ENZYME HISTOCHEMISTRY OF *TOXOPLASMA GONDII**

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At present very little is known about the energy metabolism of *Toxoplasma gondii* except that Fulton and Spooner[1] showed that a suspension of organisms utilizes glucose and oxygen. The organism is difficult to study because it is an obligate intracellular parasite. Not only does multiplication require an intracellular locus, but gradual death of organisms occurs when they are extracellular for any appreciable period of time. Since *Toxoplasma* can thrive in almost any mammalian cell or any cell in tissue culture, the factors which are provided by the cell must be simple and nearly universal. It is the purpose of this study to employ enzyme histochemistry to ascertain the presence, activity and location within the organisms of enzymes associated with respiration, and to compare the apparent enzyme activities of proliferative and encysted parasites.

Tetrazolium stains permit selective demonstration of the various enzymes of glycolysis, the Kreb's cycle, and the electron transport chain in encysted as well as proliferative forms of *Toxoplasma* and permit localization of these enzymes within the organism.

METHODS AND MATERIALS

All tissues to be sectioned for enzyme stains were frozen in liquid nitrogen and isopentane or in a mechanical freezer at −78°C. They were stored at −78°C until used. Cryostat sections from 8 μ to 12 μ in thickness were then cut. The sections were removed from the knife with a finely drawn capillary tube and placed on warm slides. They were allowed to dry for a period of 40 seconds before enzyme staining was employed. The basic medium used to demonstrate the dehydrogenase enzymes was composed of 0.25M phosphate buffer, 0.05M magnesium chloride, 0.01M NaCN, and 0.3 mg/ml p-nitroblue tetrazolium. The substrates were added to a final concentration of 1M and the co-enzymes to 5 mg/ml, and staining was done as previously described.[2]

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Toxoplasma *in peritoneal fluid.* Twenty- to twenty-five-gram mice were injected with RH strain *Toxoplasma* intraperitoneally (i.p.). The animals were sacrificed three days after injection,

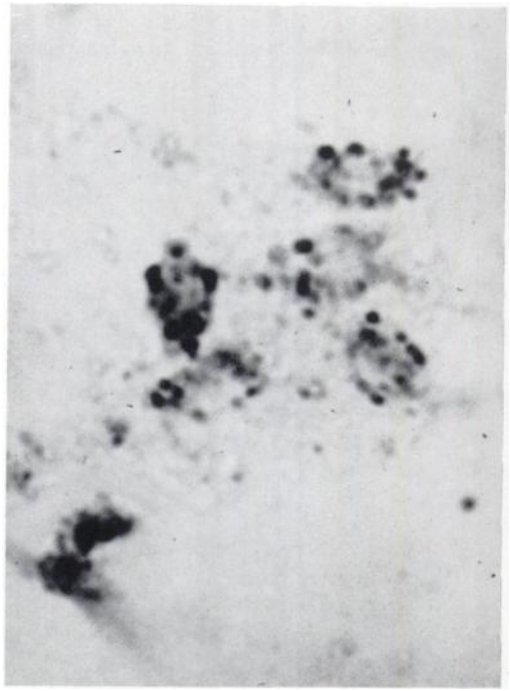

FIGURE 1. Proliferative *Toxoplasma gondii* of mouse peritoneal exudate stained for NADH diaphorase. Stains of other enzymes are similar in distribution.

and the peritoneal fluid was removed immediately. Smears were made of the fluid and the slides were dried for a minimum of one hour.

Toxoplasma *cysts in mouse brain.* Twenty- to twenty-five-gram albino mice were injected with Beverly strain *Toxoplasma* i.p. After 14 to 28 days the mice were sacrificed and the entire brain was removed intact. A small portion of the telencephalon was homogenized to determine if *Toxoplasma* cysts were present in the brain. If cysts could be seen, the brain was kept for sectioning.

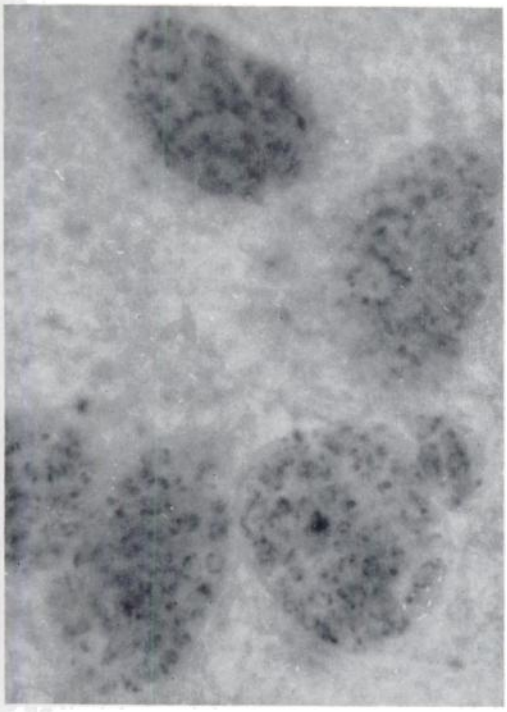

FIGURE 2. Cysts of *Toxoplasma* in frozen sections of mouse brain stained for lactic dehydrogenase. The punctate focal deposits in the organisms are much more dense than those of the surrounding brain.

Proliferative Toxoplasma *in rabbit eye.* One- to two-kilogram New Zealand white rabbits were injected between the sclera and the choroid in both eyes with RH *Toxoplasma.* The animals were sacrificed after three days, and the eyes were frozen in liquid nitrogen and isopentane or a mechanical freezer at −78°C and stored at −78°C until sectioned. Procedures for drying and staining were similar to those used in brain sections. The location and identity of organisms in both the eye and brain were determined by fluorescein labeled antibody stain.

RESULTS

The results of the stain of the peritoneal exudate smears of *Toxoplasma gondii* indicate that the glycolytic enzymes (lactic, glucose-6-phosphate and -glycerophosphate dehydrogenases) and the Kreb's cycle enzymes (malic dehydrogenase, succinic dehydrogenase, and the electron transport enzymes NADH diaphorase and NADPH diaphorase) are all represented in a qualitative manner in *Toxoplasma.* Their primary site of activity is localized in discrete deposits which seem to vary from six to eight in number (Fig. 1). These areas of tetrazolium deposits were supra-vitally stained with Janus green B[3, 4] and found to absorb the oxidized dye, indicating that the enzymes were limited to the mitochondria.

TABLE 1

Comparative intensity of enzyme staining in proliferative and encysted toxoplasma

Stain	Intensity of stain	
	Proliferative organisms	Encysted organisms
NADH diaphorase	+++	++++
NADPH diaphorase	+++	++++
Lactic dehydrogenase	+++	++++
α-glycerophosphate dehydrogenase	++	++++
Glucose-6-phosphate dehydrogenase	+++	+++
Malic dehydrogenase	+++	++++
Succinic dehydrogenase	+	++
Succinic dehydrogenase*	+++	++++
Control	0	0

* Reaction enhanced with phenazine methosulfate.

No stain could be seen in the cytoplasmic area of the organisms, but the mitochondrial deposits were so prominent that the organism could be identified by these when encysted. The localization of cysts in the mouse brain was easy using these stains because of the dense blue deposition caused by the enzymes of the mitochondria. The heavy concentration of stain in the cyst contrasted sharply with the pale stain of the surrounding brain tissue (Fig. 2). In the eye, organisms were difficult to see amid the heavy deposits of tetrazolium in the retina, except where the number of organisms was very large. The comparative intensity of enzyme staining of proliferative and encysted organisms is tabulated (Table 1).

CONCLUSIONS

All the metabolic enzymes which were studied—lactic, glucose-6-phosphate and -glycerophosphate dehydrogenases, malic and succinic dehydrogenases, and NADH and NADPH di-

aphorases—were present in *Toxoplasma gondii*. In this organism the enzymes were bound within the mitochondria and the large area of cytoplasm was free of enzymes, although in many cells these enzymes are not bound to mitochondria. Proliferative as well as encysted organisms contained all the enzymes studied. The presence of these enzymes suggests that the metabolic requirements which make this organism unable to survive extracellularly are probably not directly associated with energy production.

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