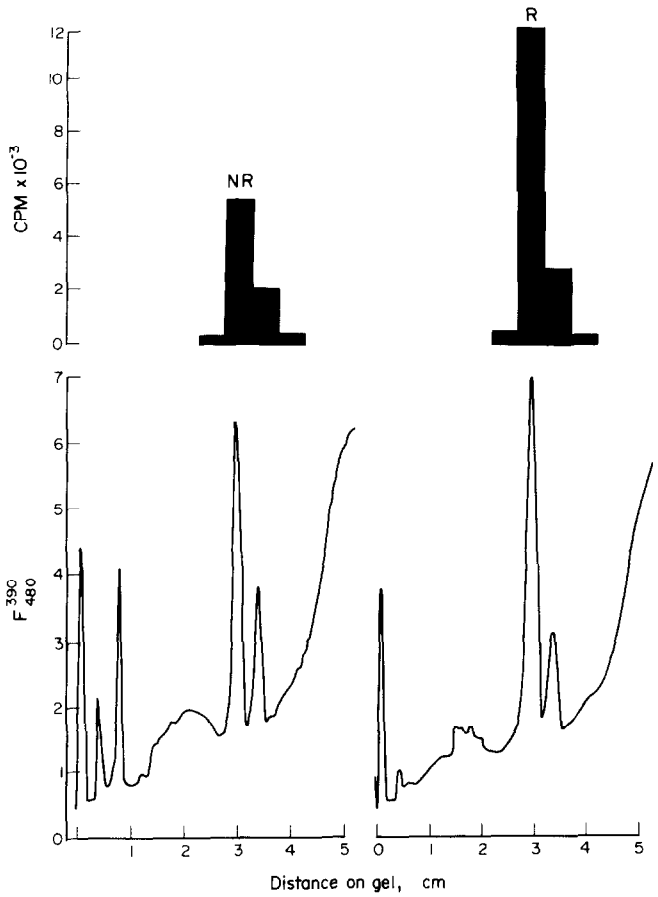

Fig. 3. Lower panels: fluorescent gel scans of synovial cell medium collagen before reduction (left), and after reduction (right). Upper panels: the radioactivity (c.p.m.) of [^{14}C]proline in 5 mm gel slices.

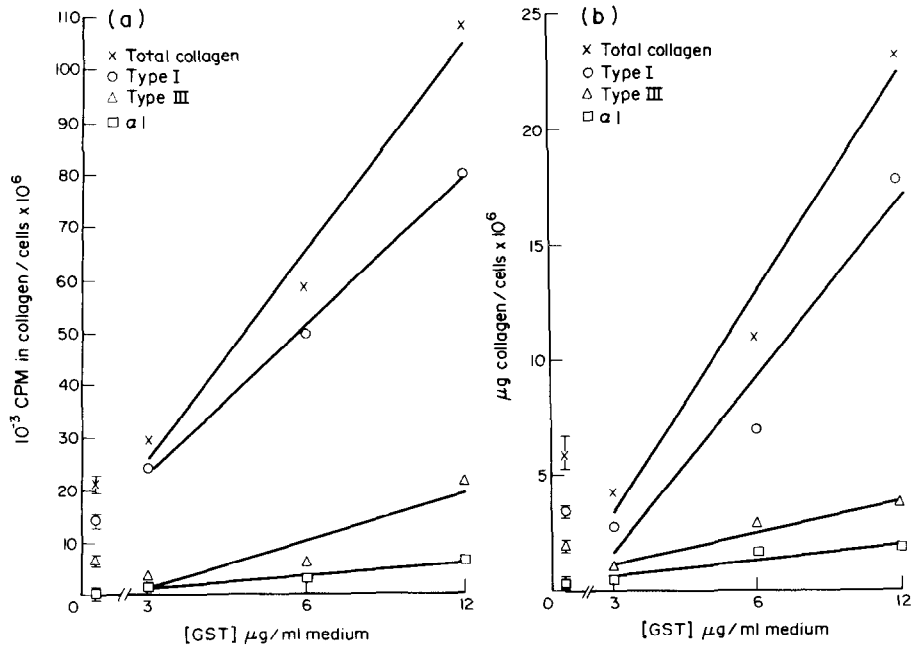

Fig. 4. Effects of GST on total collagen and collagen types based on measurement of (a) [^{14}C]collagen and (b) MDPF collagen after separation by SDS–PAGE. The control levels are the means ± S.E. for three flasks. Key: (×) total collagen, (○) type I, (△) type III, and (□) α1 (other).

Table 2. Effects of gold sodium thiomalate on the percentage of type III collagen produced by synovial cells in culture*

GST (μg/ml)	Separated by SDS–PAGE Percent collagen type III F^{390}_{480}	c.p.m.
0	30.87 ± 3.14	31.70 ± 6.27
3	9.90 ± 3.38†	13.07 ± 0.87†
6	8.30 ± 2.88†	8.83 ± 3.33†

* Values are means ± S.E. from three flasks of the amount of type III collagen as a percentage of total collagen detected by fluorescence (F^{390}_{480}) or c.p.m. [^{14}C]proline incorporated into collagen α chains. Cells were preincubated with GST for 15 days. Fresh medium without GST but containing BAPN and ascorbate was then added and the medium collagen was extracted 5 days later. [^{14}C]proline was added only during the last 48 hr of this period.
† $P < 0.05$.

titated by measuring the incorporation of [^{14}C]proline into the respective collagen α chains after separation by SDS–polyacrylamide gel electrophoresis (PAGE) (Fig. 3). In this separation procedure, type III collagen remains a trimer because of disulfide bonds, whereas type I collagen migrates as 100,000 mol. wt chains. After reduction, both migrate as α chains. Thus, the increase in the α1 band after reduction represents the radioactivity due to the presence of type III collagen.

The total amount of collagen which accumulates in the medium after a 5-day incubation was determined by coupling the medium extract with MDPF, which resulted in a fluorescent protein conjugate. The collagen components were separated by SDS–PAGE and quantitated by fluorescent gel scanning [22]. The relative migration of medium collagen chains was unaffected by GST exposure. The addition of known amounts of purified calf skin α1 (I)

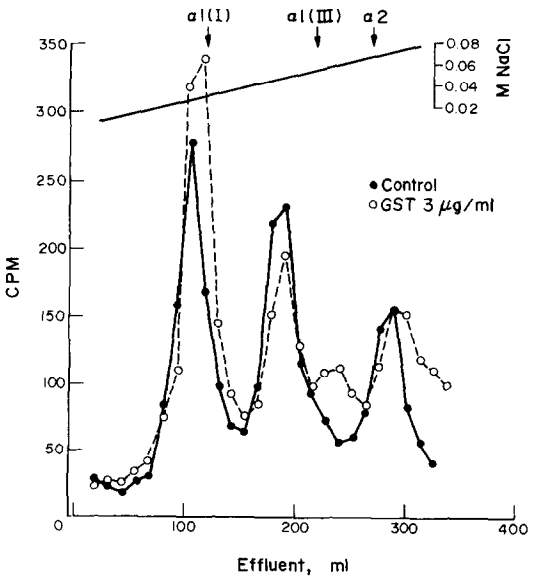

Fig. 5. Carboxymethyl cellulose chromatogram of media extracts from control and GST-treated cells. Key: (●) control, and (○) 3 μg GST/ml medium for 15 days.

(used as an internal standard) to synovial cell media collagen extracts resulted in stoichiometric increases in the fluorescent MDPF-α1 peak area. The reduction of disulfide bonds by either sodium hydroxide or β-mercaptoethanol resulted in similar increases in the α1 peak when these were measured by either fluorescence or radioactivity, as described previously [22].

As shown in Fig. 4, there is a dose-dependent increase in the amount (calculated per cell) of collagen α chains found in the medium 5 days after the termination of a 15-day GST exposure. A similar increase in the incorporation of [^{14}C]proline was observed when these cells were incubated with [^{14}C]proline during the last 48 hr of this experiment (3 days after termination of GST) (Fig. 4). This increase in both synthesis and accumulation is predominantly type I collagen (Fig. 4).

When GST (3 or 6 μg/ml) was added to medium for 15 days, the percentage of type III collagen found in the synovial cell medium (by the fluorescent assay) decreased ($P < 0.05$) (Table 2). Similarly, the rate of synthesis of type III collagen (by label incorporation) was decreased in the period following a 15-day GST exposure. However, the change in the commitment of these cells to type III collagen synthesis was not dose dependent. At 3 μg/ml of GST, the cell number (Fig. 3) and total collagen synthesis (Fig. 4) were similar to control, but the percentage of type III collagen (Table 2) was decreased ($P < 0.05$). Between 3 and 12 μg GST, the total amount of type III collagen synthesized per cell remained similar to control levels (Fig. 4), but the amount of type I collagen synthesized per cell increased in a dose dependent manner. In previous experiments [24] using 15-day exposures to higher concentrations of GST (50 μg/ml), the cell number was decreased to such an extent that not enough medium collagen was synthesized for collagen heterogeneity quantitation.

In order to confirm the above GST-induced changes in the synthesis of collagen types I and III by synovial cells, pooled medium extracts were fractionated by carboxymethyl cellulose (CMC) chromatography to separate the radioactive α chains. Figure 5 shows the chromatograms obtained from synovial cell medium collagen. The arrows represent the peak positions of the purified type I and type III calf skin collagen α chains used as standards. The medium collagen obtained from cells exposed to 3 μg/ml of GST for 15 days in the experiment described above (Table 2 and Fig. 4) was fractionated on CMC-chromatography (Fig. 5). This experiment confirmed the decrease in radioactivity in α1 (III) and the increase in radioactivity in α (I) peaks which were previously detected when individual medium extracts were fractionated on SDS–PAGE. The GST-induced change in the percentage of type III collagen found after separation of the α chains from pooled samples is quantitatively the same as the changes in the mean values reported in Table 2.

DISCUSSION

Several controlled clinical studies have established the efficacy of chrysotherapy in inducing the remis-

sion of rheumatoid arthritis [14,15,25,26]. The present study demonstrates that in cell culture GST, at pharmacologically relevant concentrations, has a direct effect on cells derived from the synovial tissues of patients with rheumatoid arthritis. Serum gold concentrations of rheumatoid arthritic patients during chrysotherapy are between 1 and 3 μg/ml, which is equivalent to 2–6 μg/ml of GST. Synovial tissue gold concentrations are approximately 20–30 μg/g tissue, which are equivalent to 40–60 μg/ml of GST. Thus, the concentrations used in these experiments were intermediate between the serum and tissue concentrations observed during chrysotherapy [27–32]. Lipsky and Ziff [12] have shown that the gold moiety of GST inhibits lymphocyte proliferation (thiomalic acid was without effect, and gold chloride inhibited proliferation). We have observed none of the effects reported here for GST when sodium thiomalate was added to the media and conclude therefore, in accord with Lipsky and Ziff [12], that the gold moiety is responsible for the observed changes in synovial cell morphology, proliferation and collagen synthesis. At the highest concentration of GST (100 μg/ml) no significant difference in the total number of cells was found in the culture system after 5 days of incubation; however, between 5 and 20 days a decrease in cell number was observed. This delayed response is due to the slow doubling time of these cells, resulting in the inability to detect a change in the growth curve at this time point. The DNA synthesis in these cells was inhibited by 50 μg/ml of GST after 2 days. Both cell number and [^{3}H]thymidine incorporation data indicate that the exposure of synovial cells to GST produced a dose-dependent inhibition of cell proliferation, which was also time-dependent in these cultures.

The synovial cell explant culture is a heterogeneous population of cells. The cells used in these experiments have morphological and growth characteristics similar to those reported by others [33–36]. Morphological changes following exposure to GST are apparent and include the development of large perinuclear phagolysosomes that have been described previously in synovial cells and in macrophages of the synovium *in vivo* following gold therapy [37,38]. The detailed description of the formation of these GST-induced morphological changes in tissue culture is the subject of a separate manuscript now being prepared for publication.

Hance and Crystal [39] reported recently that the ratio of collagen type I/III was independent of cell density or passage number in lung fibroblasts. The data reported here identify a shift in the heterogeneity of collagen synthesized following the exposure of cells to GST for 15 days. In this experiment, the cells were confluent when [^{14}C]proline was added during the post-exposure incubation. This protocol was also used in an experiment where [^{14}C]proline was added to cells following 10 days of GST exposure. We observed the same GST-induced shift in total collagen and in the percentage of type III collagen synthesized in these cells which were labeled and harvested during the late log-phase of growth. Therefore, we conclude that this alteration in the ratio of collagen type I/III synthesized by human synovial cells following GST exposure is also independent of cell density.

The quantitation of medium collagen, by the fluorescent method used here, represents the accumulation of [^{14}C]proline into collagen α chains in the synovial cell medium over a 5-day period. The measurement of total accumulation circumvents interpretation problems with regard to the influence of pool size changes and amino acid transport deficiencies on label incorporation which may be altered by GST [13]. The radioactive values represent the incorporation of [^{14}C]proline into collagen α chains during a 2-day incubation and subsequent separation of the radioactive collagen α chains by both SDS–polyacrylamide gel electrophoresis and carboxymethyl cellulose chromatography. We found that the exposure of human synovial cells to GST caused a decrease in the percentage of type III collagen synthesized (as detected by both separation techniques) and also in the percentage of type III collagen which accumulates in the medium (based on the quantitation of media collagen by fluorescent gel scanning techniques). In view of this, and the premise that the production of both type I and type III collagen is dependent on the same precursor pools, we conclude that the direct effect of gold on amino acid transport or pool specific activity is not responsible for the changes in collagen synthesis reported here.

GST treatment did not alter the electrophoretic migration of the α chains produced (Fig. 3), and it did not significantly alter the elution profile of α chains from the carboxymethyl cellulose columns (Fig. 5). The small peak of radioactivity appearing at 240 ml of effluent on Fig. 5 was not observed in all samples from gold-treated cells. The amount of radioactivity in this uncharacterized peak is a small percentage of the total and, therefore, does not significantly alter the type I/III ratio.

We do find slight but consistent differences in the percentages of type III collagen detected by these two separation methods.* In the present study, the mean of 31.7 per cent reported in Table 2 resolved as 39 per cent type III collagen when these samples were pooled and separated by CMC chromatography. It is possible that some of the collagen eluting from CMC in the α1 (III) position may be α [A] or α [B] chains, but these are minor components of the total collagen matrix [40] and their contribution to the total collagen pool could not account for the differences observed here. We observed that GST decreased the percentages of type III collagen to the same extent, using both separation techniques, which clearly demonstrates that a drug-induced shift in collagen heterogeneity resulted from the 10 or 15-day exposure periods used in these experiments. This argues against the possibility that the decrease in type III collagen observed by SDS–PAGE is due to GST reduction of disulfide bonds in the NR sample before it is electrophoresed. A recent abstract reported that bleomycin produced a relatively small shift in type III collagen synthesis in cultured lung and skin fibroblasts [41]. The data reported here represent the first published report of a large drug-induced shift in collagen heterogeneity in cultured human cells.

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The biochemical mechanism by which gold compounds alter proliferation and collagen synthesis in synovial cells can only be postulated from these experiments. The cell population generated by this culture technique is heterogeneous [35], so the dose-dependent effect of GST on proliferation and, therefore, also on collagen synthesis could be due to a selectivity for certain cell populations. The alteration in collagen types observed here could be due to direct stimulation of the synthesis of type I collagen, but we cannot rule out the possible inhibition of intracellular degradation [42]. Granulocyte collagenase has been shown to preferentially degrade type I as compared to type III collagen [43]. Therefore, intercellular inhibition of a similar enzyme may result in the secretion of increased amounts of type I collagen.

A pannus can be considered a form of granulation tissue. Gay *et al.* [44] demonstrated by immunofluorescence that type III collagen is the early collagen laid down in granulation tissue followed by type I collagen. In contrast, the predominant collagen type in normal scar is type I collagen [45,46]. A pannus responds to inflammation by generating granulation tissue, and further maturation results in the formation of scar tissue. Thus, it is conceivable that the alteration of the composition of the synovial matrix is related to or dictates the functional nature of the synovium in rheumatoid arthritis. Recent preliminary experiments by Dayer* indicate that a blood mononuclear cell factor may stimulate proline incorporation into collagen, with the preferential stimulation of a protein tentatively identified as type III procollagen. The data reported here establish that GST has a direct effect on synovial cell proliferation, and on the amount and nature (genetic composition) of the extracellular matrix they produce. This shift in synthesis or accumulation of type I collagen over type III collagen may represent a switch from a proliferative granulation tissue to end stage scar tissue. This forced maturation may in fact be responsible for aborting the progressive proliferative component of the rheumatoid arthritis lesion, and may represent one of the important mechanisms responsible for the therapeutic efficacy of gold therapy in the treatment of rheumatoid arthritis. Various studies now indicate that organic gold compounds produce a variety of different biological effects. The biological systems influenced by gold include complement activation [8], macrophage and neutrophil phagocytosis [9,10], inhibition of neutrophil chemotaxis [11], and inhibition of lymphocyte proliferation and immunoglobulin synthesis [12,13], in addition to the direct effect of this compound on synovial cell proliferation and the synthesis of collagen as reported here. The fact that organic gold therapy impinges on several target systems involved in the generation of the rheumatoid process may be a critical factor accounting for its tolerable or acceptable therapeutic index, since an overwhelming effect on any one of these critical functions would be associated with unacceptable toxicity to the patient. Thus, we suggest that the proven therapeutic benefit derived from chrysotherapy in the management of rheumatoid arthritis is dependent on a spectrum of pharmacological effects expressed on the different biological components contributing to the rheumatoid process.

* J. M. Dayer, personal communication.

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