EFFECTS OF MICROSPORIDA ON SNAIL AND TREMATODE TISSUE

JAMES R. PALMIERI, P.C. LAI, JOHN T. SULLIVAN and ANNE CALI*

University of California, ICMR, Institute for Medical Research, and *Rutgers University, Newark, New Jersey, U.S.A.

INTRODUCTION

Approximately 15 species of trematode-infecting Microsporida have been identified to date (Canning, 1975). Bulla and Cheng (1976) report over 525 named species of Microsporida and about 200 unnamed. Microsporida have some economic importance particularly in the silk and honey industries, and they have been found to infect man. Margileth *et al.*, (1973) reported a species of Microsporida from a human infant based upon formalin-fixed autopsy tissue. Sprague (1974) reported a new species of Microsporida, *Nosema connori*, from autopsy tissue, this species was named in honor of Dr. Daniel H. Connor, Chief, Infectious Diseases Branch of the Armed Forces, Institute of Pathology, Washington D.C. who first recognized the organism as a microsporidian.

The use of Microsporida as a biological control agent of trematode parasites is now in its infancy stage. At the Institute for Medical Research, Kuala Lumpur, Malaysia, studies on Microsporida have been carried out on this topic since 1968, and we are presently working with several species of Microsporida as biological control agents.

Before field studies can be initiated, extensive experimental laboratory investigations must be carried out. This report describes some techniques in use in our laboratory and some preliminary observations.

This study was supported in part by grant AI 10051 (UC ICMR) to the Department of International Health, School of Medicine, University of California, San Francisco, from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U.S. Public Health Service.

MATERIALS AND METHODS

Spores of Microsporida are exposed to laboratory reared snails containing experimentally developed larval trematodes. Snails are starved for 48-72 hours, and then placed in a 2.5-inch Petri plate for approximately 12 hours with 1-in^2 piece of lettuce to which 2 drops of Microsporida spore inoculate has been applied. After exposure, snails are moved to a 7-liter aquarium containing boiled rainwater and are fed a diet of washed uncooked lettuce.

Our laboratory experiments are designed to determine the natural life cycle, host and tissue specificities of Microsporida, the ultimate goal being a system in which control can be exerted on the intramolluscan larval stages of trematodes.

In our laboratory, *Lymnaea rubiginosa* snails were experimentally infected with the trematodes *Fasciola gigantica*, *Echinostoma audyi* and *Tracheophilus* sp. singly and in combination. Later, infected snails were separately exposed to spores of each of 3 species of Microsporida of the genus *Nosema*. Although each species of *Nosema* varied in its host and tissue specificities as well as developmental times, certain effects of the Microsporida on snail and trematode tissues were usually noted.

RESULTS AND DISCUSSION

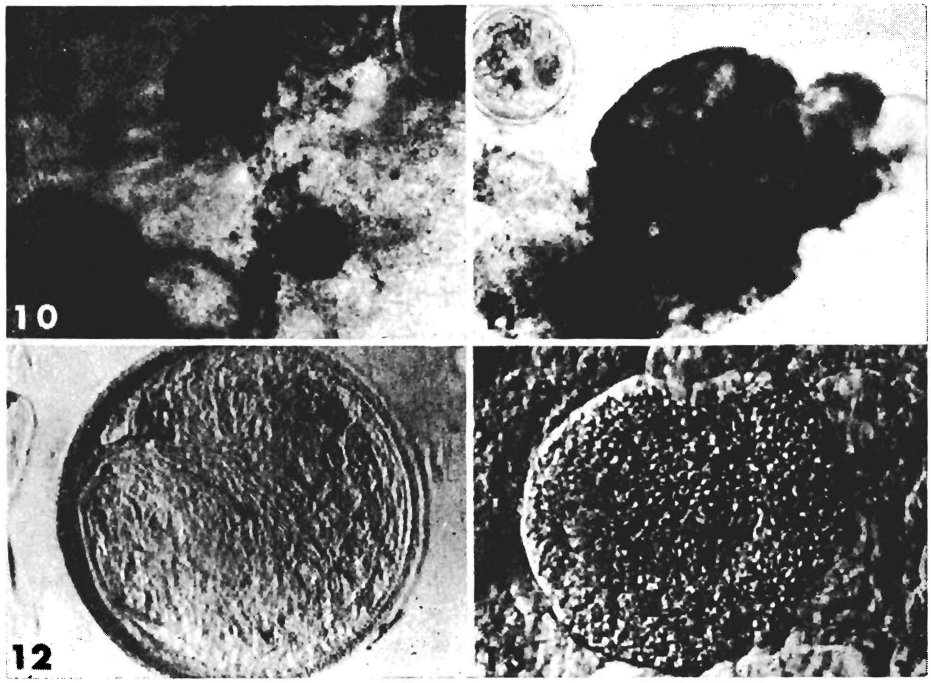
Experimental exposure of *Nosema* sp. to snails infected with trematode larvae have resulted in positive infections in *Fasciola*

gigantica, *Echinostoma audyi* and *Tracheophilus* sp.

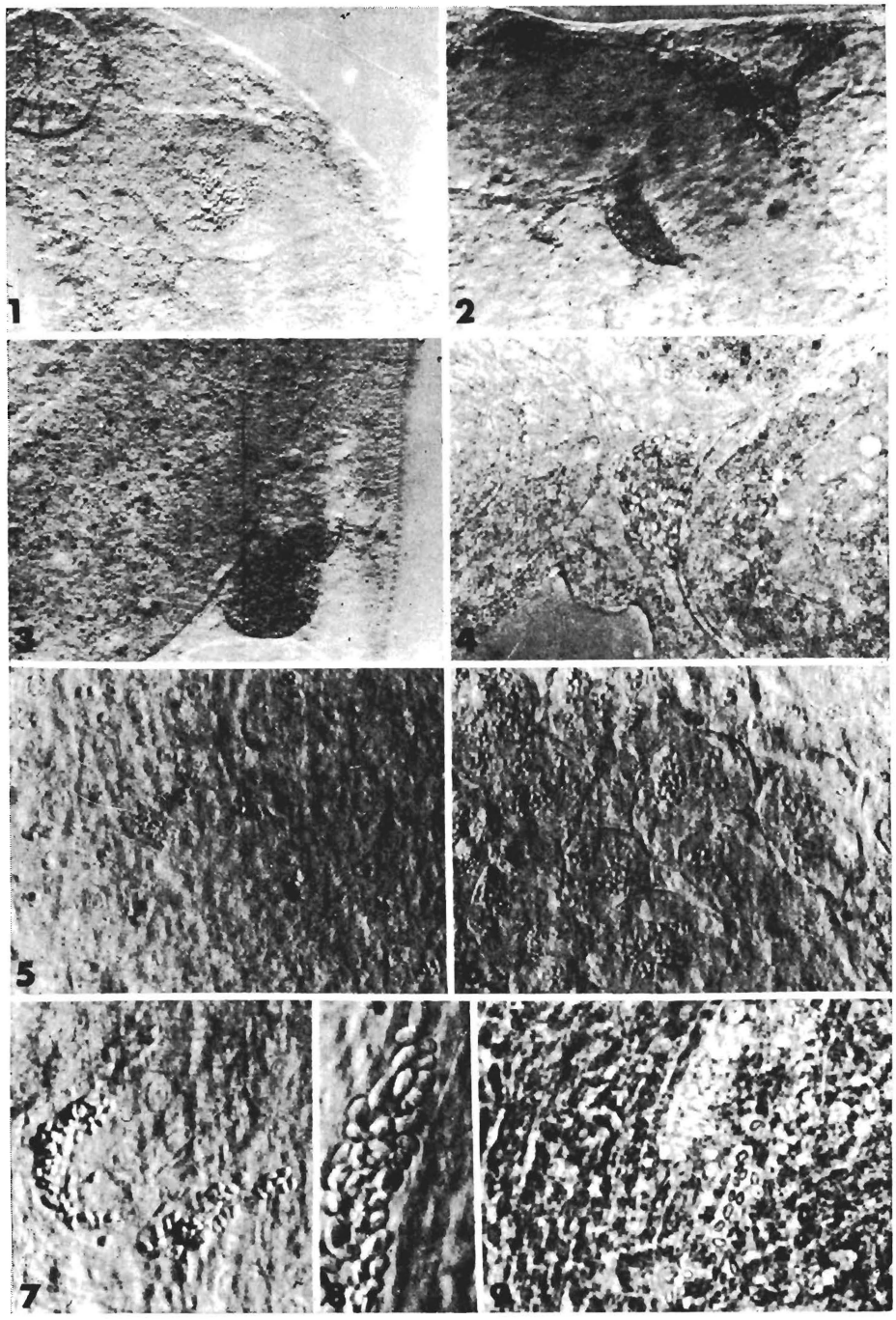
Examination of host and parasite tissues infected with Microsporida, at the light and ultrastructural level, has revealed that the developing stages of *Nosema* sp. attach the mother and daughter sporocysts, rediae, developing cercarial embryos as well as the metacercariae and disrupt their normal development such that in some cases cercarial release is reduced or prevented (Figs.1-4, 10-12). Microsporida were found to develop intracellularly in the subtegumental, embryonic and parenchymal cells of the larval trematodes where they break down these host-cell membranes. It is this rupturing which probably accounts for the spread of infection of Microsporida to surrounding tissues.

In all cases, each of the 3 species of Microsporida used were found to fully develop in molluscan tissues in both snails which contained larval trematode infections as well as those without trematode infections (Figs. 5-9, 13). Although heavy spore infections were commonly found in the blood sinus spaces of the gizzard, spores were also found in the head-foot, reproductive and digestive organs and in the digestive gland. In all cases the gizzard tissue was found to harbor developmental stages of all the species of *Nosema* used. Snail tissue reaction to microsporida infection was not observed.

It is theorized that experimental transmission of the 3 species of Microsporida took place as follows: 1) transmission to snails takes place when ingested spores excyst in the



Figs. 10-13—Microsporida spores in trematode and snail tissues. 10. Sac of spores of Microsporida in a degenerate redia of *Tracheophilus* sp. in the pericardial cavity of *L. rubiginosa*. 133x. 11. Redia of *Tracheophilus* sp. filled with spores of Microsporida. 113x. 12. Spores of Microsporida in the metacercaria of *Tracheophilus* sp. 220x. 13. A large intracellular mass of spores of Micro sporida in the mantle of *L. rubiginosa*. 447x.



Figs. 1-9—Spores of Microsporida in trematode and snail tissues. 1. Spores of Microsporida in the gut of *F. gigantica* redia. 267x. 2. Microsporida multiplying in sacs on the surface of the gut of a *F. gigantica* redia. 333x. 3. Spores of Microsporida in clusters and sacs on the surface of the gut of a *F. gigantica* redia. 250x. 4. A group of spores of Microsporida in the redia of *Tracheophilus* sp. 347x. 5. Small groups of spores of Microsporida infecting the ventricle of *L. rubiginosa*. 450x. 6. Intracellular groups of spores in the mantle wall of *L. rubiginosa*. 300x. 7. Spores of Microsporida in the sinuses of the mantle wall in *L. rubiginosa*. 513x. 8. Higher magnification of same. 1200x. 9. Spores of Microsporida in the tissue of snails whose parents were exposed to spores of Microsporida. 413x.