

BRIEF NOTE

THE INABILITY OF A TRYPANORHYNCHID CESTODE TO UTILIZE CO₂ PRODUCED DURING UREA CATABOLISM¹

PETER W. PAPPAS, Department of Zoology, Ohio State University, Columbus, OH 43210

OHIO J. SCI. 78(3): 152, 1978

Cestodes of the order Trypanorhyncha appear freely permeable to urea (Read *et al* 1959, Simmons *et al* 1960), and at least 2 members of this order (*Lacistorhynchus tenuis* and *Pterobolhrium lintoni*) have significant urease activity (Simmons 1961). It is not known, however, whether these cestodes actually utilize the CO₂ produced by urease. This paper reports on the apparent inability of one of these cestodes, *L. tenuis*, to utilize this CO₂ as a carbon source.

L. tenuis was obtained from naturally infected *Mustelus canis*. The cestodes were removed from the spiral valve and rinsed thoroughly in a saline solution containing 300 mM urea and buffered with 10 mM tris(hydroxymethyl)-amino-methane-maleate at pH 7.0 (Read *et al* 1959). Worms were randomized into groups of 5, pre-incubated in 15 ml of fresh buffered saline-urea at 20°C for 15 min, and then transferred to 10 ml of buffered saline-urea containing 0.1 μ Ci/ml of ¹⁴C-urea (New England Nuclear). Worms were incubated for up to 1 hr, removed, rinsed thoroughly in buffered saline-urea, and killed in 70% ethanol.

Worms were homogenized in 20 volumes of 70% ethanol and the homogenate centrifuged at 2,000 x g for 15 min. The resulting pellet was rinsed repeatedly with 70% ethanol, until free of soluble radioactivity, extracted once with 10 volumes of chloroform:methanol (2:1), and dissolved in 10 volumes of 1 N NaOH. The ethanol supernatants (and original ethanol extract) were combined and partitioned against acidified chloroform; the resulting aqueous layer was removed,

evaporated to dryness under N₂, and reconstituted with 0.1 N HCl. The acidified chloroform and chloroform:methanol extract of the tissue pellet were combined, evaporated to dryness under N₂, and reconstituted with chloroform:methanol (2:1). Aliquots of the dissolved tissue pellet (containing protein, glycogen and nucleic acids), and the reconstituted lipid and ethanol-soluble fractions (the latter containing soluble carbohydrates and amino acids) were assayed for radioactivity using a Nuclear-Chicago, D-47 gas-flow counter. The reconstituted ethanol extracts were also analyzed using a Technicon automatic amino acid analyzer in conjunction with a Packard Model 3022 flow analyzer scintillation spectrometer.

Simmons (1961) demonstrated significant urease activity in intact *L. tenuis* when the worms were incubated in buffered saline (at 20°C) containing ¹⁴C-urea for time periods ranging from 5 to 20 min. Incubations were conducted in a respirometer flask with 2 side arms, and NH₄ and CO₂ were "trapped" with N₂SO₄ and KOH, respectively. Under these conditions the rate of urea hydrolysis, as determined by 5 min incubations, was 316 μ moles hydrolyzed/g wet wt/hr. In my study, analyses of worms incubated for up to 1 hr demonstrated that no radioactivity was incorporated into either the ethanol-insoluble or lipid components. Analyses of the ethanol extracts showed that all radioactivity was in the form of labeled urea. Therefore, using experimental conditions nearly identical to those described by Simmons (1961) (except for the respirometer flask) to assay urease activity in intact *L. tenuis*, the present experiments were un-

¹Note received October 3, 1977 and in revised form December 1, 1977 (#77-74).

able to detect incorporation of the carbon moiety produced during urea catabolism. Thus, the physiological significance of the urease activity found in *L. tenuis* remains unknown.

Acknowledgments. Materials and facilities for part of this work were provided by the Marine Biological Laboratory, Woods Hole, MA. This work was supported, in part, by a Research Grant (AI-01384) from the N.I.H. to the late Dr. Clark P. Read.

LITERATURE CITED

- Read, C. P., L. T. Douglas and J. E. Simmons, Jr. 1959 Urea and osmotic properties of tapeworms from elasmobranchs. *Exp. Parasitol.* 8: 58-75.
- Simmons, J. E., Jr. 1961 Urease activity in trypanorhynch cestodes. *Biol. Bull.* 121: 535-546.
- Simmons, J. E., Jr., C. P. Read and A. H. Rothman 1960 Permeation and membrane transport in animal parasites: Permeation of urea into cestodes from elasmobranchs. *J. Parasitol.* 46: 43-50.