

PHENOTHIAZINE COMPOUNDS AS POTENTIAL ANTIAMOEBIIC AGENTS

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AMOEBIASIS caused by *E. histolytica* is estimated to affect more than 10% of world population¹. Several drugs effective against trophozoites are available^{2,3} without having any appreciable effect on the spectrum or prevalence of disease. The existing drugs like emetine and metronidazole have side effects necessitating research for alternative drugs. Polyamine metabolism has been recently explored as an alternative target for chemotherapy of protozoa including *E. histolytica*⁴⁻⁷. Eukaryotic systems including protozoa contain the protein calmodulin which is involved in cell growth, division, differentiation and modulation of various regulatory enzymes⁸⁻¹⁰. Calcium has been shown to be involved in the flagellation of *Naegleria gruberi*¹¹ and aggregation in *Dictyostelium discoideum*¹². Inhibition of erythrocyte transmembrane calcium by ionophore A23187 have been reported to kill *P. falciparum in vitro*¹³. The essential role of calmodulin-related process in protozoal metabolism is indicated by the antiprotozoal action of phenothiazines against *Tetrahymena*¹⁴, *Trypanosomes*¹⁵, *Leishmania*¹⁶ as well as free living amoebae¹⁷. Ockert¹⁸ has reported that children in a psychiatric unit treated with chlorpromazine had lower incidence of *Giardia* infection and indicated that chlorpromazine may have cytotoxic action on amoebae. The present communication reports the growth inhibitory effects of trifluoperazine (TFP) and chlorpromazine (CPZ) on *E. histolytica* in axenic culture and suggests that phenothiazine compounds may have potential as anti-amoebic agents.

An axenic culture of *E. histolytica* NIH-200 was grown in Diamonds TPS-1 monophasic medium¹⁹ as modified by Imam by incorporating ribonucleic acid (2.5 mg/ml). The inhibitors trifluoperazine (Eskay Lab Ltd., Bombay) 1 mg/ml; chlorpromazine hydrochloride (Sigma Chemical Co., St. Louis, MO) and ethylene glycol-oo'-bis (2-aminoethyl) NNNN'-tetraacetic acid (Fluka) were dissolved in modified TPS-1 medium, sterilized by filtration through millipore filters (0.22 μ m) and added to 10 ml cultures contained in screw capped tubes to desired concentration. The tubes were inoculated and incubated at 37°C. At desired intervals, tubes were chilled to dislodge the

amoebae adsorbed to the surface of tubes, mixed and counted by haemocytometer.

Results on the growth inhibition of *E. histolytica* by TFP and CPZ are presented in figures 1 and 2 respectively. TFP exhibited appreciable growth inhibitory action at low concentration (2–5 μ M); increase in concentration potentiated the effect but complete inhibition required about 20 μ M TFP. With chlorpromazine only, slight growth inhibition was observed 1–2 μ M; 5 μ M permitted some growth after a long interval. However growth was completely blocked at 10 μ M and higher concentrations. The calcium chelator EGTA had practically no effect on amoebic growth in micromolar concentration; 5–10 mM partially inhibited growth (table 1). Further increase in concentration (25–50 mM) resulted in rounding up of amoebae but some growth was observed in all cases. These results therefore establish the growth inhibitory action of phenothiazine compounds on *E. histolytica*, CPZ being somewhat more effective than TFP. The inhibitory action does not seem to be associated with calcium levels *per se* and the action of phenothiazine may be manifested via calmodulin-dependent reactions of amoebic growth and cell division. The cal-

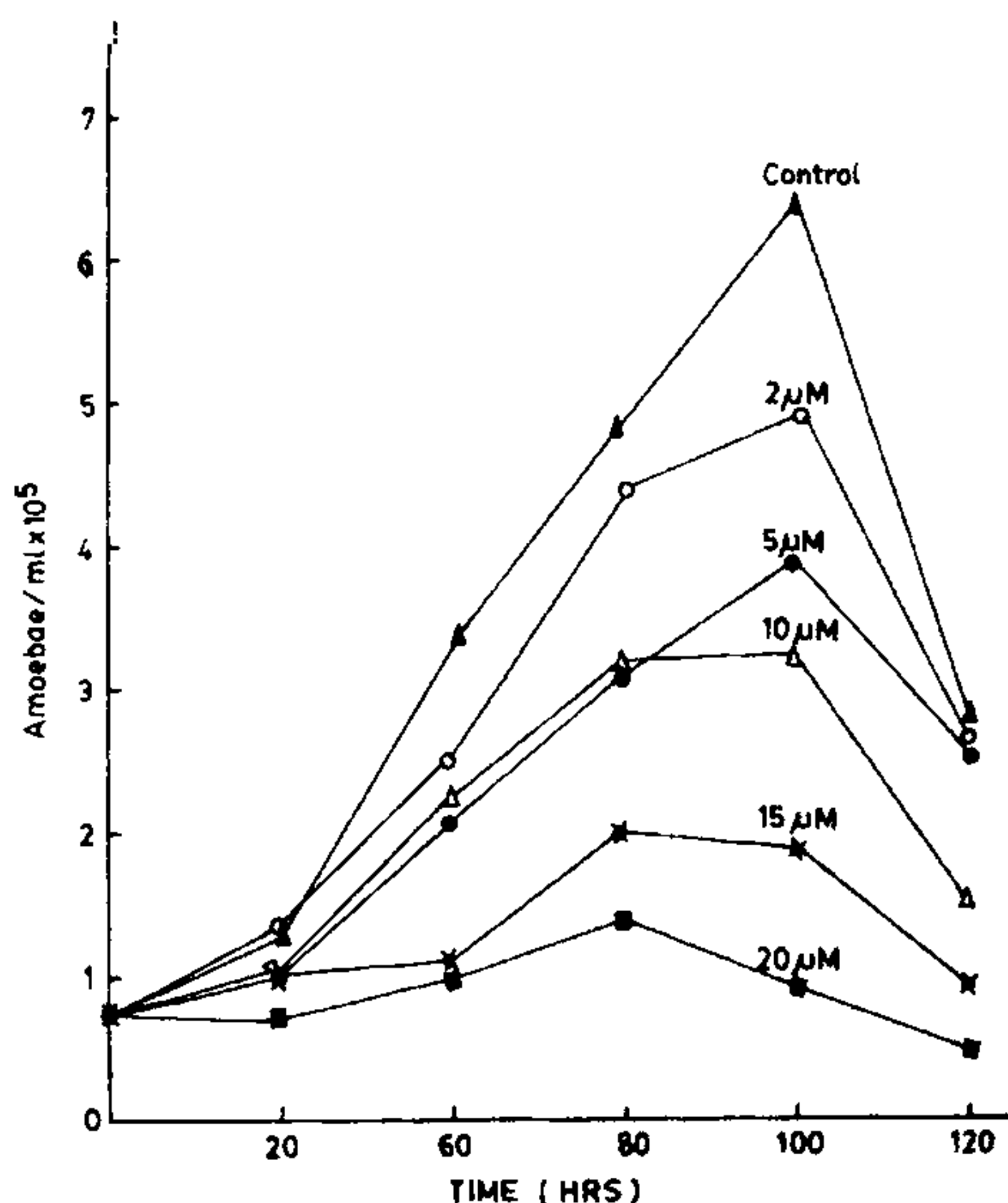


Figure 1. Effect of trifluoperazine on the growth of *E. histolytica* NIH-200.

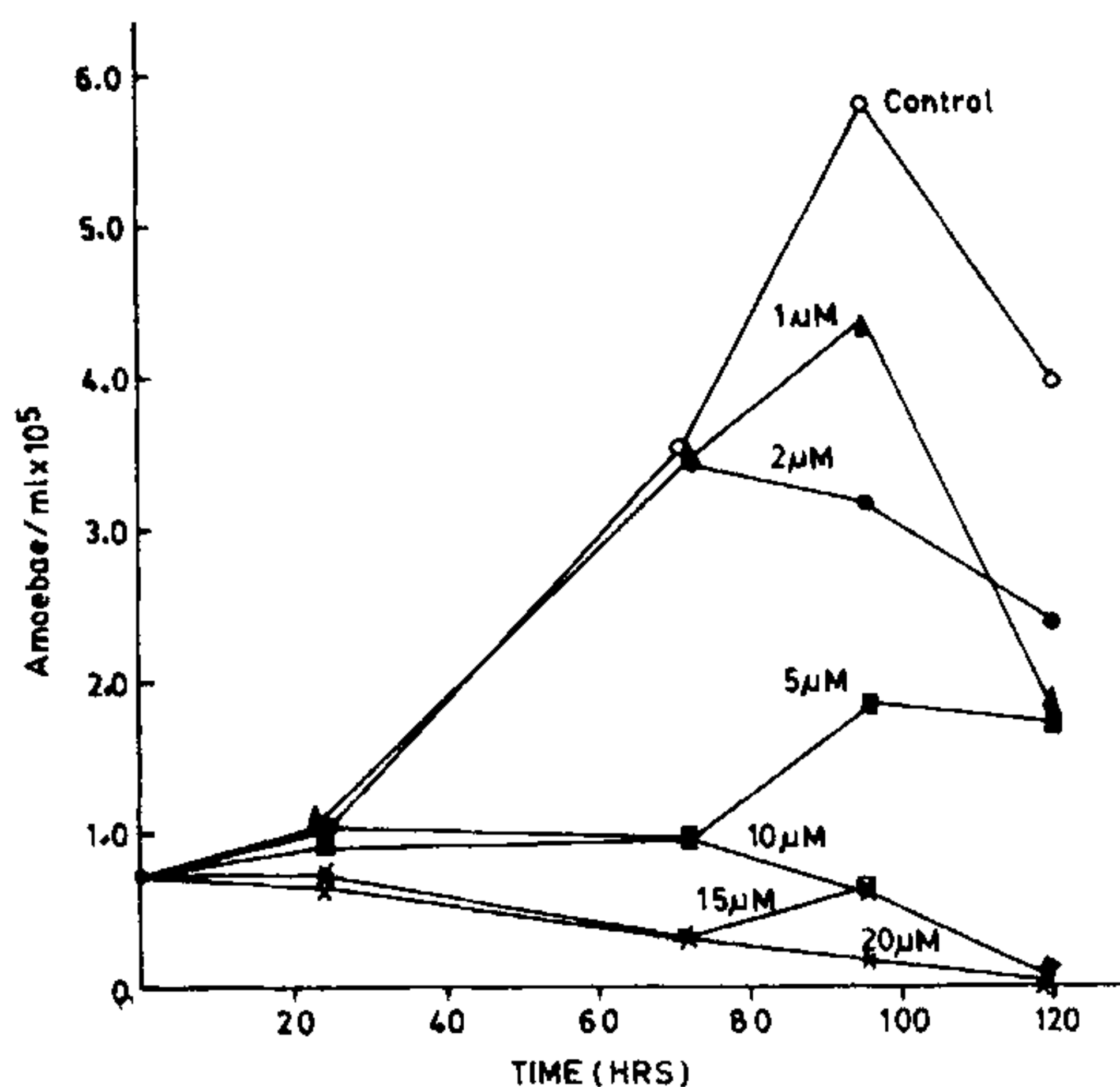


Figure 2. Effect of chlorpromazine on the growth of *E. histolytica* NIH-200.

Table 1 Effect of EGTA on the growth of *E. histolytica* NIH-200 (initial inoculum 1×10^5 amoebae/ml)

Concentration (EGTA) (mM)	Time (hr)			Amoebae/ml $\times 10^{-5}$
	24	48	72	
Nil	++	++++	5.51	
1	++	++++	5.02	
2	++	++++	4.97	
5	++	+++	2.45	
10	++	++	1.50	

modulin of *Tetrahymena* and *Trypanosomes* has been characterized¹⁵ and appears to be different from higher organisms. Partial cure of *Leishmania* infection by CPZ has been reported¹⁶. Further work is needed on the characterization of protozoal calmodulin and related reactions of calcium metabolism to understand the precise mode of antiprotozoal action of phenothiazine. Suitably modified phenothiazines and other modulators of calcium metabolism hold promise in antiprotozoal chemotherapy.

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PHOTOSYNTHETIC BACTERIA FROM THE COASTAL BOARD ECOSYSTEMS

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THE role of photosynthetic bacteria in the synthesis of organic matter in aquatic biotopes is well-recognized¹. Following the early work of Warming², who reported on the mass development of purple sulphur bacteria on the Danish Coast, many papers have appeared on the distribution of photosynthetic bacteria³⁻⁵. Generally,