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Properties of Melarsamine Hydrochloride (Cymelarsan) in Aqueous Solution

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The antitrypanosomal drug melarsamine hydrochloride (MelCy) (trade name, Cymelarsan) is a melaminophenylarsine made by conjugation of one equivalent of melarsen oxide and two equivalents of cysteamine. Immediately after it was dissolved in water, the compound was found to exist as an equilibrium mixture containing MelCy (43%), MelCy which had lost one cysteamine moiety (MelCy −1; 24%), melarsen oxide (33%), and free cysteamine. Small amounts (<1%) of the oxidation products derived from the last two components were also formed (cystamine and sodium melarsen). On incubation at room temperature, the MelCy content decreased steadily, with an associated increase in the melarsen oxide and sodium melarsen contents. After 5 days in solution at room temperature, 27% of the arsenical agent was MelCy, 14% was MelCy −1, 42% was melarsen oxide, and 17% was sodium melarsen. Since H_2O_2 production was detectable in MelCy or cysteamine solutions and the addition of small amounts of exogenous H_2O_2 readily converted the trivalent melarsen oxide to the pentavalent sodium melarsen, it is hypothesized that the nonenzymatic conversion of cysteamine to cystamine produced H_2O_2, which then oxidized melarsen oxide to sodium melarsen. Similar time course experiments showed that melarsonyl potassium and melarsoprol were more stable in solution.

African trypanosomiasis, which is caused by *Trypanosoma brucei gambiense* and *T. brucei rhodesiense*, remains a major tropical health problem, with an estimated 50 million people at risk of infection (16). Infection of animals with the related species *T. brucei brucei*, *T. evansi*, and *T. equiperdum* restricts the rearing of livestock over an area of approximately 10^7 km^2 (16). Treatment of the secondary, cerebral stage of trypanosomiasis has relied primarily on the melaminophenylarsine melarsoprol (MelB; Fig. 1), which was introduced in 1947 (6). MelB is insoluble in water and is administered by intravenous injection as a propylene glycol solution (1). This arsenical agent is frequently associated with a number of serious side effects, particularly an arsenical agent-induced encephalopathy (7, 12), and the solvent is a powerful irritant that often causes thrombophlebitis (1). Water-soluble arsenical agents such as melarsonyl potassium (MelW; Fig. 1) have also been synthesized, but showed little improvement over MelB (1).

Recently, a new, water-soluble trivalent arsenical agent, melarsamine hydrochloride (MelCy; Fig. 1) (trade name, Cymelarsan), has been shown to be very effective against *T. brucei brucei*, *T. evansi*, and *T. equiperdum* in camels, buffalo, goats, and pigs and in vitro (10, 11, 17–19). The drug was also found to be effective against diminazene aceturate-resistant *T. brucei brucei* and *T. evansi* (17), is at least 2 to 2.5 times more effective than MelW (18), and has also been useful as part of combination therapies in mouse models of secondary trypanosomiasis (8, 9). On the basis of the results of those experiments, MelCy has been licensed for use against trypanosomiasis in animals. In the study described here, we examined the properties of MelCy in solution and found that the compound is unstable and that several dissociation products are formed.

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MATERIALS AND METHODS

Compounds. MelCy, trivalent melarsen oxide, MelW, MelB, and pentavalent melarsen (as the sodium salt) were all provided by Specia, Rhône-Poulenc (Paris, France). Cysteamine and cystamine were acquired from the Sigma Chemical Co. (Gillingham, United Kingdom), and high-pressure liquid chromatography (HPLC)-grade 1-propanol was acquired from BDH (Poole, United Kingdom). Camphor sulfonic acid and fluorescamine were obtained from the Aldrich Chemical Co. (Gillingham, United Kingdom). All water was filtered and deionized by the Millipore Milli-Q50 system (Watford, United Kingdom).

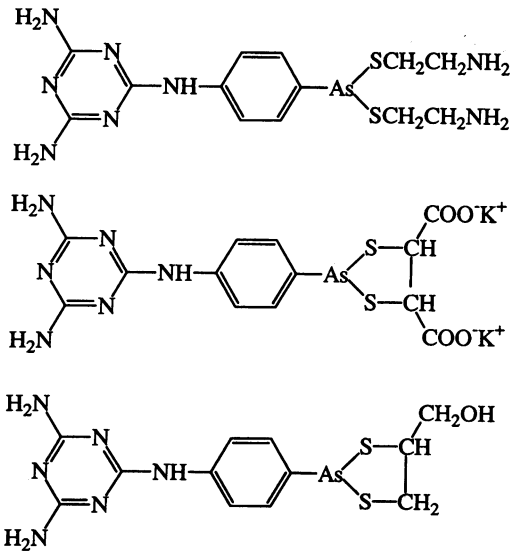

FIG. 1. The structures of (from top to bottom) MelCy, MelW, and MelB.

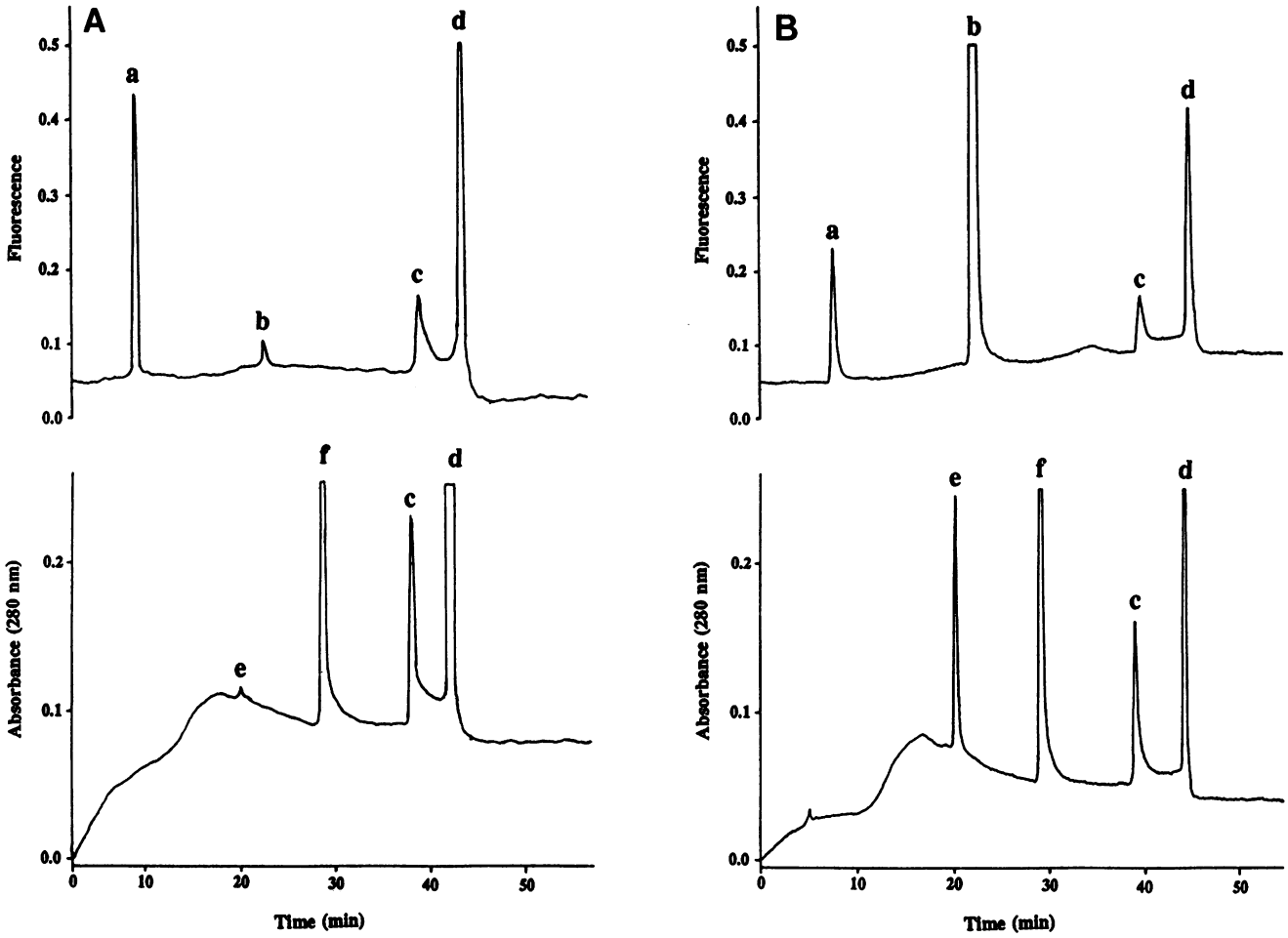

FIG. 2. HPLC analysis of MelCy in distilled water. A total of 10 nmol was injected and analyzed by HPLC by using both UV and fluorescence detection as described in the text. A total of 10 nmol of MelCy was analyzed immediately after dissolution (A) and after 114 h at room temperature (B). The labelled peaks are cysteamine (a), cystamine (b), MelCy −1 (c), MelCy (d), melarsen (e), and melarsen oxide (f).

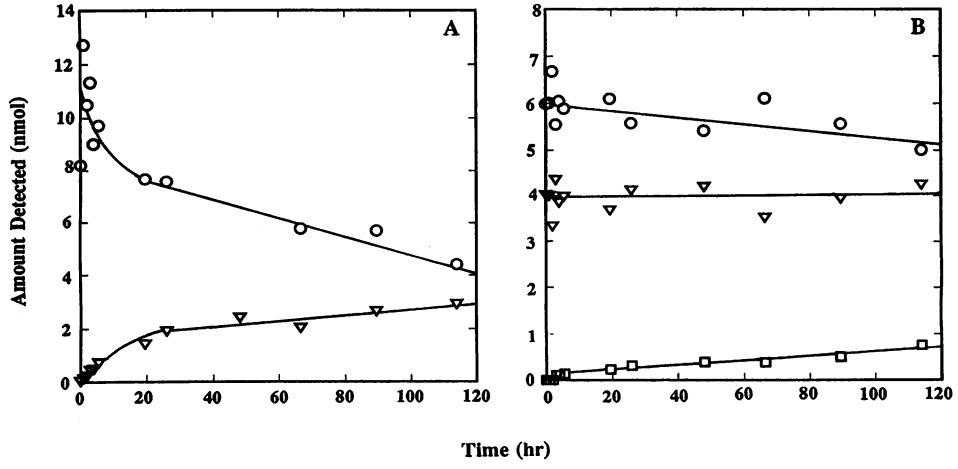

FIG. 3. Time course of MelCy decomposition. A total of 10 nmol was analyzed by HPLC at various intervals after dissolution. (A) The fluorescent (amine detection) channel showing cysteamine (open circles) and cystamine (open triangles) contents over time. (B) The UV (melaminophenyl ring detection) channel showing the MelCy plus MelCy −1 (open circles), melarsen oxide (open triangles), and melarsen (open squares) contents over time.

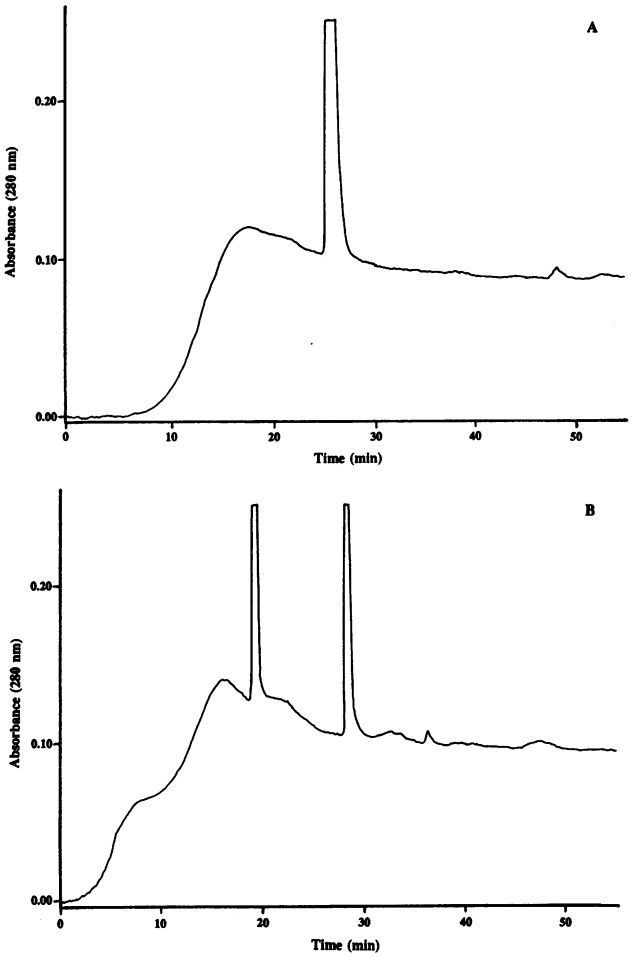

FIG. 4. The conversion of melarsen oxide to melarsen by hydrogen peroxide. Melarsen oxide was analyzed by HPLC by using UV detection in the absence (A) or presence (B) of H_2O_2 following a 60-min incubation at room temperature. In both cases, a total of 10 nmol was injected.

HPLC analyses. All HPLC analyses were performed with Beckman model 110B pumps (High Wycombe, United Kingdom) equipped with a model 167 variable-wavelength spectrophotometer and an Altex 210A manual injection valve (Beckman Instruments) with a 10-μl loop. All data were collected, stored, and analyzed by using Beckman System Gold operating software. The chromatographic conditions used were substantially those published previously (2). Briefly, runs were initiated in 90% buffer A (0.25% lithium camphor sulfonate [pH 2.65], prepared by diluting a 1% stock, [pH 2.00] with saturated LiOH) and 10% buffer B (0.25% lithium camphor sulfonate in 25% 1-propanol), held in this buffer mixture for 5 min, and then subjected to a linear gradient (10 to 55% buffer B over 45 min for MelCy or 10 to 85% buffer B over 75 min for MelW and MelB). The phenyl moieties of the compounds were detected by UV spectrophotometry at 280 nm, and the primary amine of the cysteamine moiety was detected by postcolumn derivatization with 200 μg of fluorescamine per ml in redistilled acetone and 0.1 M sodium borate (pH 9.65); this was followed by fluorometry at 390-nm excitation and 482-nm emission. The UV and fluorescence detectors were connected in series. The column used for all analyses was a Beckman octadecylsilane column (250 by 4.6 mm), with a 5-μm particle size and a flow rate of 1.0 ml/min.

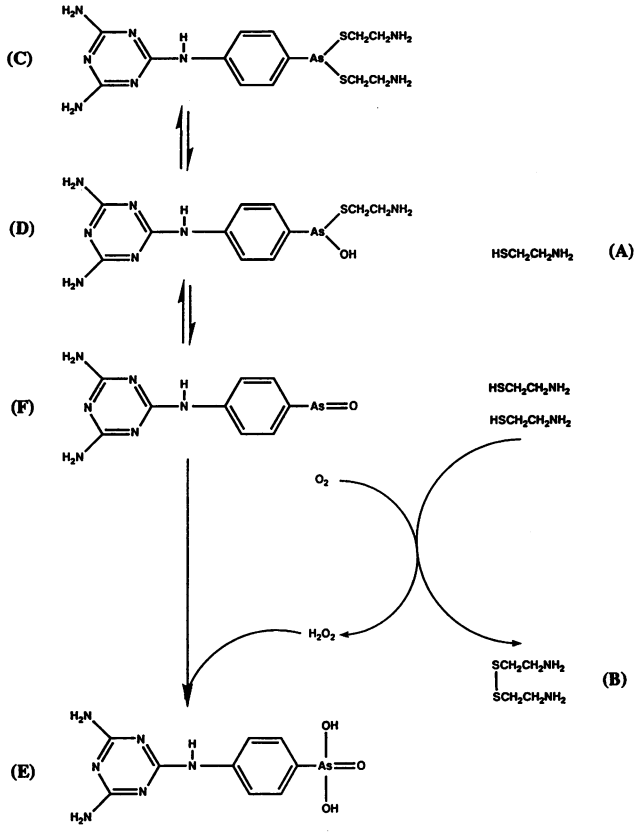

FIG. 5. The pathway of dissociation and decomposition of MelCy in aqueous, aerobic solution. The labelled compounds are cysteamine (A), cystamine (B), MelCy (C), MelCy −1 (D), melarsen (E), and melarsen oxide (F).

Experimental solutions. Stock solutions (all 10 mM) of MelCy were made in distilled water or 60 mM sodium phosphate–44 mM NaCl buffer (pH 7.4), stock solutions of melarsen oxide were made in dimethylformamide, stock solutions of melarsen were made in 0.1 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.8), stock solutions of MelB were made in 20% propylene glycol, and stock solutions of MelW, cysteamine, or cystamine were made in distilled water. In mixing experiments, aliquots from all stock solutions used were diluted in 20% propylene glycol to accommodate compounds insoluble in aqueous media. Linear standard addition curves were determined for melarsen oxide (0.05 to 6.0 nmol per injection), cysteamine (0.5 to 8.0 nmol per injection), melarsen (0.1 to 1.25 nmol per injection), and cystamine (0.05 to 4.0 nmol per injection). For stability studies, stock solutions were kept at room temperature and were periodically sampled and diluted to 1 mM in distilled water, and 10 nmol was analyzed by HPLC. The effect of exogenous H_2O_2 was analyzed by HPLC following the addition of 1 μl of H_2O_2 (30% [vol/vol]) per ml to 1 mM melarsen oxide in 20% propylene glycol. Endogenous H_2O_2 formation was measured enzymatically (13) in solutions of cysteamine and MelCy (1 mg/ml in phosphate saline buffer [pH 7.4]).

RESULTS AND DISCUSSION

HPLC analysis of 10 nmol of MelCy freshly dissolved in water or phosphate saline buffer showed that multiple com-

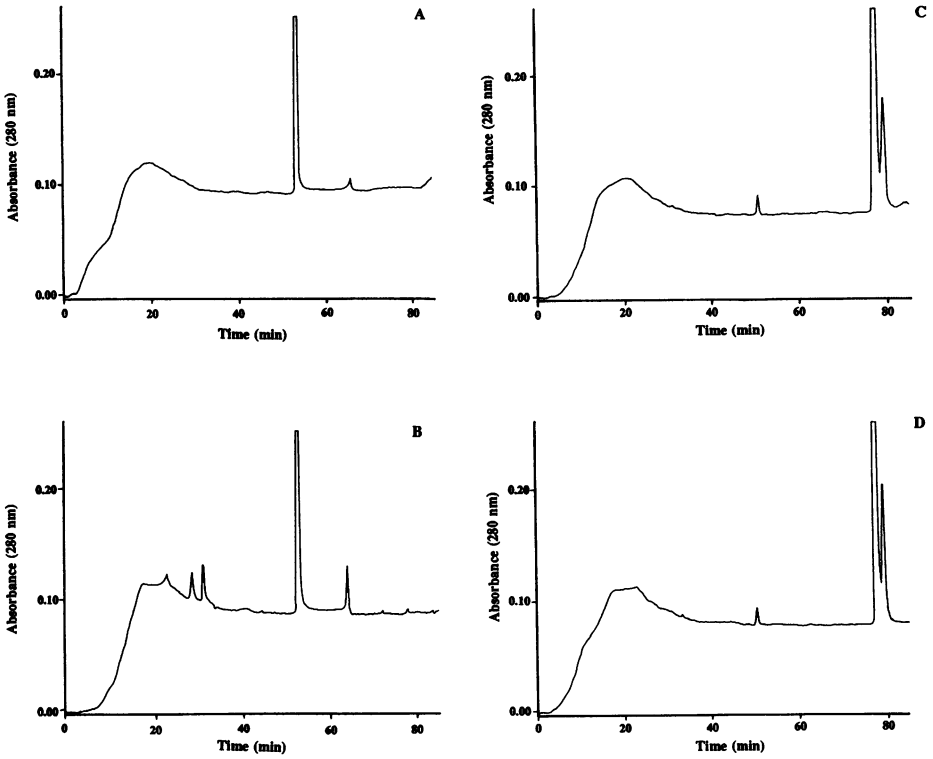

FIG. 6. The stabilities of MelW and MelB over time. MelW or MelB was analyzed by HPLC by using UV detection as described in the text. For all cases, a total of 10 nmol was injected. (A) MelW at 0 h; (B) MelW at 93 h; (C) MelB at 0 h; (D) MelB at 93 h.

pounds were present (Fig. 2A). The peaks labelled c and d in Fig. 2 clearly retained both the phenyl ring, as measured by UV spectrophotometry at 280 nm (lower trace), and the cysteamine portion of the compound, as measured by fluorometry (upper trace). In contrast, the compounds indicated by the peaks labelled a and b were positive only for amino groups, and the compounds indicated by the peaks labelled e and f were positive only for phenyl groups. By coelution studies, peaks a and b were identified as cysteamine and cystamine, respectively, and likewise, peaks e and f were found to coelute with melarsen and melarsen oxide, respectively. Peak d was calculated to be 2.09 ± 0.06 (n = 31) times as fluorescent per UV unit as peak c. Therefore, peak d appeared to be intact MelCy, whereas peak c was MelCy which had lost one cysteamine (MelCy −1).

Immediately upon dissolution in distilled water, the initial relative disposition of the arsenical compound was found to be 43% MelCy, 24% MelCy −1, 33% melarsen oxide, and <1% melarsen. Over time, this balance changed, such that the relative content, after 114 h, was 27% MelCy, 14% MelCy −1, 42% melarsen oxide, and 17% melarsen (Fig. 2B). In a similar manner, there was a noticeable decrease in the cysteamine/cystamine ratio over time. The time-dependent degradation of MelCy and the formation of cystamine and melarsen can clearly be seen in Fig. 3.

Solutions of melarsen oxide are known to be stable in the presence of oxygen (15) (data not shown). In contrast, the trivalent melarsen oxide present in aerobic solutions of MelCy was converted in a time-dependent manner to pentavalent melarsen (Fig. 3). Since solutions of both 1 mg of cysteamine and MelCy per ml produced H_2O_2 (23 and 2.5 nmol/ml, respectively, after 45 min at room temperature), it was possible that the H_2O_2 produced endogenously could be converting melarsen oxide to melarsen. This possibility was confirmed by the addition of an eightfold excess of H_2O_2, which resulted in the conversion of approximately 50% of the melarsen oxide to melarsen within 1 h (Fig. 4). In addition, incubation of MelCy in water under anaerobic conditions inhibited the formation of cystamine and melarsen (data not shown).

All of these results are consistent with the scheme given in Fig. 5. Immediately upon dissolution, MelCy forms an equilibrium mixture of MelCy, MelCy −1, melarsen oxide, and cysteamine. On storage under aerobic conditions, cysteamine is oxidized to form cystamine and H_2O_2, which, in turn, oxidizes melarsen oxide to melarsen. Removal of cysteamine and melarsen oxide from the mixture perturbs the equilibrium conditions, resulting in further dissociation of MelCy and MelCy −1. It appears the MelCy has a higher stability constant than melarsen oxide-glutathione or melarsen oxide-cysteine conjugates, because the last two compounds cannot be detected by HPLC (5).

Because MelCy was unstable in the presence of oxygen, similar experiments were also performed with MelW and MelB. Like MelCy, MelW was unstable, but it appeared to break down at a much slower rate (Fig. 6A and B). The four peaks resulting from the decomposition of MelW were melarsen oxide, sodium melarsen, and two unidentified compounds. Over the same time period, there was no detectable degradation of MelB (Fig. 5C and D). The two peaks at 78 and 80 min represent the separation of MelB isomers, because trivalent arsenic has an approximately tetrahedral conformation (5). In addition, one peak, at 51 min, was always detected in MelB solutions and was most likely due to the presence of a minor impurity.

These results have important implications for the use of MelCy as a therapeutic agent. It is theorized that melarsen oxide is the active form of all melaminophenylarsines and that it acts by binding trypanosomal thiols, such as trypanothione (5, 19). However, melarsen oxide is generally considered to be too toxic for direct administration (6), and is therefore administered as less toxic dithiol conjugates. These compounds must first be converted to melarsen oxide to have direct activity. MelCy, which is more readily converted than MelW or MelB to melarsen oxide in solution, might be expected to have greater activity. This is indeed the case when comparing MelCy, MelW, and MelB as treatments against acute infections in which the central nervous system is not involved (4, 18). However, Jennings (9) has shown that MelCy is only a marginally better treatment against a model of cerebral trypanosomiasis, which may reflect difficulties in permeability across the blood-brain barrier. In addition, *T. brucei brucei* selected for resistance to sodium melarsen is also resistant to melarsen oxide, MelW, MelB, and MelCy (4) (data not shown), primarily because of alterations in a common transport system (3). Given the above observations, MelCy is unlikely to be of any advantage in the treatment of arsenic-resistant trypanosomiasis, but it may have other solubility or pharmacokinetic properties that are superior to those of MelB.

Pentavalent melarsen is much less active than the trivalent melarsen oxide (4), presumably because of the need for metabolic reduction to melarsen oxide for activity (15). Because the melarsen content of a solution of MelCy increases on storage, we would recommend that MelCy be injected immediately after dissolution to minimize the accumulation of the less active pentavalent arsenical agent.

The nature of MelCy in solution indicates that the pharmacology of the compound is likely to be rather complicated. The existing pharmacokinetic values (14) determined by monitoring total arsenic by atomic absorption and total trypanocidal activity by bioassay are thus incomplete and need to be reexamined by techniques that are capable of separating multiple arsenical compounds from biological samples.

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