

normal saline in the ratio of 1:4. From this diluted sample a single amoeba was picked up (under low-power, 10X) using a micro-Pasteur pipette and was immediately put into the culture tubes containing 0.2 ml of medium. Likewise, amoebae were picked up from both the cases. Ten amoebae were picked up from each case.

Pus sample (about 0.5 ml) from amoebic liver abscess (ALA) was directly put into the 0.5 ml of liquid medium and all tubes were incubated at 37°C. After 48 h of incubation the tubes were centrifuged at 500 rpm for 5 min, the old medium was replaced by fresh medium and were again incubated at 37°C. These were regularly subcultured after every 48 h for 3–4 weeks thereafter the amoebae were transferred to culture tubes containing 0.5 ml of medium and were sub-cultured after every 96 h. By this time, the number of amoebae increased by 8–10 folds. Finally, the clones were transferred to 5 ml of medium along with 0.2 ml of 0.25% solution of Chlorostrep (Chloromycetin and Streptomycin) to check bacterial growth. The amoebae grew well in this medium though their rate of multiplication varied from clone to clone as they were isolated from different categories. Six clones were initiated from ALA sample in the same way as was described for NDAC and AAD cases.

The present medium and method is well suited for initiating and maintaining clone cultures of *E. histolytica*. The advantage of the present media over Robinson's media used by Farri is that the same egg-slants can be used 3–4 times to pool the overlay for cloning. Moreover, clones isolated from different categories grew well irrespective of their origin. Gillin and Diamond⁴ made colonies of *E. histolytica* on semisolid agar suspension. The amoebae grew well in the suspension-forming colonies. But to pick up a single colony from the tube having many colonies seems difficult. Das used axenic cultures of *E. histolytica* and other amoebae for cloning in perspex-cavity side. The clones grew well in cavities with TyI-S-33 medium but to know the exact biology of *E. histolytica* clones are required from indigenous strain.

In the present study, a success rate of 80–83% was obtained for acute cases and 60% for chronic cases. Farri reported a success rate of up to 80% with some strains when 48-h-old cultures were used. In the present study 24-h-old-cultures from acute cases and 72-h-old cultures from chronic cases did well for cloning.

The medium and methodology reported in this communication is recommended for initiating and maintaining clones of *E. histolytica* with a success rate of 80 to 83%.

3. Farri, T. A., *Trans. Roy. Soc. Trop. Med. Hyg.*, 1978, 72, 205.
4. Gillin, F. D. and Diamond, L. S., *J. Protozol.*, 1978, 25, 539.
5. Rees, C. W., Key, I. D. and Shaffer, J. G., *Am. J. Trop. Med. Hyg.*, 1960, 9, 162.
6. Phillips, B. P., *Science*, 1950, 2, 8.
7. Boeck, W. C. and Drabohlav, J., *Am. J. Hyg.*, 1925, 5, 371.

18 January 1990

Effect of polyamine precursors and α -difluoromethylornithine on the course of *Trypanosoma evansi* infection in experimental albino mice

Damayanthi

Department of Zoology, Kakatiya University, Warangal 506 009, India

The exogenous supply of arginine and ornithine to *Trypanosoma evansi*-infected mice prolonged mean survival time of the host although a three-fold increase in parasitaemia was noticed. Administration of the α -difluoromethylornithine, a specific suicide inhibitor of polyamine biosynthesis, reduced the parasitaemia and prolonged the survival time of the host. The possible implications of these results in relation to polyamine biosynthesis are discussed.

TRYPANOSOMIASIS is one among the six tropical diseases selected by the World Health Organization for its control and chemotherapy. The antigenic variation of trypanosomes is hampering the vaccine development against these parasites. Chemotherapy has, therefore, become important for the control of this disease¹. There is an increasing emphasis on the exploitation of trypanosome metabolism for the rational screening of antimetabolites for chemotherapy, particularly with reference to growth-promoting metabolites². Polyamines are considered to be important growth factors in proliferating cells and microorganisms³. It has been suggested that they play a vital role in trypanosome growth and metabolism⁴. Since arginine and ornithine are the important precursors in polyamine biosynthesis, an attempt is made to study the influence of these amino acids and α -difluoromethylornithine (DFMO) on *in vivo* growth of *Trypanosoma evansi*, an important pathogenic blood protozoan parasite of cattle in India.

T. evansi collected from the blood of infected cattle was maintained in laboratory animals through syringe passage. Experimental infection was initiated by the intraperitoneal (i.p.) injection of 10⁵ parasites. The details about the methods of trypanosome separation from blood and inoculation are described elsewhere⁵. Inbred Swiss albino mice (30–35 g body weight) were used in the present study. Fifteen male mice were

1. Das, S. R., *Curr. Sci.*, 1972, 41, 736.

2. Das, S. R. and Meerovitch, E., *Curr. Sci.*, 1981, 50, 817.

RESEARCH COMMUNICATIONS

divided into three equal groups. The first group served as infected controls. Arginine and ornithine were administered (i.p.) to the second and third infected groups respectively. The amino acids were administered daily for seven days. The first dose was given along with the parasites.

In a second set of experiment, 20 mice were arranged in four groups. The first group was used as infected controls. The second and third infected groups were administered ornithine and DFMO respectively. The fourth group was administered both ornithine and DFMO. The dosage of the chemicals administered is given in Table 1. The treatment was given for four days, starting 24 h after inoculation of parasites. The blood from tail vein of mice was daily checked for parasites. After the onset of infection, the parasitaemia, pre-patent period, patent period and total survival time were noted. Plasma concentrations of the polyamines were not determined.

Table 2 shows that exogenous supply of arginine and ornithine altered the course of *T. evansi* infection in mice. Administration of these amino acids prolonged the patent period, total survival time of the host and promoted parasitaemia to three-folds when compared to untreated controls. The infection appeared one day earlier in treated animals than those of the controls.

Table 1 reveals that the total survival time was prolonged in DFMO and ornithine-treated animals when compared to controls. The parasitaemia increased in ornithine-treated mice whereas decreased in DFMO-treated mice when compared to untreated controls. The co-administration of ornithine and DFMO further prolonged the survival time of animals. However, there was no change in terminal parasitaemia compared to untreated controls.

Polyamines are found in all prokaryotic and eukaryotic organisms. These biogenic amines play an important role in cellular growth and differentiation. The other functions of polyamines as regulators of metabolism are not yet understood⁶. Putrescine and spermidine are the major polyamines found in trypanosomes. These amines are not only important for the multiplication of trypanosomes but also play important role in parasite metabolism such as activation of key respiratory enzyme α -glycerophosphate dehydrogenase and DNA polymerase⁴.

Trypanosomes synthesize polyamines by a single route, i.e. decarboxylation of ornithine. Ornithine decarboxylase (ODC) is the key enzyme in the biosynthesis of putrescine which is converted into spermidine in subsequent reaction². It appears that trypanosomes prefer *de novo* synthesis of polyamines,

Table 1. Effect of ornithine and α -difluoromethylornithine on the course of *T. evansi* infection in experimental mice.

Group	Daily dose (μ m)	Total treatment period (days)	Pre-patent period (days)	Patent period (days)	Total survival period (days)	Parasitaemia tryps/ml blood
Untreated and infected control	—	—	3	3.4 \pm 0.7	6.4 \pm 0.6	(5.5 \pm 1.6)10 ⁷
Ornithine-treated and infected	50	4	2	9.2 \pm 1.2	11.4 \pm 2.8	(16.2 \pm 3.8)10 ⁷
DFMO-treated and infected	50	4	3	7.8 \pm 1.6	10.8 \pm 1.6	(3.2 \pm 1.3)10 ⁷
DFMO + ornithine-treated and infected	50 + 50	4	3	35.4 \pm 14.4	38.4 \pm 14.4	(5.5 \pm 1.02)10 ⁷

Values are mean \pm S.D. of 10 observations of two separate experiments.

Table 2. Effect of exogenous arginine and ornithine on the course of *T. evansi* infection in experimental mice.

Group	Daily dose (μ m)	Total treatment period (days)	Pre-patent period (days)	Patent period (days)	Total survival period (days)	parasitaemia tryps/ml blood
Untreated and infected controls	—	—	3	3.4 \pm 0.5	6.4 \pm 0.5	(6.1 \pm 1.0)10 ⁷
Arginine-treated and infected	75	7	2	13.6 \pm 2.2	15.6 \pm 2.2	(16.2 \pm 4.2)10 ⁷
Ornithine-treated and infected	75	7	2	11.8 \pm 2.6	15.4 \pm 3.2	(17.8 \pm 3.7)10 ⁷

Values are mean \pm S.D. of 10 observations of two separate experiments.

since uptake of exogenous polyamine is limited in these parasites⁷. Exogenous supply of these biogenic amines has not altered the course of *Trypanosoma brucei* infection in mice². *In vitro* studies revealed that exogenous arginine and ornithine stimulate polyamine synthesis in trypanosomes⁷. The increased parasitaemia levels by the administration of exogenous arginine and ornithine observed in the present study may be attributed to increased production of polyamines. The arrest of trypanosome multiplication by the administration of DFMO along with ornithine supports this theory as DFMO is a specific suicide irreversible inhibitor of ODC.

1. WHO, *Tropical Disease Research*, Seventh Programme Report, UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, Geneva, 1985.
2. Nathan, H. C., Bacchi, C. J., Hutner, S. H., Rescigno, D., McCann, P. P. and Sjoerdsma, A., *Biochem. Pharmacol.*, 1981, **30**, 3010.
3. Tyms, A. S., Williamson, J. D. and Bacchi, C. J., *J. Antimicrobial Chemother.*, 1988, **22**, 403.
4. McCann, P. P. *et al.*, in *Advances in Polyamine Research*, Vol. 3, (eds Calderera, C. H., Zappia, V. and Bachrach, U.), Raven Press, New York, 1981, p. 97.
5. Damayanthi, *Indian J. Comp. Anim. Physiol.*, 1985, **1**, 16.
6. McCann, P. P., Bacchi, C. J., Nathan, H. C. and Sjoerdsma, A., in *Mechanisms of Drug Action* (eds Singer, T. P. and Undarza, R. N.), Academic Press, New York, 1983, p. 159.
7. Bacchi, C. J., Vergara, C., Garofalo, J., Lipschik, G. Y. and Hutner, J. H., *J. Protozool.*, 1979, **26**, 484.

ACKNOWLEDGEMENTS. I am grateful to CSIR, New Delhi, for financial assistance, and to Merrel Dow Research Institute, Cincinnati for providing DFMO as gift. Thanks are due to Prof. N. Chari for encouragement.

27 March 1989; revised 29 August 1990

Catecholamines are present in hen's egg yolk in fairly stable form: elevated adrenaline indicates stress

R. P. Moudgal, J. N. Panda and Jagmohan

Division of Physiology and Reproduction, Central Avian Research Institute, Izatnagar 243 122, India

Levels of the catecholamines adrenaline, noradrenaline and dopamine in egg yolk did not change significantly on incubation of yolk for 30 min at 41°C. Enzymes that metabolize these amines may be absent or, if present, they may be in inactive state. Levels of these amines declined on exposure to heat, although considerable amounts still remained in the yolk of boiled eggs. Handling of the birds, increased density of birds in cages, and social stress induced marked elevation of adrenaline in egg yolk. The findings may be of importance to the poultry industry.

ADRENALINE and noradrenaline are potent stimuli for

increased metabolic rate (thermogenesis), lipolysis, hyperglycaemia, and anxiety and fear in human beings¹⁻⁵. They have also been implicated in modulation of cardiovascular^{5,6}, reproductive⁷⁻¹⁰, platelet-aggregation¹¹ and several other functions^{5,12}. Dopamine is well known for its role in the brain, but also acts in the kidney, inhibiting aldosterone¹³⁻¹⁵. In birds, catecholamines have been implicated in stress^{16,17} but the elevation of catecholamines has usually been assumed, through fall in skin temperature following vasoconstriction, and increase in heart rate, blood pressure and blood glucose¹⁸. Handling of birds, increase in density of birds in cages, and social changes induce stress in birds^{19,20}. Here we show the presence of catecholamines in yolk of hen's eggs and that these amines are quite stable in yolk on incubation or heating, or boiling of eggs. These results may be important in regard to consumer interest. We also show that there is marked elevation of adrenaline in yolk under stressful conditions. The catecholamine synthesis blocker α -methyl-*p*-tyrosine caused a dose-dependent reduction in levels of all three catecholamines.

The catecholamines were determined in 0.5 g of thoroughly mixed yolk, as for ovarian follicles²¹. Recovery of catecholamines added to acid butanol matched recovery of standard catecholamine added to yolk extract (Table 1). So acid butanol was used as extraction medium for determination of catecholamine in yolk. Table 2 gives catecholamine content of egg yolk after incubation for 30 min at 41°C and heating of thoroughly mixed yolk, in each case from the same eggs, for 5 or 10 min, in test-tubes of the same size (60 × 15 × 0.5 mm) placed in boiling water, and of yolk of boiled eggs. Incubation of yolk for 30 min at 41°C did not result in significant drop in levels of the catecholamines. Considering their very short half-life in the circulation, it is likely that the metabolizing enzymes for these catecholamines may be absent in yolk or, if present, may be in an inactive state. While there was a significant drop in levels after heating of yolk or boiling of eggs, considerable quantities were still present.

The effect of the catecholamine synthesis blocker α -methyl-*p*-tyrosine was also studied. Control birds were injected vehicle alone. Table 3 shows that the administration of vehicle alone caused significant increase in adrenaline content of egg yolk, indicating that the handling of birds for injection caused stress to the birds and an increase in yolk adrenaline. The catecholamine synthesis blocker caused a dose-dependent reduction in yolk catecholamine.

The yolk catecholamines were also determined six days after two birds were put together in the same standard-size individual-bird cage and again after separating them and putting them in separate cages. The increase in cage density and the social change, both considered to act as stressors^{19,20}, caused significant