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NOTES

Experimental Infection in Mice with *Treponema hyodysenteriae*

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Nineteen of 22 female mice (CF1 strain) inoculated intragastrically with *Treponema hyodysenteriae* developed cecal and colonic lesions consisting of catarrhal inflammation, edema, and occasional hemorrhage.

Attempts to induce lesions in mice and rabbits with pathogenic *Treponema hyodysenteriae* have failed (6; C. S. Phin, M.V.M. thesis, University of Glasgow, Bearsden, Glasgow, Scotland). Guinea pigs (GP) inoculated intragastrically with isolate B234 of *T. hyodysenteriae* developed colonic lesions similar to those of swine dysentery (SD) (7). However, to induce lesions in GP, high numbers of *T. hyodysenteriae* were needed as well as a special diet and a 72-h fasting period. The CF1-strain mouse has been shown to be susceptible to enteric infection with *Salmonella* (5). This host is inexpensive, easy to house, and readily available from a number of commercial companies. Because of these features, a study was conducted to evaluate the mouse as an alternate host for SD.

Forty-five specific pathogen-free female mice (CF1 strain), 30 to 38 days old, were obtained from Charles River Laboratories, Portage, Mich. Mice were housed in disposable plastic cages (five per cage). The bedding (wood shavings) was not changed after inoculation of the mice. Mice were fed a commercial mouse pellet ration, ad lib., during the 30-day experimental period, except during the fasting period.

Beta-hemolytic isolate B234 of *T. hyodysenteriae*, pathogenic for pigs, was supplied by Joann M. Kinyon, Iowa State University, Ames (10). After one passage through mice, isolate B234 was isolated from an infected mouse cecum, and the isolate was referred to as T8. Isolate T8 of *T. hyodysenteriae* was pathogenic. Weakly beta-hemolytic isolates B359 and B1555a of *T. innocens* (9), nonpathogenic for pigs, were supplied by Joann M. Kinyon, Iowa State University (10).

T. hyodysenteriae isolates B234 and T8 and *T. innocens* isolates B359 and B1555a passaged less than 18 times in vitro were cultured in Trypticase soy broth (8) as described by Joens et al. (7).

Mice were inoculated intragastrically with 1 ml of isolate B234, T8, B359, or B1555a for 2 consecutive days after a 72-h fasting period. The average number of viable cells was 5.2×10^7, 1.2×10^8, 1.2×10^8, or 1×10^7, respectively. Control mice received 1 ml of Trypticase soy broth. Inoculation of mice was accomplished via a 5-ml syringe with an 18-gauge needle and plastic catheter tubing (reorder no. 6179; Becton, Dickinson & Co.). Two hours after the second inoculation, mice were returned to an ad lib. ration.

T. hyodysenteriae and *T. innocens* were detected in fecal samples by streaking plates containing Trypticase soy agar, 5% citrated bovine blood, and 400 μg of spectinomycin per ml and incubating the plates at 42°C under an atmosphere of 50% H_2 and 50% CO_2. Plates were examined three times at 2-day intervals for the presence of beta-hemolytic or weak beta-hemolytic zones (11). At necropsy, the cecum and small intestine of each mouse were opened aseptically and examined for *Salmonella* as described (7).

Mice were killed during the study when either *T. hyodysenteriae* or *T. innocens* was detected in feces by both phase-contrast microscopy and culture, or at 30 days. Tissue specimens from cecum, colon, small intestine, and liver were fixed in 10% phosphate-buffered Formalin. Tissues were embedded, sectioned, stained (7), and examined by light microscopy for lesions and the presence of *T. hyodysenteriae*.

In a preliminary experiment, five mice fasted for 72 h and inoculated intragastrically with only

TABLE 1. *Results of intragastric inoculation of mice*

Inoculum	No. of mice	Data			
		Clinical[a]		Necropsy[b]	
		Mucoid feces	Shedding[c]	Colonic lesions	*T. hyodysenteriae*
Isolate B234	18	11	18	15	17
Isolate T8	4	4	4	4	4
Isolate B359[d]	5	0	0	0	NA[e]
Isolate B1555a[d]	5	0	0	0	NA
Trypticase soy broth[f]	8	0	0	0	0

[a] Number of mice showing signs of infection.

[b] Number of mice with lesions and *T. hyodysenteriae* in the crypts.

[c] Number of mice from which *T. hyodysenteriae* or *T. innocens* was detected by direct plating of fecal material on Trypticase soy agar–5% citrated bovine blood–400 μg of spectinomycin per ml.

[d] *T. innocens* isolates.

[e] NA, Not applicable.

[f] Control group.

one dose of isolate B234 (approximately 1.5×10^7 cells) did not shed *T. hyodysenteriae* or develop colonic lesions. Subsequent mouse exposure to *T. hyodysenteriae* by intragastric inoculation consisted of one dose on 2 consecutive days.

Of 22 mice inoculated twice with *T. hyodysenteriae*, 15 developed mucoid feces. *T. hyodysenteriae* was detected by phase-contrast microscopy in fecal samples from 21 of 22 mice before necropsy. Shedding of *T. hyodysenteriae* was detected by culture of feces from 17 of 22 mice at 2 to 7 days postinoculation and from feces of all 22 infected mice before necropsy (Table 1).

At necropsy, hyperemia of areas of the cecum and colon was detected in 19 of 22 infected mice.

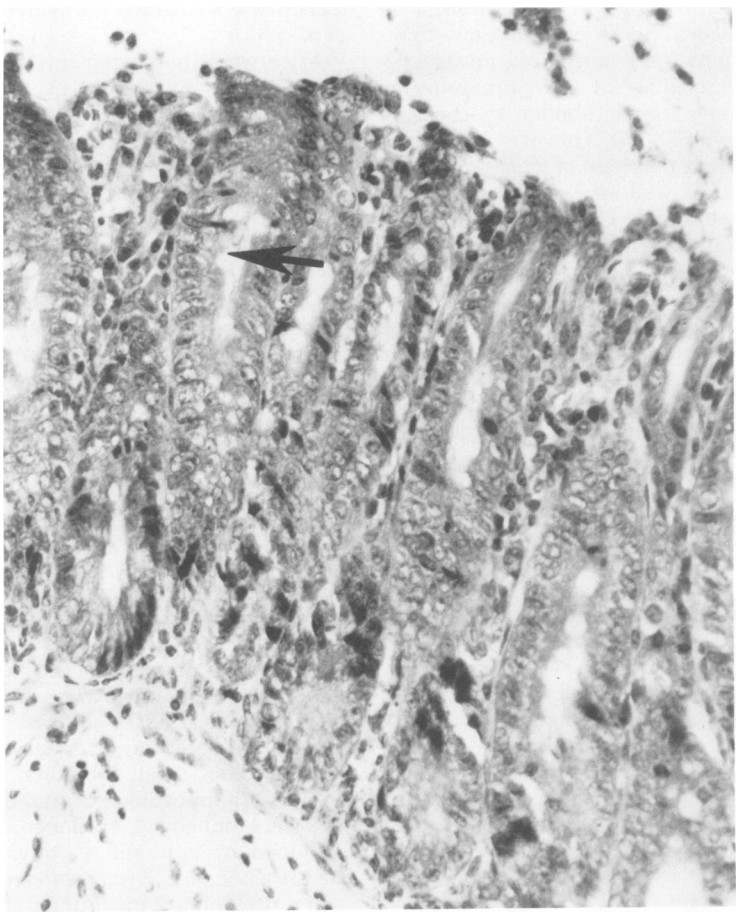

FIG. 1. *Swollen cecal mucosa with some crypt dilatation (arrow) and erosion of epithelium lining the cecal lumen (top). Hematoxylin and eosin stain, ×300.*

Catarrhal inflammation of the mucosal surface was noted in the ceca of 19 mice. Two of the mice inoculated with isolate B234 and all four inoculated with isolate T8 had ceca with areas of petechial hemorrhage.

Microscopic examination of cecal and colonic tissues of infected mice revealed mucosal edema, hyperemia, crypt dilatation, and some epithelial erosion (Fig. 1). *T. hyodysenteriae* was present in the crypts of 21 of 22 infected mice as determined by histological examination (Fig. 2).

T. innocens was not detected by culture from mouse cecal tissue at necropsy. Lesions were not noted in mice inoculated with *T. innocens*. All eight control mice remained normal throughout the study (Table 1). Tissues collected at necropsy were negative for *Salmonella* sp.

The mice used in this study appeared to be very susceptible to infection after two intragastric inoculations with *T. hyodysenteriae* isolate B234 or T8. Mice receiving only one oral dose did not become infected. The minimum infective dose may have been achieved only after mice were inoculated for 2 consecutive days. The failure of previous workers (6; Phin, M.V.M. thesis) to induce lesions in mice similar to those of SD may have resulted from the use of different mouse strains, the use of different routes of inoculation (6), or the use of only a single inoculation.

Clinical signs of infection in mice were evident at 2 to 14 days postinoculation, which is equivalent to the incubation period of SD. Mucus but no blood was observed in the feces of infected mice; however, petechial hemorrhages were noted on the mucosal surfaces of the ceca of some mice at necropsy. This characteristic was also observed by Joens et al. (7) in GP infected with *T. hyodysenteriae*. The gross colonic lesions observed at necropsy were similar to those observed in swine with SD (1, 3, 4) and in experimentally infected GP (7). Microscopic lesions of the large intestine also resembled those of swine with acute SD (1-3) and of GP (7) infected with *T. hyodysenteriae*.

The relative ease with which mice were in-

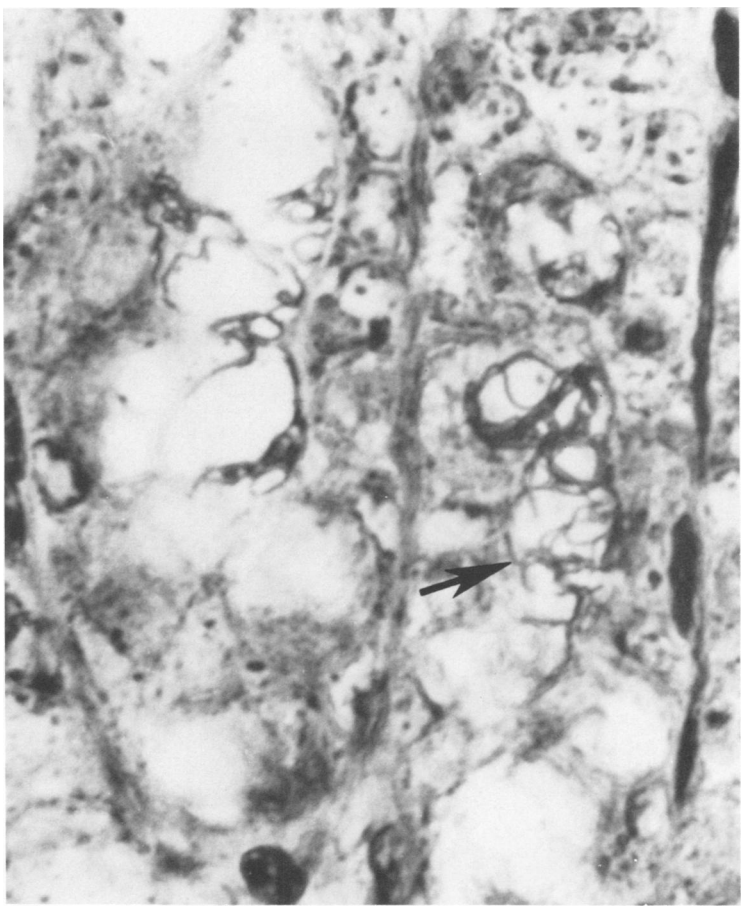

FIG. 2. *Cecal crypts containing numerous T. hyodysenteriae (arrow). Warthin-Starry stain, ×600.*

fected with *T. hyodysenteriae* suggests that studies with mice may provide a tool with which to study various aspects of the infectious process and so contribute to our understanding of SD.

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