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Research Note

Cryptosporidium in Cell Culture

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Cryptosporidium sp., a coccidial protozoan of the intestinal tract, occurs in a wide range of vertebrates. Of veterinary interest is its presence in goats, calves and chickens (Current and Long, 1983). In these animals it causes severe diarrhea (Anderson and Hall, 1982). In the immunocompetent human cryptosporidiosis is a self-limiting disorder, and is manifested by diarrhea and malabsorption (Reese *et al.*, 1982, Current *et al.*, 1983). Recognition of cryptosporidiosis as an important clinical entity came about as a result of the severe, life-threatening diarrhea and consequent water loss seen in immunosuppressed patients, and particularly in those with acquired immonodeficiency syndrome (AIDS), as reported by Guarda *et al.* (1983).

Recent experimental studies reported in the literature have added important information to the morphology and life cycle of *Cryptosporidium* sp. Current and Long (1983) obtained complete development of the parasite in the microvillous region of endoderm cells of the chorioallantoic membrane of the chick embryo. Current and Haynes (1984) reported on the complete development of the parasite in cell culture. The cell lines used included human fetal lung and primary chicken kidney and porcine kidney cells.

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Reported here is the complete development of the parasite in a different cell line and by the use of different culture methods. The cell line from the fibroblast of human foreskin (LFS) was used. It was laboratory derived, frozen and maintained in the virology laboratory of the Loma Linda University Medical Center for a period of five years.

In this study the cells were monolayered on 12 mm sterile glass coverslips placed in individual wells in a 24-well tissue culture plate. Excysted sporozoites were inoculated onto each coverslip. Excystation and culture procedures used were as described by Current and Haynes (1984), except that the culture

Table 1 Developmental stages of *Cryptosporidium* in human foreskin cell culture at selected times after inoculation of sporozoites. Seen in culture (+). Not seen (0)

Time after inoculation (hours)	Meronts		Macro-/micro-gametes	Oocysts
	Type 1	Type 2		
12	+	0	0	0
14	+	0	0	0
18	+	0	0	0
24	+	+	0	0
48	+	+	+	0
72	+	+	+	+
96	+	+	+	+
120	0	+	+	+
144	0	+	+	+
168	0	0	0	+
192	0	0	0	+

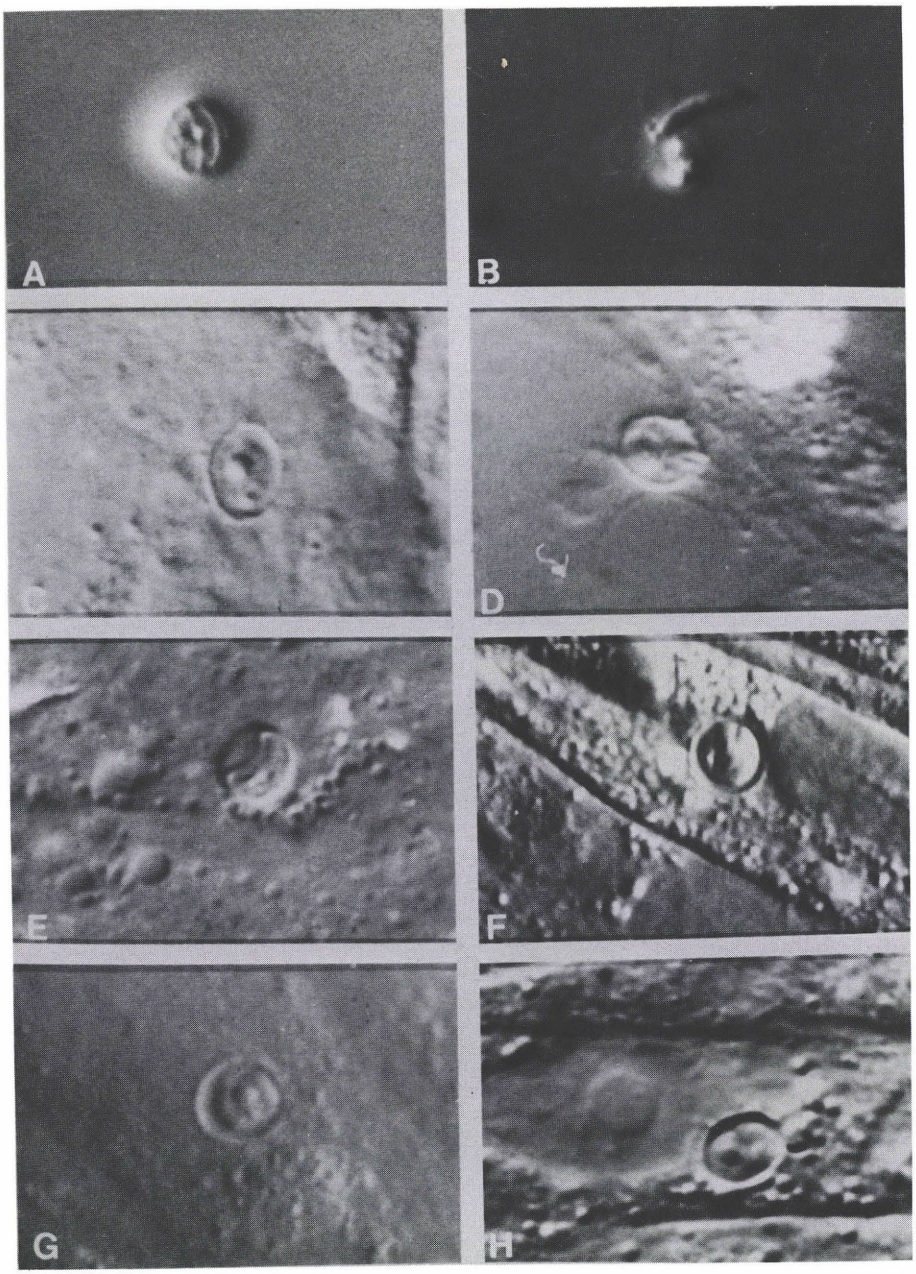

Fig. 1 Representative stages of *Cryptosporidium* in human foreskin cell culture. Photomicrographs of live parasite stages taken under the Nomarski interference contrast microscope (1250X). (A) an oocyst showing 3 sporozoites; (B) a sporozoite above a yeast cell; (C) an immature type I meront with a central refractile body and nuclei around the periphery; (D) a type II merozoite in the process of leaving the host cell; (E) a mature type I meront with merozoites; (F) a mature type II meront with merozoites; (G) a macrogamete with a prominent refractile body; (H) an oocyst at 7 days post-inoculation.

medium was supplemented with 10% instead of 2% fetal calf serum. Oocysts used in this study were maintained in 2.5% potassium dichromate (see Current and Haynes, 1984) provided by the senior author. The initial *Cryptosporidium* inoculum was from an AIDS person, passed through goats three times, and through calves six times.

The results are shown in Table 1 and in Fig. 1. Fig. 1 shows (A) an oocyst with sporozoites, and (B) a free sporozoite above a yeast cell. The first indication of the presence of the endogenous immature type I meront (with 6 or 8 merozoites) was at 12 hr after inoculation (Fig. 1-C). Its presence through 12–96 hr may imply that it undergoes recycling (merogony) according to the life cycle proposed by Current and Long (1983). A mature type I meront with merozoites is seen in Fig. 1-F. A type II meront (with 4 merozoites) is shown in Fig. 1-D, with a pair of merozoites in the process of leaving the host cell. It was present in the cell culture at 24–144 hr after inoculation. A macrogamete with a prominent refractile body (Fig. 1-G) was present at 48 hr after inoculation and an oocyst (Fig. 1-H) was first seen at 72 hr. The one shown in Fig. 1-H was at 7 days post-inoculation. These oocysts were present 8 days post-inoculation which represents the end of the observation period.

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細胞培養法による *Cryptosporidium* の培養

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本培養においては，培養細胞として Loma Linda 大学で分離維持している人包皮線維芽細胞由来の系統細胞を使用し，脱殻ならびに培養法は，培養液の処方を多少変えたほかは，Current and Haynes (1984)の方法に従った．*Cryptosporidium*は，ＡＩＤＳ患者から由来し，山羊ならびに仔牛に継代されてきたものである．培養の結果，メロント，メロゾイト，マクロガメート，オーシストなどの形成を観察した．