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Influence of Mannose and Its Derivatives on the Growth of *Trypanosoma brucei gambiense in vitro*

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Abstract: In the previous experiment mannose specifically inhibited the growth of blood trypomastigotes of *Trypanosoma brucei gambiense in vitro* over new born mouse brain cells. On the search of its mode of action, effect of mannose derivatives and other monosaccharides on the growth of trypanosomes both on feeder layer and feeder layer free systems was assayed. Different concentrations of each of the monosaccharides and mannose derivatives were added into culture media and used for the cultivation of trypanosomes. Most of the monosaccharides and mannose derivatives tested had no pronounced effect on the growth of the parasites, except α-D-(+)-mannose and 2-Deoxy-D-glucose which specifically inhibited the growth of bloodstream forms on feeder layer and feeder layer free cultures. Furthermore, transmission electron microscopy revealed denaturation of glycosomes, nuclei and kinetoplasts in those parasites cultured in medium containing α-D-(+)-mannose or 2-Deoxy-D-glucose. Nevertheless, surface coat, cell membrane and subpellicular microtubules were intact. Possible mode of action of mannose and 2-Deoxy-D-glucose is discussed.

Key word: *Trypanosoma brucei gambiense, In vitro* cultivation, Mannose derivatives, Monosaccharides, Feeder layer, Feeder layer free system

Brain cells derived from a new born ICR mouse have proved to support and promote continuous growth of bloodstream forms of *Trypanosoma brucei* species (Mhando *et al.*, 1986b). The hallmark of continuous growth of trypanosomes in the feeder layer system is the appearance of clusters either on the surface or between the feeder layer cells (FLC). Many attempts to search for mechanisms or factors which support and promote the growth of these parasites have been carried out but up to date they have not yet been fully analysed (Tanner, 1980; Mhando *et al.*, 1987). While trying to search for these mechanisms which support the growth of trypanosomes, data obtained from the previous

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work, revealed that among the monosaccharides tested only α-D-mannose specifically inhibited the growth of trypanosomes on the feeder layer cells, though the underlying mode of action has still remained unclear (Mhando, 1988).

In an attempt to determine the biochemical effect of mannose in trypanosomes, mannose derivatives and other sugars were assayed for the growth inhibition of parasites both on feeder layer and feeder layer free systems. In addition, transmission eletron microscopy was performed on the parasites incubated with those compounds capable of inhibiting the growth of trypanosomes so as to evaluate their morphological structures.

Bloodstream forms of *Trypanosoma brucei gambiense* Wellcome strain [Tbg (W)] were used in the experiments. The history of this strain has been described (Mhando *et al.*, 1986 a). Trypanosomes obtained from blood of infected ICR mice were separated from blood components by chromatography method (Lanham and Godfrey, 1970). The parasites were once washed and resuspended in the culture media. Cultivation of trypanosomes was carried out as previously described (Mhando, 1988). Briefly, 24 h. prior to parasite inoculation each tissue culture dish (Nunc 60×15 mm) was planted with 2×10^5/ml brain cells obtained from a new born ICR mouse (NMBC) and incubated at 37℃ in 5% CO_2 incubator. A density of 3×10^5/ml trypanosomes (5ml) was inoculated into each tissue culture dish with confluent monolayer cells or without feeder layer cells (feeder layer free system) and incubated at 37℃ in 5% CO_2 incubator. Eagle's Minimum Essential Medium (MEM) with Earle's salt (GIBCO) containing penicillin 200 U/ml and streptomycin 100μg/ml was supplemented with 5% new born bovine serum and 5% foetal bovine serum and used for cultivation in feeder layer system, while a mixture of equal volumes of MEM and Leibovitz's medium (L15) (on publication) supplemented with 2,9-dimethyl-4,7-diphenyl-1,10-phenonthroline-disulphonic acid (BCS) (Yabu *et al.*, 1988) and 10% foetal bovine serum was used in feeder layer free system. Initiation of the cultures was carried out either by centrifuging the supernatant fluid and return all the pelleted trypanosomes into culture dishes or by replacing half of the old medium by fresh one every day until trypanosomes showed propagation or completely disappeared from the cultures. In those cultures where growth of trypanosomes became established in FLC, whole medium was changed every day and daily trypanosome density was estimated from the culture supernatant fluid. For the inhibition assay, 5 ml aliquots of each culture medium containing various concentrations of each of the reagents [α-D-mannose pentaacetate, D-mannose-6-phosphate, methyl-α-D-mannoside, D-mannosamine hydrochloride, L-(−)-mannose, α-D-(+)-mannose-1-phosphate, D-mannitol, α-D-talose, N-acetyl neuraminic acid, 6-deoxy-L-galactose, 2-deoxy-D-glucose, D-mannono-1, 4-lactone and D-mannose] were used for the cultivation of parasites as described above. In another experiment trypanosome growth inhibitory effect of α-D-mannose was compared to that of 2-deoxy-D-glucose in feeder layer free system. Parasites obtained from normal culture medium and those from medium treated with α-D-(+)-mannose/2-deoxy-D-glucose were three times washed with sodium phosphate buffered saline [PBS (−)] and fixed in 2% glutaraldehyde in 0.1 M PBS(−) pH 7.3 at 4℃ for 2 h. The fixed parasites were further processed for transmission electron microscopy.

Inhibition of trypanosome growth was observed with α-D-(+)-mannose and 2-deoxy-D-glucose, but no specific inhibitory effect was observed with the rest of mannose derivatives or other sugars, except that at 1.25 mM or greater D-mannosamine hydrochloride and D-mannose pentaacetate apparently inhibited the growth of trypanosomes. However, their mode of actions were considered to be different from those of mannose and 2-deoxy-D-glucose, for the fact that, they inhibited the growth of both bloodstream and insect forms and in addition, they had pronounced toxic effect on FLC as revealed by detaching and floating of the cells (Table 1). There is a remarkable similarity in the growth inhibition of trypanosomes by α-D-mannose and 2-deoxy-D-glucose both in feeder layer and feeder layer free systems (Fig. 1).

Electron micrographs show a prominent dense boundry to the trypanosome body which consists of a cell membrane and an external cell coat, both in non-treated and mannose/2-deoxy-D-glucose treated trypomastigotes. Immediately within this layer subpellicular microtubules were found to be normal in both cases. However, there were signs of denaturation of glycosomes, nuclei and kinetoplasts in mannose/2-deoxy-D-glucose treated trypanosomes (Fig. 2). This effect of mannose was considered to be specific rather than natural death of the parasites, because within 24 h it affected the organelles of trypomastigotes while the cell membrane and subpellicular microtubules were intact.

Table 1 Effect of mannose derivatives and other reagents on the growth of *Trypanosoma brucei gambiense* on feeder layer cells

	Reagents	Concentration (mM)						
		0.625	1.25	2.50	5.00	10.00	25.00	50.00
1	α−D−Mannose pentaacette	−	*	*	**	**	***	NT
2	D−Mannose−6−phosphate	−	−	−	−	−	−	−
3	Methyl−α−D−Mannoside	−	−	−	−	−	−	−
4	D−Mannosamine hydrochloride	−	*	**	**	***	***	NT
5	L−(−) Mannose	−	−	−	−	−	−	−
6	α−D−(+) Mannose−1−phosphate	−	−	−	−	−	−	−
7	D−Mannitol	−	−	−	−	−	−	−
8	α−D−Talose	−	−	−	−	−	−	−
9	N−acetylneuraminic acid	−	−	−	−	−	−	−
10	6−Deoxy−L−galactose	−	−	−	−	−	−	−
11	2−Deoxy−D−glucose	−	±	+	+	++	+++	+++
12	D−Mannono−1, 4−Lactone	−	−	−	−	−	−	−
13	D−Mannose	−	+	+	+	++	+++	+++

+: Inhibition of trypanosome growth (Parasites disappeared after ±−7 days, +−5 days, ++−4 days, +++−2−3 days)
−: No inhibition observed
*: Toxic to parasites and feeder layer cells (Parasites died within *−30 min. **−20 min, ***−10 min)
NT: Not tested

It has therefore, been established in these experiments that mannose specifically inhibited the growth of bloodstream forms of trypanosomes. Taking into account the same mode of trypanosome growth inhibition *in vitro* and the same morphological structure changes after treatment with α-D-(+)-mannose and 2-deoxy-D-glucose it is speculated that the inhibitory effect of mannose might resemble that of 2-deoxy-D-glucose which inhibits metabolism of trypanosomes partly by affecting some enzymes in the Embden Meyerhof-Parnas pathway (Seed *et al.*, 1965). However, this evidence is not yet conclusive, more detailed knowledge on the utilization rate of mannose by blood trypomastigotes may be necessary.

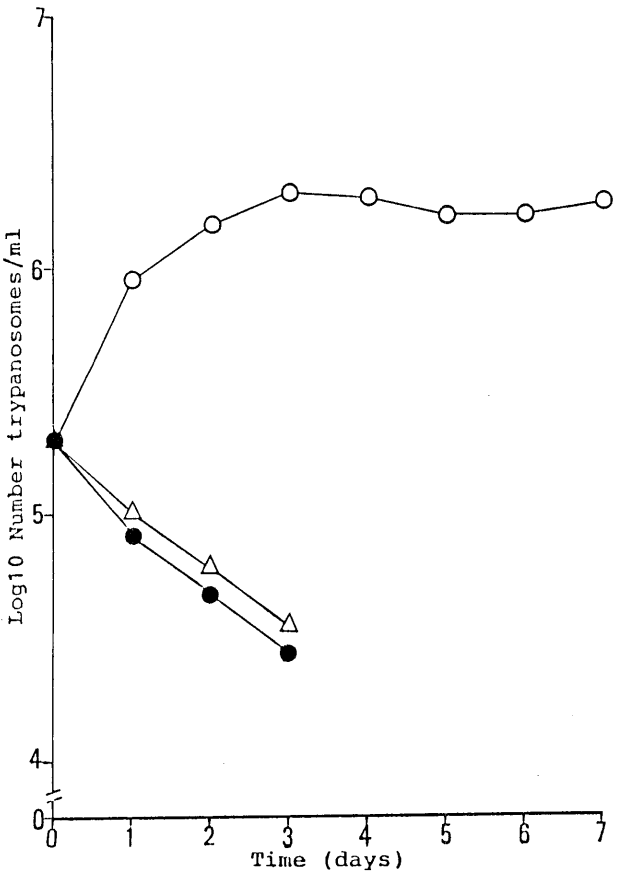

Fig. 1. The effect of α-D-(+)-mannose (△), 2-deoxy-D-glucose (●) and normal culture medium (○) on the growth of trypomastigotes in feeder layer free system. Each point represents the mean log number of parasites from four replicates.

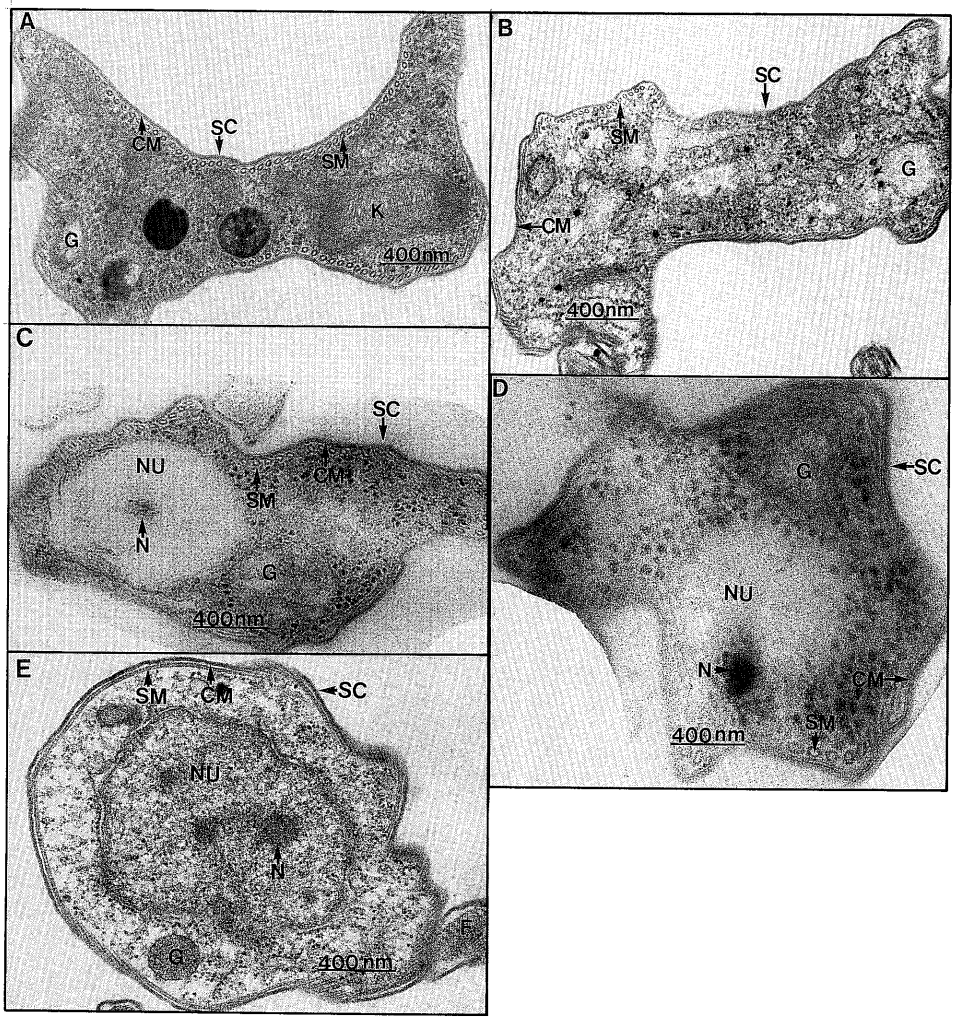

Fig. 2. Electron micrographs of trypomastigotes of *T. b. gambiense* (W) showing denaturation of glycosomes (G) A−B and nuclei (NU) C−D after incubation with culture medium containing 5 mM mannose (A & C) or 5 mM 2-deoxy-D-glucose (B & D) for 24 h. E-trypomastigote incubated with normal culture medium. SC−Surface coat, CM−Cell Membrane, SM−Subpellicular Microtubules, F−flagellum, K−kinetoplast and N−nucleolus.

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