The authors are very grateful to Dr. P.K. Rajagopalan, Director, for constant encouragement and guidance.

16 July 1982

1. Hagan, H. R., Embryology of the viviparous insects, New York, 1951.
2. Detinova, T. S., Age grouping methods in Diptera of Medical importance with special reference to some vectors of malaria, *WHO Monogr. Ser.*, 1962.
3. Polovodova, V. P., *Med. Parazit. (Moskow)*, 1949, **18**, 352.
4. Rajagopalan, P. K., Kazmi, S. J. and Mani, T. R., *Indian J. Med. Res.*, 1977, **66**, 200.

EFFECT OF METABOLIC INHIBITORS ON EXCYSTMENT PROCESS IN *ENTAMOEBA HISTOLYTICA*

R. K. Sharma*, Sheela Ghoshal and S. R. Das
Division of Microbiology, Central Drug Research Institute, Lucknow 226 001, India.
*Present Address: Department of Surgery, K.G's Medical College, Lucknow 226 001, India.

It is believed[1] that cysts of *Entamoeba histolytica* formed in human bowel do not undergo any further development unless ingested by a new host. Due to the presence of bacteria in large numbers in the caecum and the large intestine, large concentrations of excystment factors would presumably be produced in these localities.

Review of literature on anerobic amoebae belonging to the genus *Entamoeba* clearly shows that the physiological and biochemical mechanism involved in encystation is not understood. It is generally believed that encystation follows periods of vigorous growth, culminating in some changed unfavourable conditions, that result in encystation[2,3]. Various factors causing excystation have been postulated to potentiate the emergence of trophozoites from their resting cysts in *Entamoeba histolytica*[4,5]. Excystment depended on the presence and type of bacteria[10]. Although some molecular changes associated during excystment have been reported, the factor(s) that trigger and regulate the events leading to excystation have not been elucidated[6]. The mode of action of different excystment agents as well as the mechanism of excystment have not been properly understood so far. The behaviour of metabolic inhibitors or excystment process of soil amoebae[7] triggered by different excystment agents has been studied. There are also reports on the effect of metabolic inhibitors on the growth and survival of the trophic stage of *E. hystolytica*[8,9], but there is no report on cysts of *E. histolytica* so far.

Cysts from faecal samples obtained from two patients suffering from chronic amoebic dysentery were checked in normal saline 0.85% (W/V) and in Lugol's Iodine (2:1) by gross microscopic examination. The cysts were concentrated and sterilized[10]. Excystment of these cysts was studied in a mixture of five L-amino acids (Arginine monohydrochloride, Alanine, Serine, Glutamic acid and Isoleucine 0.25% each pH 6.8), and live suspension of *E. coli*. Amino acid mixture was sterilized by autoclaving. The excystment was studied by inoculating sterile cysts in Balamuth's medium and incubated at 37° C for 48 hr, where live *E. coli* was used as an excystment agent. Balamuth's media tubes were preconditioned with bacteria for 6 hr before adding both cysts and metabolic inhibitors.

In the case of amino acids as an excystment agent the Balamuth's media were replaced by the amino acid mixture to which inhibitors and cysts were added.

In order to see the effect of inhibitors on excystment, these were sterilized by millipore filtration and incorporated in different excystment agents. The final concentration of the inhibitors in excystment agent is given in table 1. When the cysts failed to excyst in excystment agent containing inhibitors, excystment agent and inhibitors were washed off and the cysts put for excystment in fresh excystment media. Control sets of experiments were always kept where inhibitors were not used.

Studies on the effect of inhibitors, on the excystment of cysts of *E. histolytica* induced by excystment agents namely live *E. coli* and mixtures of five L-amino acid have been carried out. The results presented in table 1 show that inhibitor of DNA synthesis (mitomycin C) and messenger RNA (actinomycin D) when incorporated in Balamuth's media with live *E. coli* and L-amino acid mixture could not prevent excystment of cysts of *E. histolytica*. Inhibitor of protein synthesis (cycloheximide) when incorporated in Balamuth's media with live *E. coli* did not prevent excystment whereas in the presence of amino acid mixture there was no excystment. However the cysts excysted after the inhibitor, cycloheximide was washed off. The inhibitor of oxidative phosphorylation sodium arsenite[7], prevented the excystment of cysts, completely. Cysts exposed to sodium arsenite failed to excyst in the presence of excystment agents, *E. coli* and L-amino acid mixture, even after the inhibitor was removed by repeated washings.

The present study clearly shows that the behaviour of the metabolic inhibitors on cysts, during the process of excystation was not influenced by the type of

TABLE 1

Effect of metabolic inhibitors on the excystment of cysts of E. histolytica induced by different agents

Inhibitors	Concentrations	Excystment agent							
		Live E. coli				L-amino acid mixture			
		I	IA	II	IIA	I	IA	II	IIA
Mytomycin C	1μg/ml	Ex	15	Ex	43	Ex	21.4	Ex	50
Actinomycin D	1μg/ml	Ex	25	Ex	43	Ex	22.2	Ex	33.3
Cycloheximide	2×10^{-2} M	Ex	17	Ex	16.5	Unex	Nil	Ex	10
Sod. Arsenite	10^{-2} M	Unex	Nil	Unex	Nil	Unex	Nil	Unex	Nil
Controls	—	Ex	87	Ex	87	Ex	80	Ex	80

Ex — Excysted. Unex — Unexcysted.
Expt I — Excystment in presence of excystment agent + inhibitors
Expt II — Excystment in presence of excystment agent after washing off the inhibitors
IA and IIA — Excystment percentage
L-amino acid mixture (isoleucine, arginine monohydrochloride, alanine, serine and glutamic acid)

excystment agent used (*E. coli* and L-amino acid mixture). In general the inhibition of excystment by cycloheximide and sodium arsenite shows that *de novo* protein synthesis and energy transduction mechanism of oxidative phosphorylation are essential, for both the activation of amoebae inside the dormant cysts, and their subsequent emergence from the cysts. Mitomycin C and actinomycin D have no apparent action on the activation of cysts or the emergence of amoebae.

One of us (RKS) is thankful to CSIR for financial assitance.

9 June 1982

1. Faust, E. C. and Russell, P. F., *Craig and Faust's clinical parasitology*, Lea and Febiger, Philadelphia, U.S.A., 1964.
2. Neal, R. A. *Problems in vitro culture*, Proceedings of 5th symposium of the British Society of Parasitology, Blackwells, Oxford, 1967, 9.
3. Das, S. R., Sharma, R. K. and Ghoshal, S., *Indian J. Parasitol.*, 1979, **2**, 15.
4. Singh B. N., *Ann. Appl. Biol.*, 1941, **28**, 65.
5. Hall, R. P., *Protozoology*, Prentice-Hall Inc., New York, 1953.
6. Rastogi, A. K., Sagar, P. and Agarwall, S. C., *J. Protozool.*, 1971, **18**, 506.
7. Jehan, M. and Dutta, G. P., *Protistologica*, 1977, **2**, 181.
8. Yang, C. T. W., *Am. J. Trop. Med. Hyg.*, 1959, **8**, 575.
9. Singh, K. and Dutta, G. P., *Indian J. Med. Res.*, 1976, **64**, 1185.
10. Singh, B. N., Das, S. R. and Saxena, U., *Ann. Biochem. Exp. Med.*, 1963a, **13**, 51.

ROLE OF NEUROTRANSMITTERS IN THE AESTIVATION OF THE INDIAN APPLE SNAIL, *PILA GLOBOSA* (SWAINSON)

P. MURALI MOHAN, K. YELLAMMA, K. SUBHASHINI AND K. SASIRA BABU
Department of Zoology, Sri Venkateshwara University, Tirupati 517502, India.

EARLIER investigations[1,2], on the nervous system of the aestivating snail, *Pila globosa*, revealed a decrease in the spontaneous activity and conduction velocities and an increase in the threshold values for different nerves. The decrease in electrical activity was correlated to the marked drop in the metabolism of the snail during aestivation. Murali Mohan and Babu[3] suggested an inhibitory role for glutamic acid during aestivation. As a followup, the present study attempts to elucidate the significance of the neurotransmitters acetylcholine (ACh) and glutamate during this torpid state in this snail.

The snails were aestivated by embedding them in sand in wooden boxes[1]. The ACh content[4], acetylcholinesterase (AChE) activity[4], and glutamic acid levels[5] in the central nervous system, consisting of all the ganglia, connectives and commissures, of normal and three months aestivated snails, were estimated by methods described earlier.