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CHEMICAL AND PHYSICAL FACTORS AFFECTING THE EXCYSTATION OF *CRYPTOSPORIDIUM PARVUM* OOCYSTS

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ABSTRACT: Cryptosporidium parvum oocysts were examined to ascertain excystation requirements and the effects of gamma irradiation. Oocysts and excysted sporozoites were examined for dye permeability and infectivity. Maximum excystation occurred when oocysts were pretreated with acid and incubated with bile salts, and potassium or sodium bicarbonate. Pretreatment with Hanks' balanced salt solution or NaCl lowered excystation; however, this effect was overcome with acid. Sodium ions were replaceable with potassium ions, and sodium bicarbonate was replaceable with sodium phosphate. Oocysts that received 200 krad irradiation excysted at the same rates as nonirradiated oocysts (95%), the excystation rates were lowered (50%) by 2,000 krad, and no excystation was observed by 5,000 krad. No differences were observed between the propidium iodide (PI) permeability of untreated oocysts and oocysts treated with 200 krad, while 92% of oocysts were PI positive after 2,000 krad. Most of the sporozoites exposed to 2,000 krad were not viable as indicated by the dye permeability assay. The oocysts irradiated with 200 and 2,000 krad infected cells, but no replication was observed. The results suggest that gamma-irradiated oocysts may still be capable of excystation and apparent infection; however, because the sporozoites could not reproduce they must not have been viable.

The identi®cation of the mechanisms that trigger and carry out the process of excystation is critical for understanding how sporozoites are freed from oocysts prior to initiating the penetration of the hosts' intestinal cells. In vitro excystation is a parameter that has been used to measure the viability of sporozoites within oocysts (Campbell et al., 1992; Jenkins et al., 1997). Jenkins et al. (1997) reported that the in vitro excystation assay for potential infectivity of oocysts correlated with the in vivo mouse infectivity test and the in vitro dye permeability assay.

Several studies have examined the requirements for *Cryptosporidium parvum* oocyst excystation (Fayer and Leek, 1984; Reduker and Speer, 1985; Woodmansee, 1987; Current, 1990). Robertson et al. (1993) reviewed the excystation protocols developed by different research groups and recommended that to achieve high excystation rates, *C. parvum* oocysts should be incubated with bile or bile salt for 4 hr at a temperature of 37 C, after a 1-hr treatment with acidi®ed Hanks' balanced salt solution. Like other protozoan parasites (McKerrow et al., 1993), the excystation of *C. parvum* oocysts is possibly induced by proteolytic enzymes secreted by sporozoites (Nesterenko et al., 1995; Okhuysen et al., 1996; Forney et al., 1996, 1998) or by the structure of the oocyst wall or suture being changed by external chemical or physical factors. Oocysts that are killed by heat (60 C for 5 min) or formaldehyde (10% formalin) will not excyst (Jenkins et al., 1997), which suggests that viable sporozoites may be required for inducing excystation. However, most treatments that kill sporozoites also change the protein conformation of the oocyst wall and suture. Gamma irradiation is potentially capable of inactivating the contained sporozoites without affecting the structure of the oocyst wall and suture. If excystation requires proteolytic enzymes produced by either living or nonliving sporozoites, irradiation treatments that may block the production of the proteolytic enzymes or denature the proteolytic enzymes present in the cytosol of nonliving sporozoites should prevent in vitro excystation. Sincock et al. (1998) observed that the permeability of the oocyst wall to Sytox Green was changed by gamma irradiation levels between 100 and 900 krad. However, the effects of irradiation treatments on the permeability of the oocyst wall to propidium iodide (PI), which has been used as an indicator of oocyst inactivation or death (Campbell et al., 1992; Jenkins et al., 1997), have not been examined.

The objectives of the present study were to identify the minimum pretreatment and incubation medium requirements for oocyst excystation under in vitro conditions and examine the effects of sporozoite inactivation by gamma irradiation on excystation. A dye permeability assay (Anguish and Ghiorse, 1997) using PI was performed to determine whether the permeability of the oocyst wall to PI would be changed by exposure to gamma irradiation. In addition, a cell culture infectivity assay was performed to examine the effects of gamma irradiation on sporozoite viability.

MATERIALS AND METHODS

Oocysts

Cryptosporidium parvum oocysts were obtained from naturally infected 7±14-day-old calves in Tompkins County, New York. A sucrose/Percoll flotation method was used to extract oocysts from calf feces

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