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Incidence of *Clostridium perfringens* in the Livers of Conventional and Gnotobiotic Mice[1]

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Clostridium perfringens type A has been found in 12% of livers examined from newly slaughtered bovine animals (Canada and Strong, J. Food Sci. **29**:862, 1964). The organism has also been recovered from the livers of apparently healthy dogs (Wolbach and Saiki, J. Med. Res. **21**:267, 1909; Berg et al., Proc. Soc. Exptl. Biol. Med. **24**:433, 1926; Schweinburg and Sylvester, Proc. Soc. Exptl. Biol. Med. **82**:527, 1953). Schatten (Surgery **36**:256, 1954) presented evidence that, in dogs, *C. perfringens* may pass from the gastrointestinal tract to the liver via the portal vein.

The incidence of *C. perfringens* in excised liver tissue and in feces from four types of mice was determined: conventional, conventional (*C. perfringens*, force-fed), gnotobiotic (*C. perfringens* monoflora), and germ-free. The strain of mice used was Ha/ICR (Schmidt).

The 14 conventional mice were quartered in a laboratory animal room and fed (ad lib) a standard commercial diet. Fecal droppings on the fifth day from all animals were pooled and examined for *C. perfringens* in the following manner. A 1-g fecal sample was added to 99 ml of sterile 0.1% peptone water. Fractions of this dilution and further dilutions in 0.1% peptone water were plated in SPS agar (Angelotti et al., Appl. Microbiol. **10**:193, 1962). Colonies appearing on the medium, which resembled *C. perfringens*, were transferred to identification media. A portion of liver from each mouse was also examined for *C. perfringens*.

The second lot of conventional mice were maintained under conditions identical to those described above, except that, on the first day of arrival in the laboratory, each mouse was force-fed ca. 0.2 ml of an actively growing, *C. perfringens* thioglycolate culture. Each of three type A strains of the organism was fed to five mice. On the fifth day, pooled fecal droppings and a portion of liver from each mouse were cultured for *C. perfringens*.

Thirty-four germ-free mice quartered in plastic film isolators were force-fed cultures of *C. perfringens*. When these gnotobiotic-monoflora mice were removed from the isolator, the length of time that they were associated with *C. perfringens* varied from 2 to 12 days. Seven germ-free mice were also included in the study as a control measure. Fecal populations were determined, and a portion of liver from each mouse was cultured for *C. perfringens*.

Portions of liver from the 14 conventional mice, the 15 conventional mice which were force-fed *C. perfringens*, the 34 gnotobiotic-monoflora mice, and the 7 germ-free mice were examined for *C. perfringens* in the following manner. The mouse was anaesthetized with ether, and the body surface was thoroughly swabbed with 70% ethanol. Sterile implements were used to make an incision on the underside, exposing the peritoneal cavity. A portion of liver was removed from the living animal and added to tubed thioglycolate medium. After 48 hr of incubation at 37 C, any resulting growth in the liquid medium was subjected to confirmatory tests for *C. perfringens*.

The recorded fecal population of *C. perfringens* and the observed incidence of this organism in the liver of the four types of mice are presented in Table 1. The data indicate that *C. perfringens* is inherently associated with the intestinal tract and liver of the conventional mouse. The magnitude of the *C. perfringens* fecal population was increased after force-feeding of the organism to conventional mice. The strains of *C. perfringens* type A tested were capable of passing through the alimentary tract of the living, healthy gnotobiotic mouse to its liver. This transfer was accomplished in 48 hr. It seems most unlikely that the cultures of *C. perfringens* which developed from the excised liver originated from environmental contamination, since identical techniques were used to obtain portions of liver from both the gnotobiotic-monoflora (*C. perfringens*) and the germ-free mice. In every case, *C. perfringens* was isolated

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TABLE 1. *Fecal population and incidence in the liver of Clostridium perfringens in mice*

Type of mouse	No. of mice	Avg fecal population	Incidence in liver
		cells/g	%
Conventional	14	2.75×10^4	100
Conventional (*C. perfringens*, force-fed)	15	1.75×10^5	73
Gnotobiotic (*C. perfringens* monoflora)	34	2.99×10^7	100
Germ-free	7	0	0

from the livers of monoflora mice, and no microbial growth was demonstrated in medium inoculated with portions of liver from the germ-free mice. Likewise, it seems doubtful that the liver became contaminated from postmortem invasion by intestinal bacteria, since the mice were living at the time the liver was excised. The removal of the liver required less than 2 min.

Although it is quite convincing that, with gnotobiotic-monoflora mice, bacteria from the intestinal contents may contaminate the liver, these findings may or may not be applicable with conventional mammals. The intestinal wall of the germ-free mouse is very thin and enlarged. Thus, it may not provide as good a physical barrier to bacteria as the intestinal wall of conventional animals. Other defense mechanisms of the germ-free mouse are also poorly developed.

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