Synthesis of antimony complexes of yeast mannan and mannan derivatives and their effect on *Leishmania*-infected macrophages

Geny CANTOS,* Clara L. BARBIERI,† Marcello IACOMINI,* Philip A. J. GORIN* and Luiz R. TRAVASSOS†‡

*Department of Biochemistry, Federal University of Paraná, Curitiba, PR 81531, and †Department of Microbiology, Immunology and Parasitology, Paulista School of Medicine, São Paulo, SP 04023, Brazil

Antimony(Sb)–yeast mannan complexes were synthesized as a strategy to introduce Sb into macrophages infected with *Leishmania* amastigotes. The complexes were taken up by endocytosis after specific recognition by α -D-mannosyl receptors on the macrophage membrane. About 90 % of the intracellular parasites were destroyed by Sb–mannan *in vitro*, whereas the corresponding Sb concentration used as the pentavalent antimonial drug glucantime destroyed about 60 % of the amastigotes.

None of the Sb complexes prepared with mannan acid or basic derivatives was as effective as the simple Sb–mannan complex in clearing macrophage infection by *Leishmania* (*L.*) *amazonensis*. The leishmanicidal effect of Sb–mannan was also demonstrated *in vivo* with infected hamsters. The alternative use of Sb–mannan complex in the treatment of human leishmaniasis is envisaged on the basis of parasite-killing efficiency and the use of a low antimony dose.

INTRODUCTION

Leishmaniasis is a parasitic disease affecting 12 million people in the world (Modabber, 1987). Treatment has been achieved with antimonials, amphotericin B and pentamidine, which, however, can cause severe toxic effects. Toxicity occurs probably because these drugs, at the concentrations used, affect host cells other than the macrophages harbouring the parasites. Liposome inclusion of clinically useful drugs has been more effective than free drugs for treatment of experimental leishmaniasis (Black et al., 1977; Alving et al., 1978; New et al., 1978, 1981), but this method has limitations. Selective destruction of intracellular amastigotes of *Leishmania* (*L.*) *amazonensis* by lysosomotropic amino acid esters, which presumably enter macrophages by passive diffusion, has also been described (Rabinovitch et al., 1986; Alfieri et al., 1989).

The effective intracellular killing of parasites by selective drug delivery to macrophages based on the specificity and high efficiency of receptor-mediated endocytosis has been attempted. The leishmanicidal drug is coupled to a carrier substance which is recognized by macrophage receptors (Mukhopadhyay et al., 1989; Chaudhuri et al., 1989; Chakraborty et al., 1990). In the present investigation, the leishmanicidal effects of antimony (Sb) complexes of yeast mannan and mannan derivatives were compared with those of glucantime, a pentavalent antimonial drug currently used in the chemotherapy of leishmaniasis. α -D-Mannosyl-binding receptors are well characterized in macrophages (Stahl et al., 1980; Stahl & Gordon, 1982). We therefore hypothesize that these receptors could mediate binding and accumulation of soluble Sb–mannan and Sb–mannan derivatives inside *Leishmania*-bearing macrophages, thus contributing for clearance of the parasitic infection.

MATERIALS AND METHODS

Preparation of yeast mannan

Yeast mannan was prepared as previously described (Gorin and Spencer, 1970) by extraction of baker's yeast with hot 2 % KOH

followed by treatment of liberated polysaccharide with Fehling solution, which precipitated mannan as its Cu complex. After deionization, the polysaccharide was recovered and precipitated in methanol, yielding 2.2 % mannan (based on wet weight of yeast).

Preparation of CM-(carboxymethyl)mannan

The method of Karabinos and Hindert (1954) was used. Mannan (5 g) in 30 % NaOH (13.3 ml) with propan-2-ol (16 ml) was treated, dropwise with stirring, with a solution of monochloroacetic acid (6 g) in a little propan-2-ol. The temperature was then raised to 55 °C, and after a given time (see below), the soluble material was decanted and added to methanol containing 30 % of water (170 ml). Sufficient acetic acid was added to neutralize the excess alkali and the precipitate was filtered off, washed with ethanol and dried. Various preparations were carried out, maintaining the temperature at 55 °C for different times. The degree of substitution (DS; mol of substituent/mol of anhydromannose unit) was determined by methods involving conversion of the copper salt into CuO (McLaughlin and Herbst, 1950), conversion of the sodium component of CM-mannan to NaCl, acid washing (Eyler et al., 1947) or treatment with resin, followed by titration, conversion into the uranyl derivative and thence into U_3O_8 (Francis, 1953). The mean DS values were 0.08 (0.33 h), 0.25 (0.67 h), 0.4 (1.25 h), 0.53 (2.5 h), 0.74 (3.5 h) and 0.79 (5 h). For the run of 0.67 h, 5.25 g of derivative was obtained.

Sulphate esters of mannan

By means of the method of Whistler and Spencer (1961), a solution of mannan (5 g) in formamide (20 ml) and pyridine (50 ml) at 0 °C was treated dropwise with stirring with chlorosulphonic acid (13.5 ml; 0.45 mol/mol of anhydromannose) and maintained at 4 °C overnight. Ice/water was added, followed by aq. 10 % $NaHCO_3$ until effervescence ceased, and then by extraction with diethyl ether. The solution was dialysed, then

Abbreviations used: DS, degree of substitution; FCS, fetal-calf serum; PBS, phosphate-buffered saline.

‡ To whom correspondence and requests for reprints should be sent.

freeze-dried, giving mannan sulphate (5.43 g). The DS of the sulphate was determined by conversion into H_2SO_4 and its Ba^{2+} salt measured turbidimetrically (Dodgson and Price, 1962) or gravimetrically, or treated with resin followed by acid titration. By using a variety of molar proportions of the chlorosulphonic reagent (mol/mol of anhydromannose in parentheses) the DS values were: 0.29 (0.45), 0.4 (0.62), 0.65 (1.25), 1.29 (1.87) and 1.65 (2.5).

Mannan phosphates

As described by Paschall (1964), $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (2.8 g; 2 mol/mol) and $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ (4.18 g; 2 mol/mol) were dissolved in water (15.9 ml), final pH 6.1 at 35 °C. Mannan (5 g) was dissolved, the solution evaporated, and the residue left at 100 °C for 15 h and then for 1, 2, 3 and 5 h at 155 °C. The product was dissolved in water, dialysed and freeze-dried. The yield was 5.64 g after incubation for 4 h. The DS values were determined spectrophotometrically using sulphomolybdate and molybdovanadate (Chen et al., 1956) and by treatment with cationic ion-exchange resin followed by titration. The DS values thus obtained were 0.06 (1 h), 0.1 (2 h), 0.16 (3 h) and 0.25 (5 h).

DEAE-mannan

By using the method of Bullock and Guthrie (1965), to a solution of mannan (5 g) and NaOH (3.3 g) in water (17.4 g), 2-chlorotriethylamine hydrochloride (2.9 g) in water (25 ml) was added dropwise, the temperature being maintained at 0 °C. The solution was kept at 85 °C for 1, 2, 3, 4 and 6 h in separate experiments, and the solutions were then dialysed and freeze-dried. The yield was 5.46 g in the one that was incubated for 4 h. The DS values were determined by treatment with strongly basic ion-exchange resin and titration of the product. They were: 0.11 (1 h), 0.13 (2 h), 0.19 (3 h), 0.24 (4 h), 0.32 (5 h) and 0.32 (15 h).

Complexation of mannan and mannan derivatives with Sb

Polysaccharide (1 g) in water (50 ml) at 0 °C was treated with stirring with various proportions of SbCl_5 (0.78, 1.1, 1.56 and 2.34 mol/mol of anhydromannose) for 15 min and the solution then adjusted to pH 7.0 with aq. 10% (w/v) NaHCO_3 , and heated at 60 °C for 1 h. Insoluble material was removed and the supernatant treated with methanol to a concentration of 70%. The precipitate was then isolated. In another process, the solution was dialysed instead of being treated with methanol.

Estimation of Sb content in complexes

Two different methods were used. (a) The sample was acidified with H_2SO_4 and a solution of KI and 25% H_2SO_4 added. Absorbance was measured at 425 nm using a blank and a standard of potassium antimony tartrate (Vogel, 1981a). (b) The sample was acidified with concentrated HCl to 4 M, aq. 10% tartaric acid was added with KI and, after 5 min, the solution was titrated with 0.1 M $\text{Na}_2\text{S}_2\text{O}_3$ (Vogel, 1981b). The Sb contents of the various preparations are summarized in Table 1.

^{13}C -n.m.r. spectroscopy

Solutions of substituted mannans in $^2\text{H}_2\text{O}$ were examined at 70 °C. Chemical shifts (δ) are expressed in p.p.m. relative to tetramethylsilane (= 0) determined in a separated experiment.

Leishmania cells

Amastigotes of *Leishmania amazonensis* were transferred every 4–6 weeks to golden hamsters by inoculation into the footpads. Amastigote suspensions were prepared by homogenization of the excised lesions. The infiltrated tissue was homogenized in RPMI 1640 containing 10% fetal calf serum (FCS), and a suspension of amastigotes was obtained after cell disruption by four passages through 22-gauge needles and centrifugation at 250 g for 10 min. The supernatant fluid was centrifuged at 1400 g for 10 min and the pellet resuspended in RPMI with 10% FCS.

Macrophage cultures

Macrophages were collected in phosphate-buffered saline (PBS) from the peritoneal cavities of mice. About 4×10^5 cells were allowed to attach to round 12 mm-diameter cover-glasses. These were rinsed with PBS and placed in 16 mm-diameter wells of Costar plates containing 0.5 ml of RPMI with 10% heat-inactivated FCS, 100 units/ml penicillin and 100 µg/ml streptomycin, and incubated in an atmosphere of air/ CO_2 (19:1) at 37 °C. After 24 h, the macrophage cultures were infected with the parasites in a proportion of three amastigotes per cell. Infected macrophages were used in the experiments with Sb-mannan 48 h after infection. Most (98%) macrophages were infected, showing large parasitophorous vacuoles harbouring amastigotes, a typical feature of the infection by species from the *Leishmania mexicana* taxonomic complex.

Sb-mannan solutions for endocytosis experiments

Solutions of the Sb-mannan complexes were prepared in PBS by overnight incubation at 37 °C followed by centrifugation at 450 g for 5 min. The supernatant fluids were adjusted to pH 7–7.2, sterilized by filtration (Costar LB filter; 0.45 µm pore size) and the final concentration of polysaccharide was estimated by sugar colorimetric assay (Dubois et al., 1956).

Treatment with Sb-mannan of hamsters infected with *L. amazonensis*

Hamsters (4 to 8 weeks old, weight 125 to 150 g) were infected in their footpads with 2×10^6 *L. amazonensis* amastigotes isolated from donor-hamster footpads as described above. After 6 weeks the hamsters were inoculated subcutaneously near each footpad lesion with Sb-mannan (containing 19.5 µg of Sb) on days 1–5 (total of 390 µg of Sb/hamster). Control animals received mannan at the same concentration used for Sb-mannan (48 mg, total), as well as glucantime (from a commercial source; 0.3 g/ml) at a dose effective in destroying 99% parasites in hamsters (3.27 mg/day per footpad) during 5 days (65.4 mg total). Two hamsters were used for each drug. Animals were also inoculated intraperitoneally using the same final drug concentration. The size of the lesions was measured with a dial caliper on the indicated days.

RESULTS

Baker's-yeast mannan and derivatized (DEAE, CM, sulphate and phosphate) mannans were complexed with Sb_2O_3 and the resulting drugs tested *in vitro* and *in vivo* for their leishmanicidal properties.

Complexing of Sb with mannan and derivatives

In order to see how the mannan substitution process would affect complexing with Sb, the positions of substitution were determined. The use of conventional methods, such as methylation analysis, was not possible, since phosphate and sulphate groups are unstable under the alkaline conditions used. For the CM- and DEAE-mannans, partly *O*-methylated CM or DEAE derivatives could be obtained and characterized by g.l.c.-m.s., but suitable standards were not available. The method of preference was ^{13}C -n.m.r. spectroscopy of the intact derivatives.

In the case of CM derivatives of cellulose (Parfondry & Perlin, 1976), the ^{13}C -n.m.r. signals were very broad, and it was necessary to examine the mixture of CM-glucoses formed on hydrolysis. Substitution took place mainly at OH-2, with a small proportion at OH-6 and very little at OH-3. Comparison of the ^{13}C -n.m.r. spectrum of mannan with that of its CM derivative showed substitution at O-2 with new signals at δ 101.2 (β -shift of -2.5 p.p.m. of C-1 signals at δ 103.7 of C-1s of non-reducing end-units and adjacent 3-*O*-substituted ones; see Gorin, 1973b) and δ 82.2 of equal area (α -shift of 10.2 p.p.m. of the C-2 signal at δ 70.2). Evidence for substitution at OH-6 was obtained by virtue of a new signal at δ 73.5 (β -shift of -1.4 p.p.m. of C-5 signal at δ 74.9. (A very small signal at δ 76.6 could be due to substitution of OH-4 (α -shift of 8 p.p.m. of the C-4 signal at $\sim \delta$ 68).

Substitution of carbohydrates occurs mainly on primary hydroxy groups (Turvey, 1965). Such derivatives have an i.r. absorption band at 1240 cm^{-1} (Lloyd, 1962) that was also observed in the spectrum of the mannan. ^{13}C -n.m.r. studies on the sulphation of methyl 3-*O*-methyl α -D-mannopyranoside at OH-6 showed that the C-5 signal suffered a β -shift of -2.0 p.p.m. (Honda et al., 1973). Such a shift occurred with the C-5 signal of the mannan originally at δ 74.8.

Additional signals were detected in the ^{13}C -n.m.r. spectrum of DEAE-mannan at δ 100.7 (C-1 of non-reducing end and adjacent 3-*O*-substituted units, suffering a β -shift of -3.0 p.p.m. from δ 103.7) due to substitution at OH-2, and δ 73.8. (β -shift of -1.2 p.p.m. of C-5 signal originally at 75 p.p.m.), due to substitution at OH-6.

In the case of phosphorylation, an additional signal at δ 64.4 arose, due to phosphorylation at OH-6 (α -shift of 1.7 p.p.m.; see Gorin 1973a).

Antimony complexes of mannan and its derivatives were prepared starting with various proportions of Sb_2O_3 as reagent. Final isolation was effected by methanol precipitation or by

Figure 1 Mouse macrophages infected with *L. amazonensis* treated with Sb-mannan (750 $\mu\text{g/ml}$; 6.6 $\mu\text{g/ml}$ Sb)

(a) Control cells; (b) treated cells after 3 days of incubation.

dialysis. The final products, although originally soluble in water, could not be completely redissolved.

Leishmanicidal effect of Sb-mannan *in vitro*

Mouse peritoneal macrophages were infected with *L. amazonensis* amastigotes for 48 h as described above and treated with different Sb-mannan concentrations. Infected macrophages used as controls were treated with PBS, mannan and glucantime.

Figure 1(a) shows the infected macrophages at the start of the Sb-mannan treatment. Very large parasitophorous vacuoles were seen harbouring many amastigotes. Figure 1(b) shows infected macrophages after 3 days of treatment with 500 $\mu\text{g/ml}$ of Sb-mannan (6.6 $\mu\text{g/ml}$ of Sb). After this period the large parasitophorous vacuoles and their amastigotes disappeared and the macrophages recovered their normal morphology. The cell viability was followed by Trypan Blue exclusion.

The kinetics of *L. amazonensis* amastigote destruction in infected macrophages treated with different Sb-mannan concentrations is shown in Figure 2. As can be observed, Sb administered as Sb-mannan destroyed three times more parasites than glucantime at the same Sb concentration. Mannan alone did not show any effect on the intracellular amastigotes.

Effect of Sb-mannan derivatives

None of the mannan derivatives complexed with Sb were as effective as Sb-mannan in clearing the *Leishmania* infection *in vitro*. The best complexes for this purpose were those of mannan phosphate and CM-mannan, which, however, cleared only 72 % and 70 % respectively of the infected macrophages, as compared with 90 % with Sb-mannan at the same concentration (5 mg/ml)

Table 1 Sb contents of complexes of mannan and its derivatives

'Pp' and 'Dia' refer to respectively experiments in which the Sb complex was prepared via precipitation with methanol and by dialysis. Sb (mol/mol), mol of SbCl₃/mol of anhydromannose

Substrate (Sb complex)	Percentage of Sb present					
	Sb (mol/mol) ... 0.78		1.56		2.30	
	Pp	Dia	Pp	Dia	Pp	Dia
Mannan	3.1	1.5	6.4	2.5	9.7	3.6
CM-mannan	2.9	0.5	4.8	0.9	8.6	1.3
Mannan sulphate	2.5	0.3	4.3	0.8	7.9	1.1
Mannan phosphate	2.8	0.4	5.1	0.9	8.5	1.2
DEAE-mannan	2.6	0.5	4.7	1.0	8.8	1.4

Figure 2 Treatment of *L. amazonensis*-infected mouse macrophages with different Sb-mannan concentrations

The phagocytic index is the product of the percentage of infected macrophages times the average number of amastigotes per macrophage. ●, PBS; ○, mannan (750 µg/ml); ▲, Sb-mannan 500 µg/ml (4.4 µg/ml Sb); △, Sb-mannan 750 µg/ml (6.6 µg/ml Sb); ■, glucantime (6.6 µg/ml Sb). The results were obtained by counting at least 200 macrophages per coverslip in triplicate.

Figure 3 Inhibition of the leishmaniasis effect of Sb-mannan by increasing concentrations of mannan

●, Control *L. amazonensis*-infected macrophages; ○, treatment with Sb-mannan at 750 µg/ml; ▲, Sb-mannan and mannan (250 µg/ml); △, Sb-mannan and mannan (500 µg/ml); ■, Sb-mannan and mannan (1 mg/ml). 'Phagocytic index' is defined in Figure 2.

in the same period. The relative effectiveness of the mannan derivative complexes at 2 mg/ml and little difference in Sb content (Table 1) was: Sb-mannan phosphate = Sb-CM-mannan > Sb-mannan sulphate. The DEAE-mannan complex was even more effective than Sb-mannan at 1 mg/ml, but caused morphological alterations or destroyed the macrophages at 2–5 mg/ml. Samples were prepared with 0.78 mol of SbCl₅/mol of anhydromannose.

Table 2 Treatment of hamster footpad lesions with Sb-mannan and glucantime

Lesion size was measured as described in the Materials and methods section. Results are means of two determinations. Normal footpad diameter was 3.5 mm.

Treatment	Time after treatment (days)...	Lesion size (diameter in mm)			
		0	11	22	33
Non-treated control		8.1	8.5	8.5	8.7
PBS		8.0	8.5	8.1	7.7
Mannan (48 mg)		8.3	8.9	8.0	7.5
Sb-mannan (390 µg of Sb)		6.4	4.3	4.2	5.1
Glucantime (65.4 mg of Sb)		7.3	4.2	3.7	4.2

Inhibition of the leishmanicidal effect by mannan or mannose

To test whether the Sb-mannan uptake by macrophages is dependent on an α -mannosyl receptor, a competition experiment with mannan or mannose was performed. The results clearly showed that, on increasing the mannan concentration, there was a proportional decrease in the intracellular parasite destruction caused by the Sb-mannan complex (Figure 3). Similar results were obtained when the test was carried out with mannose at 1 mg/ml.

Efficacy of Sb-mannan in treating hamsters infected with *L. amazonensis*

Golden hamsters were infected with *L. amazonensis* amastigotes and the subcutaneous treatment with Sb-mannan was started 45 days afterwards as described above. There was a significant size reduction of the footpad lesions in hamsters treated with Sb-mannan (Table 2), similar to the reduction observed in animals treated with glucantime (80% reduction in relation to untreated or mannan-treated controls). In this protocol the final antimony concentration administered for Sb-mannan and for glucantime was 390 µg/hamster and 65.4 mg/hamster respectively. Intra-peritoneal drug administration did not reduce the footpad lesions either with Sb-mannan or glucantime.

DISCUSSION

Among antimonial drugs currently utilized in the treatment of leishmaniasis, the one of preference in Brazil is the antimonate of *N*-methylglucamine (1-deoxy-1-methylamino-D-glucitol) or glucantime, whereas in other countries, particularly French-speaking ones, sodium stibogluconate (Pentostan) is the preferred drug. The basic and acid nature of these drugs possibly give rise to complexes which are more stable than those in simple polyhydroxylic systems. We therefore prepared basic and acid derivatives of yeast mannan as a step toward obtaining stable Sb complexes.

The value for the DS chosen for the DEAE-, CM-, phosphate and sulphate derivatives of mannan was 0.25 mol/mol of anhydromannose unit. Presumably, at this level, sufficient hydroxy groups would remain free to form antimony complexes, and a high proportion of mannopyranosyl groups would remain capable of binding to macrophages. These complexes were then compared with the simple Sb-mannan complex for their biological activity.

Little is known about the mechanism of complexation of Sb with polyhydroxy systems but, by analogy with the formation of

borate complexes (Boeseken, 1949), it could react with 4,6- and 2,3-dihydroxy groups of mannopyranosyl units (structures I and II):

Partial substitution of mannan at positions OH-2 and OH-6 as in the derivatives obtained in the present work reduced by 10–20% the amount of antimony complexes formed as compared with Sb-mannan. With sulphation the average reduction of Sb complexation to the polysaccharide was 24%. Contrary to what might be predicted, the Sb complexation with unsubstituted mannan was more stable than that with the mannan derivatives, as inferred from dialysed preparations of the Sb-complexes (Table 1). As compared with Sb-mannan, the Sb contents in the other complexes, after dialysis in water, were on average reduced to one-third of that of the unsubstituted mannan complex. Like the borate complex, that of Sb could be in equilibrium with mannan and antimonate in aqueous solution. Such a dissociation might be prejudicial to the action of the drug, although the possibility exists that the mannan may act as a scavenger for antimonate and that they remain in close proximity, being effective as a target. However, this remained in doubt until the 'in vitro' toxicity experiments were carried out.

As shown in the present study, the Sb-mannan complex at 750 µg/ml (6.6 µg/ml Sb) was able to destroy approx. 98% of the parasite burden of macrophages infected with *L. amazonensis*, with a leishmanicidal activity 3-fold higher than that with glucantime. None of the other Sb complexes of acid and basic derivatives of mannan were as effective, and some were rather toxic to macrophages. The cell viability in the presence of the Sb-mannan complex was preserved, as demonstrated by Trypan Blue exclusion. Differences of 7–20% in the Sb contents and the different stability of the mannan derivative complexes, as compared with Sb-mannan, could partly account for their lesser activity. Other factors, however, seem possible, such as the differential affinity for macrophage receptors, or a different efficiency in the fusion of endocytic vesicles with the parasitophorous vacuole. Toxicity could depend on the derivatives themselves or be due to small contaminants in the preparations.

That the internalization of Sb-mannan occurs by endocytosis after specific recognition by α-D-mannosyl receptors on the macrophage membrane was shown by mannan/mannose competition experiments. The inhibition of Sb-mannan incorporation by infected macrophages in the presence of increasing mannan or mannose concentrations was inferred from the corresponding reduction of the leishmanicidal effect of the Sb-complex.

The proposed mechanism of action of Sb-mannan involves: (a) macrophage uptake of the complex via recognition by an α-D-mannosyl receptor and endocytosis; (b) fusion of the endocytic vesicle carrying the complex with the parasitophorous vacuole; (c) dissociation of the complex at the acid pH of the parasitophorous vacuole with the formation of toxic Sb₂O₃; (d) destruction of the parasites without affecting macrophage viability. Although this sequence of events may involve additional steps,

those suggested are based on previous observations on *Leishmania*-infected macrophages. Thus, the occurrence of fusion between the parasitophorous vacuoles and secondary lysosomes has been demonstrated by different authors using lysosomotropic drugs (Alexander & Vickerman, 1975; Berman et al., 1981; Brazil, 1984). The low pH in the phagolysosomal compartment is not measurably altered by *Leishmania* amastigotes (Chang, 1980), which have enhanced metabolism at pH 5 (Mukkada et al., 1985). In addition, we have observed that treatment of *Leishmania*-infected macrophages with NH₄Cl, which raises the internal pH of the parasitophorous vacuole, prevents parasite destruction by Sb-mannan (results not shown). This is evidence that an acidic pH is necessary to dissociate the complex releasing Sb (Sb₂O₃) to perform the leishmanicidal effect. A prior decomposition of the complex is necessary to kill the parasites, since isolated amastigotes are not harmed by Sb-mannan.

The leishmanicidal effect of Sb-mannan could also be demonstrated in infected animals. Our 'in vivo' results showed a significant decrease in the size of the lesions after Sb-mannan treatment, although a complete reduction was not obtained. Also, a relapse was observed 44 days after treatment with either Sb-mannan or glucantime. Certainly, additional efforts will be necessary to optimize the Sb-mannan administration protocol, both in terms of doses and frequency. Our data already show, however, that Sb-mannan is comparable in efficiency to glucantime at Sb concentrations 160 times lower than that in glucantime. Higher Sb-mannan concentrations are still limited by the incomplete solubility of the complex in physiological solutions. The use of smaller mannan fragments as Sb carriers, as well as other solubilization procedures of the complex, could be alternatives to solve this problem.

The efficiency of mannan-coupled antimony in killing *Leishmania* amastigotes both *in vitro* and *in vivo* allows one to envisage the introduction of this complex as an alternative antimonial drug for treatment of human leishmaniasis, with the advantage of using low antimony doses.

We thank the Brazilian National Research Council (CNPq) for research grants and fellowships and Mr. Ted Mazurek, Plant Biotechnology Institute, N.R.C., Saskatoon, Saskatchewan, Canada, for producing the ¹³C-n.m.r. spectra.

REFERENCES

- Alexander, J. and Vickerman, K. (1975) *J. Protozool.* **22**, 502–508
- Alfieri, S. C., Shaw, E., Zilberfarb, V. and Rabinovitch, M. (1989) *Exp. Parasitol.* **68**, 423–431
- Alving, C. R., Steck, E. A., Hanson, W. L., Loizeaux, P. S. and Chapman, W. L., Jr. (1978) *Life Sci.* **22**, 1021–1026
- Berman, J. D., Fioretti, T. B. and Dwyer, D. M. (1981) *J. Protozool.* **28**, 239–242
- Black, C. D. V., Watson, G. J. and Ward, R. J. (1977) *Trans. R. Soc. Trop. Med. Hyg.* **71**, 350–352
- Boeseken, J. (1949) *Carbohydr. Chem.* **4**, 189–207
- Brazil, R. P. (1984) *Ann. Trop. Med. Parasitol.* **78**, 87–91
- Bullock, A. L. and Guthrie, G. D. (1965) *Methods Carbohydr. Chem.* **5**, 409–410
- Chakraborty, P., Bhaduri, A. N. and Das, P. K. (1990) *Biochem. Biophys. Res. Commun.* **166**, 404–410
- Chang, K. D. (1980) in *The Host Invader Interplay* (van den Bossche, H., ed.), pp. 231–234. Elsevier, Amsterdam
- Chaudhuri, G., Mukhopadhyay, A. and Basu, S. K. (1989) *Biochem. Pharmacol.* **38**, 2995–3002
- Chen, P. S., Toribara, T. Y. and Warner, J. (1956) *Anal. Chem.* **28**, 1756–1758
- Dodgson, K. S. and Price, T. (1962) *Biochem. J.* **84**, 106–109
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956) *Anal. Chem.* **28**, 350–356
- Eyler, R. W., Klug, E. D. and Diephuis, F. (1947) *Anal. Chem.* **19**, 24–27
- Francis, C. V. (1953) *Anal. Chem.* **25**, 941–945
- Gorin, P. A. J. (1973a) *Can. J. Chem.* **51**, 2105–2109
- Gorin, P. A. J. (1973b) *Can. J. Chem.* **51**, 2375–2383
- Gorin, P. A. J. and Spencer, J. F. T. (1970) *Adv. Appl. Microbiol.* **13**, 25–98

- Honda, S., Yuri, H. and Takiura, K. (1973) *Carbohydr. Res.* **28**, 150–153
- Karabinos, J. V. and Hindert, H. (1954) *Adv. Carbohydr. Chem.* **9**, 285–301
- Lloyd, A. G. (1962) *Biochem. J.* **83**, 455–459
- McLaughlin, R. R. and Herbst, J. H. E. (1950) *Can. J. Chem.* **88**, 737–740
- Modabber, F. (1987) in *Tropical Disease Research, A Global Partnership, 8th Programme Report* (Maurice, J. and Pearce, A. M., eds.), pp. 99–112, WHO, Geneva
- Mukhopadhyay, A., Chaudhuri, G., Arora, S. K., Sehgal, S. and Basu, S. K. (1989) *Science* **244**, 705–707
- Mukkada, A. J., Meade, J. C. and Glaser, T. A. (1985) *Science* **229**, 1099–1101
- New, R. R. C., Chance, M. L., Thomas, S. C. and Peters W. (1978) *Nature (London)* **272**, 55–56
- New, R. R. C., Chance, M. L. and Heath, S. (1981) *J. Antimicrob. Chemother.* **8**, 371–381
- Parfondry, A. J. and Perlin, A. S. (1976) *Carbohydr. Res.* **57**, 39–49
- Paschall, E. F. (1964) *Methods Carbohydr. Chem.* **4**, 294–296
- Rabinovitch, M., Zilberfarb, V. and Ramazeilles, C. (1986) *J. Exp. Med.* **163**, 520–535
- Stahl, P. and Gordon, S. (1982) *J. Cell Biol.* **93**, 49–56
- Stahl, P., Schlesinger, P. H., Sigardson, E., Rodman, J. S. and Lee, Y. C. (1980) *Cell* **19**, 207–215
- Turvey, J. R. (1965) *Adv. Carbohydr. Chem.* **20**, 183–218
- Vogel, A. (1981a) in *Análise Inorgânica Quantitativa* (4th edn.), p. 285, Guanabara Koogan, Rio de Janeiro
- Vogel, A. (1981b) in *Análise Inorgânica Quantitativa* (4th edn.), p. 544, Guanabara Koogan, Rio de Janeiro
- Whistler, R. L. and Spencer, W. W. (1961) *Arch. Biochem. Biophys.* **95**, 36–44

Received 9 March 1992/30 June 1992; accepted 13 July 1992