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Experimental models *in vivo* for *Campylobacter pylori*

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Contents.

Étude *in vivo* de *Campylobacter pylori*. Modèles expérimentaux.

Table des matières.

INTRODUCTION

Campylobacter pylori (C. pylori) gastritis has been induced in human volunteers, thus fulfilling Koch's postulates for gastritis. These observations have renewed interest in the gastric flora of other animal species, particularly in view of obtaining a convenient animal model for human gastritis and peptic ulcer disease.

Because of its unique location within the poorly understood gastric mucus layer, conventional antibacterial therapy has not been successful at eradicating *C. pylori*. If a new generation of agents is developed to attack *C. pylori*, animal models will be necessary to easily screen new compounds for efficacy. Animal models will also allow convenient study of pathogenetic mechanisms relating *C. pylori* to peptic ulcer disease.

ACCIDENTAL *C. PYLORI* INFECTION IN MAN

The most famous epidemic of *C. pylori* occurred in Texas when 17 out of 37 volunteers undergoing acid secretion studies developed hypochlorhydria and gastritis (17). Several volunteers were still hypochlorhydric after one year. Apart from epigastric symptoms during the first week of the illness, the disease was asymptomatic. It is believed that *C. pylori* was transmitted to these individuals via a wet *p*H electrode. Wiersinga and Tytgat (20) reported a patient with Zollinger-Ellison (ZE) syndrome who developed gastritis and hypochlorhydria and in whom *C. pylori* was detected after the gastritis developed. In this patient, acid secretion studies as well as endoscopy were also performed immediately before the infection, so again the infection may have been contracted from the *p*H electrode, or from the endoscope. In this patient, hypochlorhydria was beneficial in that it allowed remission of his ZE syndrome. Both *C. pylori* and gastritis persisted for 10 years but recently acid secretion returned and the patient again became symptomatic (G. N. Tytgat, personal communication).

In the accidental *C. pylori* infections, prolonged intubations and acid neutralization may have been common factors. As there was no source for a large inoculum in the Dallas epidemic, we can conclude that in susceptible individuals small numbers of *C. pylori* are sufficient to infect the gastric mucosa.

DELIBERATE *C. PYLORI* INFECTION IN MAN

Two deliberate self-infection experiments have been reported. In 1984, Marshall infected himself with 10^9 freshly isolated organisms after premedication with

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cimetidine (800 mg) (13). A mild upper gastrointestinal illness developed which peaked on day 8 and thereafter disappeared. *C. pylori* infection and active gastritis were documented on day 10. Symptoms included gnawing, bloating, nausea, achlorhydric vomiting and halitosis. Surprisingly, *C. pylori* had disappeared by day 14 and histology had partially returned to normal. This experiment suggested that not all *C. pylori* would lead to permanent chronic gastritis.

In 1986, Morris repeated the self ingestion experiment and developed severe colicky pain three days after ingestion (16). *C. pylori* and gastritis were noted on day 5, and hypochlorhydria on day 8. Notably, once hypochlorhydria had developed, *C. pylori* was often isolated from the gastric aspirate. After antibiotic therapy, acid secretion apparently returned to normal. Morris reports continuing chronic gastritis with *C. pylori* infection (15) but he is presently asymptomatic (October 1988).

GASTRIC SPIRAL BACTERIA IN ANIMALS

Spiral bacteria inhabit the gastric mucosa of many animals and have been studied since before the turn of the century. These organisms are presumably the cause of gastric urease which was first studied in detail by Luck and Seth (12).

Unlike *C. pylori,* gastric spiral bacteria (GSB) of animals are commensals. They are summarized in table I.

Commensal GSB's characteristically infect the gastric body, are often found within the parietal cell canaliculi, and do not cause gastritis. They are acid tolerant, protected by their urease enzyme which splits urea and generates ammonia. In the monkey and perhaps also in the cat, several types may be present in the one animal. The presence of GSB's does not necessarily prevent colonization by *C. pylori* (4).

TABLE I. — *Gastric spiral bacteria.*

Cats	Urease positive, two types: 1. Spiral, 6 μm, 6 wavelengths, multiple terminal sheathed flagella, no axial filaments, has not been cultured. 2. Similar, double axial filament, has been cultured by Lee *et al.* (10). The type 1 organism is present in most cats.
Dogs	Similar or identical to the bacteria present in cats. Type 2 cat organism may be the prevalent organism in dogs, it has been described by Lockart and Boler (11).
Macaques	Mixed infections with GSB and *C. pylori* occur (18). The GSB is similar to the type 1 cat organism. It has sheathed terminal flagella, is urease positive, and may have an axial filament (5). It has not been cultured.
Baboon	Similar to the GSB of cats and dogs but has an outer sheath around its coils (3).
Ferret	This « *C. pylori*-like organism » is unlike the GSB described above. It has been isolated by Fox (6). Similar to *C. pylori* but smaller. Has been named « *C. pylori ssp. mustelae* ». Association with gastritis is inconsistent. Can be isolated using *C. pylori* techniques. Urease, catalase and oxidase positive, has sheathed flagella which arise from the sides and ends of the organism. Has no axial filaments. It is not present in all ferrets (19).

NATURALLY OCCURRING *C. PYLORI* INFECTION IN ANIMALS

Baskerville and Newell (1) reported *C. pylori* associated histological gastritis in monkeys which was identical to that found in humans. The lesion was most common in *Macacus mulatta* (Rhesus monkey), but was also present in *Macacus fascicularis* (*cynomologous* monkey). Bronsdon and Schoenknecht (2) have found *C. pylori* in a colony of Macacus nemestrina (pigtailed Macaque), also in association with typical active chronic gastritis.

Wherever *C. pylori* has been found in animals, it has been associated with gastritis. Unlike the commensal GSB's, *C. pylori* prefers the gastric antrum and does not heavily infect the gastric body mucosa or the parietal cells.

EXPERIMENTAL *C. PYLORI* INFECTION IN ANIMALS

Attempts to infect small laboratory animals with *C. pylori* have been unsuccessful so far. Rats and nude mice were unable to be colonized by *C. pylori* orally or by injection in experiments reported by Ehlers *et al.* (4). Nevertheless, because of the convenience of a small animal model, further attempts in such animals would certainly be worthwhile.

Experimental infection of germ-free pigs has been achieved by Lambert *et al.* (9) and by Krakowka *et al.* (8). In both cases, chronic gastritis developed without a severe clinical syndrome being evident. The gnotobiotic model of these investigators is expensive and, because of the pig's ultimate size, it may be an inconvenient animal for long-term studies. Advances have now been made with the technique, and Gustavsson *et al.* (7) have been able to infect 7 week-old barrier-reared pigs.

Morgan *et al.* have utilised the gnotobiotic pig model in studies of *C. pylori* virulence (14). In a limited experiment, a non toxigenic isolate of *C. pylori* appeared to be less virulent than a toxigenic strain. In a litter of pigs given the former, gastritis with ultrastructural epithelial cell changes were seen (vacuolation), whereas the non toxigenic strain did not infect a second litter of pigs (although an antibody response was detected).

CONCLUSIONS

There is still a great demand for a convenient, cheap, and easily reproductible animal model of *C. pylori* gastritis. The present controversy surrounding *C. pylori* exists because many asymptomatic persons have *C. pylori* and the factors which convert a patient from the asymptomatic state to the symptomatic state are not known. It is likely that both host factors (blood group, secretor status, cell-mediated and humoral immunity) and factors within the infectious agent (attachment, toxin production, antibiotic resistance) are equally important.

C. pylori is very common in economically depressed and

less developed countries where it may infect the majority of children and adults. It is unlikely that effective therapy will be cheap and safe enough to eradicate the bacterium from these populations. So public health measures and a vaccine will ultimately be required. The development and testing of these measures would be most conveniently performed in an animal model.

Although successful animal models now exist in the pig and the monkey, these are not easily copied by most investigators. Attempts to infect small rodents, ferrets, cats, dogs and other laboratory animals should therefore continue.

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