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THE EFFECT OF CYANIDE ON THE UPTAKE OF GOLD BY RED BLOOD CELLS

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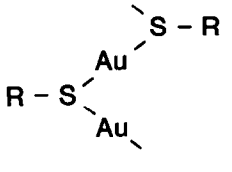
Abstract—Cyanide markedly increased the rate of uptake of gold by red blood cells when incubated with sodium aurothiomalate, a polymeric gold complex. Thiocyanate had no significant effect on gold uptake. The effect of cyanide was demonstrated to be due to the conversion of aurothiomalate to the complex ion, aurocyanide, which is rapidly taken up by red blood cells. At a low ratio (1:20) of cyanide to aurothiomalate, cyanide appeared to act as a shuttle to carry gold into red blood cells. Tobacco smoking is known to increase the concentrations of gold in red blood cells in patients treated with aurothiomalate. The present data indicate that this effect of smoking is most likely due to cyanide inhaled in tobacco smoke and not to thiocyanate, a circulating metabolite of cyanide. An effect of cyanide on the uptake of polymeric gold complexes to target cells such as polymorphonuclear leukocytes and monocytes is suggested.

Gold complexes are of considerable value in the treatment of rheumatoid arthritis. The most widely used are the polymeric complexes, aurothiomalate and aurothioglucose. In solution, the individual monomers are bonded by bridging sulphurs (Fig. 1), possibly to yield hexameric structures [1]. Recently, clinical trials have commenced on a novel complex, auranofin. Unlike the polymeric complexes, this complex is monomeric, lipid soluble [2] and is an active anti-rheumatic drug when administered orally [3, 4].

Gold complexes are considered to have intracellular sites of action since the gold becomes localised in intracellular organelles [5, 6] but little is known about the mechanism of the cellular uptake of gold complexes, particularly that of the polymeric complexes. An interesting aspect of the cellular uptake of gold complexes is the observation that approximately one third of patients treated with aurothiomalate attain significant levels of gold in red blood cells [7]. The concentrations of gold in these cells in the remaining patients are very low. Recently, it was found that this biomodal distribution of gold concentrations in red blood cells is due to the smoking habits of the patients, the cellular uptake of gold being markedly higher in smokers than in non-smokers [8, 9]. From *in vitro* studies, it was found that smokers' blood contained a circulating factor which increases the cellular uptake of gold from solutions of aurothiomalate. This factor was identified as being either thiocyanate or cyanide [8]. The latter could be produced from thiocyanate *in vitro*. We now present evidence that cyanide is the factor ultimately responsible for the increased cellular uptake of gold. Our data demonstrate that the polymeric gold complexes and cyanide interact to yield the monomeric linear complex, aurocyanide (Fig. 1) [2], which is readily taken up by red blood cells.



$(NC - Au - CN)^-$

Fig. 1. Structures of gold complexes. Upper: General structure of polymeric gold complexes. Lower: Aurocyanide complex ion.

MATERIALS AND METHODS

Materials. The gold complexes used were sodium aurothiomalate (Myocrisin, May and Baker, Dagenham, U.K.), aurothioglucose (Schering Corp., Kenilworth, U.S.A.), auranofin (Smith, Kline and French, Welwyn Garden, U.K.) and potassium aurocyanide (Engelhard Ind., Sydney, Australia). The concentrations of the polymeric complexes, aurothiomalate and aurothioglucose, are expressed in terms of the monomeric structures. Auranofin was prepared as a stock solution in methanol (4 mM) before dilution with phosphate buffered saline (0.116 g disodium hydrogen phosphate, 0.02 g potassium dihydrogen phosphate, 0.02 g potassium chloride, 0.8 g sodium chloride/100 ml). Potassium cyanide (BDH, AR grade, Poole, U.K.) was prepared as a stock solution at approximately 0.2 M. The solution was assayed by titration with silver nitrate [10] before each series of experiments. All other reagents were of analytical reagent grade.

Uptake of gold by red blood cells. Suspensions of red blood cells were prepared from healthy non-smokers after collection of blood in 1/10 vol of citrate preservative solution (2.63 g sodium citrate, 0.327 g citric acid, 0.222 g sodium dihydrogen phosphate,

5.1 g dextrose, 0.027 g adenine/100 ml). The red blood cells were isolated by centrifugation, washed five times with normal saline, suspended in phosphated buffered saline, and mixed with 1/10 vol of citrate preservative solution. The final haematocrit of the suspension of red blood cells was 35–40%. The suspensions of red blood cells were incubated with sodium aurothiomalate (5–20 μM), aurothioglucose (5 μM) and either potassium thiocyanate (100 μM) or potassium cyanide (0.1–20 μM) at 37° for up to 24 hr. At the end of the incubation period, the red blood cells were centrifuged immediately, washed five times with phosphate buffered saline and the gold content measured by atomic absorption spectrometry using a graphite furnace [11].

Uptake of cyanide by red blood cells. Suspensions of red blood cells were prepared as described above and incubated for up to 15 min with potassium cyanide solution (10 and 20 μM). At the end of the incubation period the cells were washed four times with normal saline. Aliquots of the suspension of washed red blood cells (2 ml) were incubated for 2 hr with sulphuric acid (10%, 1 ml) in Conway diffusion cells at room temperature (20°) and the liberated hydrocyanic acid collected in sodium hydroxide solution (0.5 ml, 0.1 M) in the centre well [12]. The concentration of cyanide in the sodium hydroxide solution was determined by a colorimetric method [13].

Chemical interaction between gold complexes and cyanide. The absorbance of solutions of sodium aurothiomalate (25 mM) or aurothioglucose (2 mM) was measured at 390 nm in the presence of cyanide at up to a 2:1 molar ratio of cyanide to gold complex. The interaction was also studied by determination of the concentration of free cyanide after incubation of cyanide (0–80 μM) with solutions of aurothiomalate, aurothioglucose or auranofin (20 μM) in phosphate buffered saline. The incubations were conducted in Conway diffusion cells, the cyanide trapped in the sodium hydroxide solutions and assayed as described above.

RESULTS

Uptake of gold by red blood cells. The cellular uptake of gold from sodium aurothiomate (20 μM) was slow and not significantly increased by thiocyanate (100 μM) (Fig. 2). There appeared to be an increase in gold uptake in some experiments after incubation with thiocyanate for 20 and 24 hr but, overall, there was no significant effect. Cyanide (1 μM) markedly increased the uptake of gold by red blood cells, the effect being statistically significant ($P < 0.05$) at all times from 1 hr onwards. For example, after incubation for 24 hr, the cellular uptake of gold was increased by cyanide by 21.1% from a mean of 12.7 to 33.8%. In these experiments, the concentration of aurothiomalate was 20 μM. Thus, the addition of 1 μM cyanide increased the uptake of gold by the equivalent of 4.2 μM. The effect of cyanide on the cellular uptake of gold from solutions of aurothiomalate was detectable at a cyanide concentration of 0.1 μM when the incubations were conducted for 1 hr (Fig. 3).

In the above experiments, the solution of sodium aurothiomate was added to the suspension of red blood cells immediately before the addition of cyanide or thiocyanate. Preincubation experiments indicated that the effect of cyanide was due to the formation of a complex with cyanide and not to any effect of cyanide on the permeability of red blood cells. When aurothiomalate was mixed with cyanide in a molar ratio of 1:2 for 10 min prior to the administration of red blood cells, the cellular uptake of gold was very rapid, being essentially complete after incubation for 1 min (Fig. 4). This is a nominal incubation time, the actual period of incubation being slightly longer because of the time taken to sediment

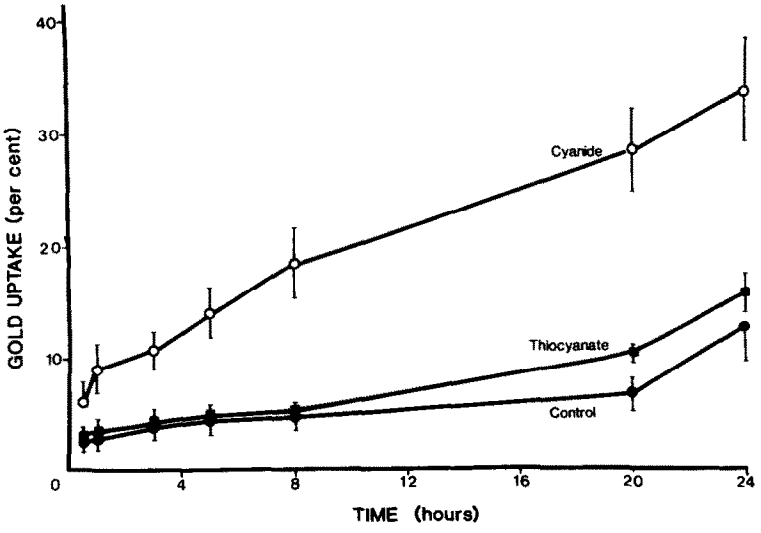


Fig. 2. The effect of cyanide (1 μM) and thiocyanate (100 μM) on the time course of uptake of gold by red blood cells. Results shown as mean ± S.E. (N = 4).

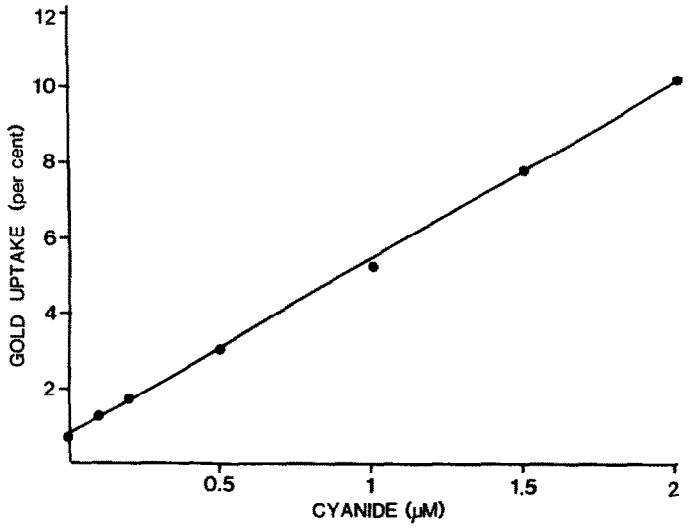


Fig. 3. The effect of cyanide on the uptake of gold by red blood cells incubated with sodium aurothiomalate (20 μM). Time of incubation, 1 hr.

the cells by centrifugation and to remove traces of extracellular gold and cyanide by washing. While there is doubt about the precise incubation periods, the cellular uptake of gold was clearly slower when cyanide was preincubated with the red blood cells before the addition of aurothiomalate (Fig. 4). In the absence of cyanide, the cellular uptake of gold was very slow, less than 1% after 1 hr. Similar results were obtained in experiments using aurothioglucose in place of aurothiomalate. The mean cellular uptake of cyanide was 64% after incubation of red blood cells with cyanide for 5 min in the absence of a gold complex and 109 and 101% when incubated for 10 and 15 min, respectively.

The cellular uptake of gold from solutions of potassium aurocyanide (5 μM) was very rapid 97.7 ± 2.7% (mean ± S.D., N = 6) after incubation with red blood cells for 5 min.

Chemical interactions between cyanide and gold complexes. The absorbance of solutions of aurothiomalate and aurothioglucose was progressively decreased by increasing concentrations of cyanide. Complete loss of absorbance was noted when the molar ratio of cyanide to aurothiomalate or aurothioglucose was 2:1 or more (Fig. 5). The loss of colour appeared to be immediate on the addition of cyanide. Aurothioglucose yielded a solution of higher molar absorptivity at 390 nm than that of

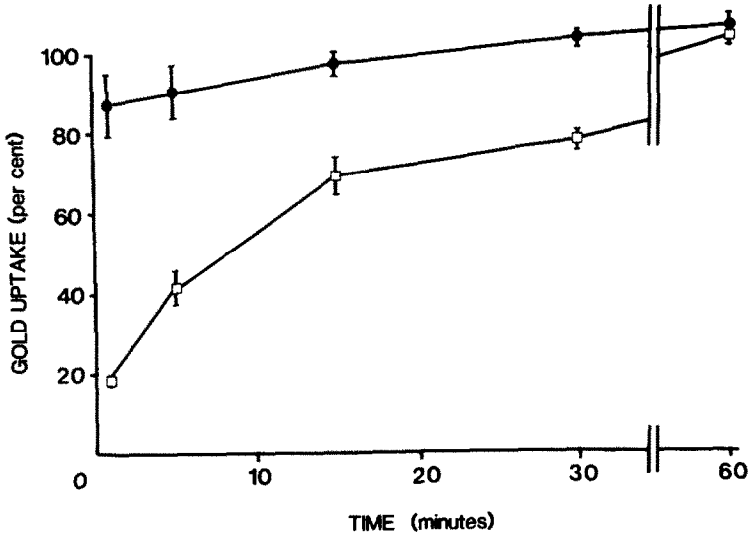


Fig. 4. The time course of uptake of gold by red blood cells incubated with sodium aurothiomalate (5 μM) and cyanide (10 μM). Cyanide preincubated with red blood cells (□); cyanide preincubated with aurothiomalate (●). Uptake of gold was less than 1% after incubation in the absence of cyanide for 60 min. Results shown as mean ± S.E. (N = 4).

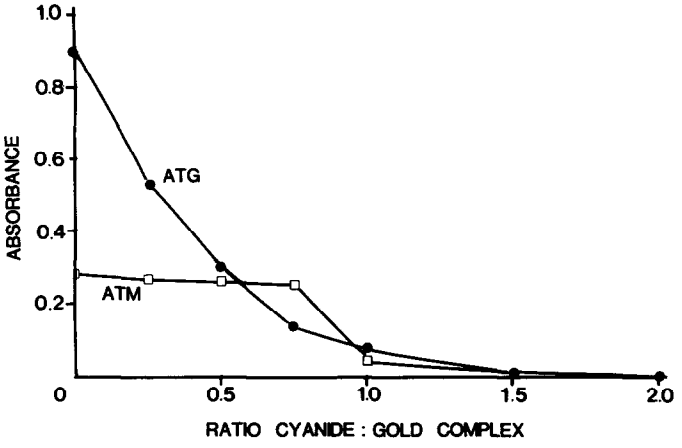


Fig. 5. The effect of cyanide on the absorbance of solutions of aurothioglucose (2 mM) and sodium aurothiomalate (25 mM) at 390 nm. Aurothiomalate (□, ATM); aurothioglucose (●, ATG).

sodium aurothiomalate and hence the experiments were conducted at a lower concentration of aurothioglucose than aurothiomalate. Potassium aurocyanide had zero absorbance at 390 nm.

Colorimetric assays demonstrated that there was very little free cyanide present when low concentrations of cyanide were mixed with aurothiomalate (Fig. 6). Above a molar ratio of cyanide to aurothiomalate of 2:1, there was a marked increase in the amount of diffusible cyanide and the absorbance due to the colorimetric reaction increased in parallel to the absorbance from cyanide alone. Similar results were obtained from the incubation of aurothioglucose with increasing concentrations of cyanide. By contrast, auranofin showed a lesser reduction in the colour developed from the diffusible cyanide, although there was still an apparent increasing proportion of free cyanide when the molar ratio of cyanide to auranofin exceeded 2:1.

DISCUSSION

The data obtained in the present study is consistent with the hypothesis that the polymeric gold complexes react with cyanide to yield the complex ion aurocyanide which is readily taken up by red blood cells. Cyanide itself is taken up by red blood cells as shown by Vesey and Wilson [14] and confirmed under the conditions used in the present studies. Cyanide is bound strongly to red blood cells and it

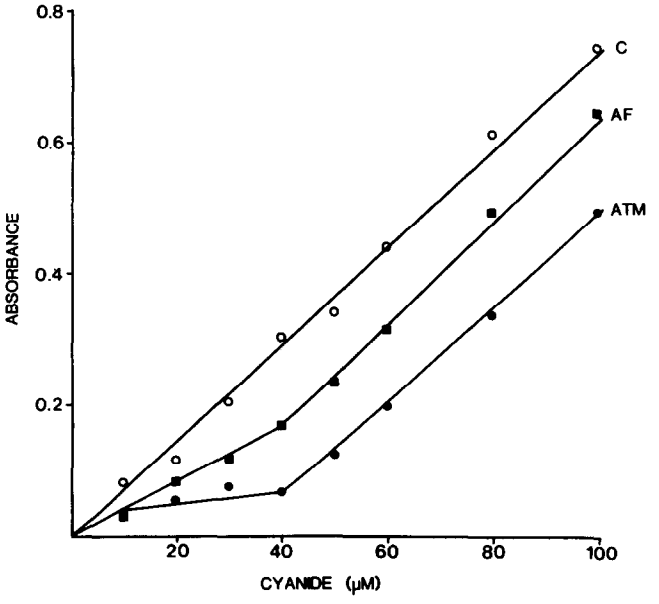


Fig. 6. The effect of auranofin (20 μM) and sodium aurothiomalate (20 μM) on the absorbance of solutions developed from the colorimetric reaction of diffusible cyanide. Control (○, C); auranofin (■, AF); aurothiomalate (●, ATM).

is not removed by repeated washing [14]. Any uptake of cyanide by red blood cells must decrease the extracellular concentration available for interaction with the polymeric gold complexes. In consequence, the pre-incubation of red blood cells with cyanide led to a slower cellular uptake of gold than when the cyanide of gold complexes were allowed to interact before the addition of red blood cells. However, the rate of uptake of gold was still very much greater when cyanide was pre-incubated with red blood cells than in the absence of cyanide, indicating that there was still sufficient extracellular cyanide to facilitate the uptake of gold.

Over a 24 hr period, the addition of cyanide (1 μM) increased the cellular uptake of gold by 4.2 μM (Fig. 2). This is over eight times the amount of gold which could be converted to aurocyanide at any one time. Thus, cyanide is probably acting as a shuttle to carry gold into red blood cells and it is unlikely that the aurocyanide complex is bound within the cell. The gold within the red blood cells may be bound to the sulphydryl groups of haemoglobin [8]. If this is correct, cyanide is transporting gold from one sulphydryl binding site (the polymeric gold complex) to another (haemoglobin) but further work is required to define the site of gold binding within the red blood cell.

Smoking enhances the uptake of gold by red blood cells in patients treated with aurothiomalate [8, 9]. Although the concentrations of cyanide in blood are not known due to interference in the available assays assays by the very much higher concentrations of thiocyanate, the marked effect of cyanide on gold uptake *in vitro* indicates that the increased uptake *in vivo* is most likely due to hydrocyanic acid inhaled in tobacco smoke. Concentrations of thiocyanate in plasma of smokers are elevated [15] through the activity of the enzyme, rhodanese, which metabolizes cyanide to thiocyanate [16]. It was observed previously that thiocyanate added to whole blood increased the uptake of gold by red blood cells when incubated with aurothiomalate. However, no consistent effect of thiocyanate on the uptake of gold by red blood cells was demonstrated in the present studies in which the uptake of gold was examined in suspensions of washed blood cells. The effect of added thiocyanate in whole blood may have been due to myeloperoxidase in polymorphonuclear leukocytes which can convert thiocyanate to cyanide [17].

All of the preceding discussion has concerned the interaction of cyanide with the polymeric complexes, principally aurothiomalate, and the resulting effects on the uptake of gold by red blood cells. The decreased concentration of free cyanide at low ratios of cyanide to auranofin demonstrates that an interaction also occurs between auranofin and cyanide. However, the interaction appears to be less marked than with the polymeric gold complexes. Auranofin is monomeric, neutral and lipid soluble. These physicochemical properties may be responsible for the ready uptake of gold by red blood cells during treatment with auranofin [18]. The pharmacokinetic properties of this drug are also consistent with greater immediate cellular uptake than produced by the polymeric complexes [19]. Thus, while auranofin can interact with cyanide, the interaction is probably of lesser importance as regards cellular uptake than the interactions with the polymeric complexes. For example, smoking does not increase the concentrations of gold in red blood cells during treatment with auranofin [20], in contrast to the marked effect during treatment with aurothiomalate [9, 10, 20].

The clinical significance of the interaction between gold complexes and cyanide is not known although several hypotheses can be put forward. Firstly, gold complexes have a variety of effects on the function of white blood cells [21, 22] and effects on these cells or their tissue equivalents may be responsible, at least in part, for their anti-arthritic effects. Polymorphonuclear leukocytes, and possibly monocytes as well, produce cyanide during phagocytosis [23]. It is possible that this production of cyanide in the local environment of the cell may increase the uptake of the polymeric gold complexes.

The effect of cyanide on the cellular uptake of the chemical forms of gold in plasma requires study. In plasma, gold is predominately bound to albumin during treatment with aurothiomalate [24, 25]. The interaction between cyanide and the gold bound to albumin has not been studied. However, an interaction between cyanide and a short lived parent drug may still significantly increase the uptake of gold by target cells during long term treatment.

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REFERENCES

1. A. A. Isab and P. J. Sadler, *J. chem. Soc. Dalton Trans.*, 1657 (1981).
2. P. Sadler, *J. Rheum.* **9** (suppl. 8), 71 (1982).
3. A. E. Finkelstein, F. R. Roisman, V. Batista, F. G. de Nudelman, E. H. de Titto, M. Mizraji and D. T. Walz, *J. Rheum.* **7**, 160 (1980).
4. B. M. Sutton, E. McGusty, D. T. Walz and M. J. Di Martino, *J. med. Chem.* **15**, 1095 (1975).
5. F. N. Ghadially, A. F. Oryschak and D. M. Mitchell, *Ann. rheum. Dis.* **35**, 67 (1976).
6. B. Vernon-Roberts, J. L. Dore, J. D. Jessop and W. J. Henderson, *Ann. rheum. Dis.* **35**, 477 (1976).
7. P. M. Smith, E. M. Smith and N. L. Gottlieb, *J. Lab. clin. Med.* **82**, 930 (1973).
8. G. G. Graham, T. M. Haavisto, P. J. McNaught, C. D. Browne and G. D. Champion, *J. Rheum.* **9**, 527 (1982).
9. D. W. James, N. W. Ludvigsen, L. G. Cleland, *J. Rheum.* **9**, 532 (1982).
10. A. I. Vogel, in *A Textbook of Quantatitive Inorganic Analysis*, 2nd ed., p. 263. Longmans, London (1951).
11. M. Harth, D. S. M. Haines and D. C. Bondy, *Am. J. clin. Path.* **59**, 423 (1973).
12. D. Feldstein and N. C. Klendshoj, *J. Lab. clin. Med.* **44**, 166 (1954).
13. P. Lundquist, J. Martensson, B. Sorbo and S. Ohman, *Clin. Chem.* **25**, 678 (1979).
14. C. J. Vesey and J. Wilson, *J. Pharm. Pharmac.* **30**, 20 (1977).
15. F. Lickint, *Z. Klin. Med.* **100**, 543 (1924).
16. B. Vennesland, P. A. Castric, E. E. Conn, L. P. Solomonson, M. Volini and J. Westley, *Fedn. Proc.* **41**, 2639 (1982).
17. B. Sorbo and J. G. Llungren, *Acta. chem. Scand.* **12**, 470 (1958).
18. D. T. Walz, D. E. Griswold, M. J. Di Martino and E. E. Bumbier, *J. Rheum.* **10**, 117 (1982).

19. D. T. Walz, D. E. Griswold, M. J. Di Martino, and E. E. Bumbier, *J. Rheum.* **7**, 820 (1980).
20. D. Lewis, A. I. A. Rogers, E. Smith and H. A. Capell, *Ann. rheum. Dis.* **40**, 619 (1981).
21. P. E. Lipsky and M. Ziff, *J. clin. Invest.* **59**, 455 (1977).
22. J. Highton, G. S. Panayi and J. Griffin, *Agents Actions* **10**, 507 (1980).
23. T. Stelmaszynska and J. M. Zgliczynski, in *Cyanide in Biology* (Eds B. Vennesland, E. E. Conn, C. J. Knowles and J. Westley), p. 371, Academic Press, London (1981).
24. E. G. McQueen and P. W. Dykes, *Ann. rheum. Dis.* **28**, 437 (1969).
25. C. J. Danpure, D. A. Fyfe and J. M. Gumpel, *Ann. rheum. Dis.* **38**, 364 (1979).