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The 1977 report by my colleagues and me [1], published in The Journal of Infectious Diseases, was the initial report of Clostridium difficile as the etiologic agent of antibiotic-associated pseudomembranous colitis (PMC). The present review includes background to the report, a brief summary of studies that were included in the report, and a postscript on highlights of subsequent developments.

Retrospective review, much of which became apparent after publication of the 1977 report, included 3 different lines of relevant investigation: studies dealing with the anatomy and clinical features of PMC, studies of C. difficile, and studies of antibiotic-associated colitis in rodent models. The first report of a pseudomembranous lesion in the intestine was published in 1893 by Finney [2]. It concerned a 22-year-old patient of William Osler who underwent gastric surgery and postoperatively developed severe diarrhea that subsequently proved to be lethal. The autopsy showed “diphtheritic membranes” in the small bowel (the pathologic sections are still in the Pathology Department at Johns Hopkins Hospital, along with the handwritten clinical notes of William Osler). PMC was a rare condition until the introduction of antibiotics, when it became a relatively common adverse effect of treatment with tetracycline and chloramphenicol. Staphylococcus aureus was the major nosocomial pathogen and was implicated because of its high frequency of recovery in stool [3].

At that time, every textbook of medicine had a section devoted to S. aureus enterocolitis, and no one challenged its validity. On the basis of this assumption, oral vancomycin became the standard treatment and seemed to work well [4]. The first study to seriously question the etiologic role of S. aureus in antibiotic-associated colitis was the report by Tedesco et al. in 1974 [5]. Tedesco, a gastroenterologist at Barnes Hospital, was struck by the large number of patients with antibiotic-associated colitis as an adverse effect of treatment with clindamycin, which had become a favored drug for treatment of anaerobic infections during the “anaerobic bandwagon.” The Barnes investigators then performed a prospective evaluation of 200 patients treated with clindamycin; 41 (21%) developed diarrhea, and 20 (10%) had PMC detected by use of endoscopy. Theirs was the first report in which endoscopy had been routinely performed in patients with antibiotic-associated diarrhea, and the observation of a 10% frequency of PMC shocked the medical community, since this was viewed as a potentially lethal adverse effect of treatment with antibiotics. One of the important observations was the absence of S. aureus, despite the relative ease of growing this organism in selective cultures.

The second series of contributing experiments concerned the rodent model of antibiotic-associated colitis. Hambra et al. [6] attempted to show the potential value of penicillin in the treatment of gas gangrene in a guinea pig model, in an effort to treat this infection after battle injuries in World War II. However, treatment with penicillin proved to be more lethal than gas gangrene in the guinea pig model; necropsy studies showed large ceca filled with hemorrhagic fluid. Subsequent work showed that guinea pigs were susceptible to a multitude of antibiotics that also proved to be lethal to hamsters. The reason for penicillin-associated deaths in these rodents remained a mystery in veterinary medicine but,
fortunately, did not deter the subsequent development of penicillin for humans. There were many attempts to define the mechanism, but a particularly important one was reported in 1974 by Green [7], who noted that stools and tissues of infected animals showed cytotoxic changes in tissue-cultured cells, suggesting that a viral infection was present, although no virus could be propagated. They concluded that antibiotic-associated cecitis in rodents was due to a latent virus.

The third line of relevant investigations concerned studies of *C. difficile*. This organism was first recognized as a component of the normal intestinal flora of newborn infants by Hall and O'Toole in 1935 [8]. They noted that the organism, which was known at the time as “*Bacillus difficilis,*” because of its fastidious growth requirements, produced a toxin in vitro that was highly lethal when injected intraperitoneally in mice. Potency was impressive; in the mouse lethality test, it was just 1–2 logs lower than botulinum toxin [9]. Nevertheless, there did not appear to be any clinical consequences in humans, because its presence in stool or even at infected sites was not associated with any defined histotoxic clostridial syndrome. The conclusion was that either the organism did not produce the toxin in vivo or humans were not susceptible. One of the most comprehensive reports of *C. difficile* was written by Hafiz at the University of Leeds, who completed his PhD thesis on it [10]. His report showed that the organism was a common component of the stool flora in many different animals, including camels, that it was widespread in the environment, and that most strains were toxogenic.

This review of data along these 3 lines of investigation shows that all 3 culminated in what might be viewed retrospectively as seminal reports in 1974; at the time, there was no way of knowing that the organism so comprehensively studied by Hafiz [10] produced the cytotoxin discovered by Green [7] that caused the complication so elegantly described in the colonoscopy studies of Tedesco et al. [5].

My own interest in this topic was based on my fellowship experience with Sydney Finegold, who fostered general interest in anaerobic infections. One of my projects was the first large prospective study of lincomycin for treatment of anaerobic infections [11], a clinical trial that Upjohn refused to support because the physicians they queried in Kalamazoo, Michigan, had never heard of anaerobic infections. Despite extensive use of lincomycin and, then, clindamycin, I had not witnessed the devastating complication of “clindamycin colitis” reported in 10% of all clindamycin recipients by Tedesco et al. [5]. In retrospect, this phenomenon is probably best explained by an epidemic of *C. difficile* at Barnes Hospital, but the epidemic was certainly not recognized at the time. Sherwood Gorbach encouraged me to pursue this as a new line of investigation using the animal model, with the goal of identifying an enteric pathogen in the model without the tedious process of culturing the stool. The 1977 publication in *The Journal of Infectious Diseases* was the result of those experiments.

Syrian hamsters were challenged with clindamycin and, predictably, developed florid cecitis that proved to be lethal, usually within 3–5 days. Cecal contents were aspirated and inoculated intracereally into a hamster that had not been treated with clindamycin. The procedure was repeated for 5 serial passages. Controls were hamsters that received intracecal injections from cecal contents of healthy hamsters. After the second passage, the cecal contents from 10 hamsters were pooled and centrifuged at 15,000 g for 30 min, the supernatant was removed, and aliquots were filtered with a 0.45-μm filter, a 0.02-μm filter, and a PM-10 membrane filter. Those experiments showed that the cecal contents from clindamycin-treated hamsters produced typical cecitis with 5 serial passages. All hamsters died within 3 days. The transferable agent passed through the 0.02-μm filter but was retained by the PM-10 membrane filter, indicating that the toxin was filterable.

The next series of experiments included efforts to neutralize the toxin. Multiple trials were performed, but the one that worked was performed with gas gangrene antitoxin. On the basis of this information, stool was cultured, using heat shock to select for clostridia. Several clostridia were recovered and grown in broth culture, and then the supernatant was used for intracecal injection. One strain reproduced this disease. The experiment was reproducible with intraceal challenge in 16 of 16 recipients, and neutralization was achieved with gas gangrene antitoxin. The implicated *Clostridium* species was then cultured from each of 5 hamsters with clindamycin-induced disease and was recovered in concentrated 10^6–10^8 CFU/g of dry weight.

The identity of this *Clostridium* species was unclear, and it was consequently named in the report by the appellation that it had acquired in the laboratory: *Clostridium BVA* 17 HF1-9. “BVA” represented the Boston VA Hospital where the work was done, “17 HF” indicated the experiment in this series of studies in which each series of experiments was given “H” for hamster and a letter until the alphabet was complete, then going to the second numerical designation. Thus, “17F” indicated that this was experiment number 442, and “1-9” indicated that this *Clostridium* species was the ninth cecal isolate tested in the first hamster of that series.

The conclusion from this series of experiments was that antibiotic-associated colitis was caused by a toxin produced by *Clostridium BVBA* 17 HF1-9. Andy Onderbonk was pretty sure it was *C. difficile*, but an attempt to confirm this in Sydney Finegold’s lab was frustrated by contamination, and we decided that we needed to go to press, because Bob Fekety and colleagues, our competition in these studies, was believed to be not far behind.

Once the cause of antibiotic-associated enterocolitis was defined in the rodent model, it was critical to link this to clinical
observations by identifying the same agent in cases of antibiotic-associated colitis in humans. There were 2 facets to the immediate work in this area that are highly relevant. The first is the work of T. W. Chang, a collaborator in the Infectious Disease Division at Tufts University. He was convinced that the etiology of antibiotic-associated colitis was viral, had pursued tissue-culture assays, and had found a cytopathic effect with the highly characteristic actinomorphic changes in all fibroblast cell lines, as previously reported by Green [7]. However, when no virus could be propagated, Chang showed that the cytotoxin could be neutralized by gas gangrene antitoxin, which fit well with our observations in the hamster model. This antitoxin is composed of antibodies to the 5 clostridia toxins implicated in gas gangrene, so the next step was to define which of the 5 clostridia toxins implicated in gas gangrene was responsible for neutralization. The results of that experiment showed that it was C. sordelli antitoxin. However, C. sordelli could not be cultivated from the hamster, and BVA 17 HF1-9 produced a toxin in vitro that was neutralized by this antitoxin, indicating that antigenic cross-reaction had occurred. The next experiment was the use of this diagnostic test on stool specimens from patients with antibiotic-associated colitis, and the report of 4 such cases was published in 1978 [12].

With the detection of a new microbe in human disease, the usual cascade of subsequent studies includes the development of a diagnostic test for recognition, use of this test to define the clinical spectrum, investigations of pathophysiology, and attempts at treatment and prevention. This work was done in 1978 to 1980 and included the initial report of 2 toxins produced by C. difficile, now designated toxin A and toxin B [13]. Studies of the carriage rate [14], the implications of multiple antibiotics causing this complication [15], the serologic response to toxin A and B [16], the definition of the clinical spectrum [17], the definition of the cytotoxic assay that became the routine test for that period [18], and the first report of the enzyme immunoassay [19] followed.

In 1980, it appeared to me that most of the important work in this area had been done and that it was time to move on. Nevertheless, multiple other investigators have subsequently substantially advanced this field. Bob Fekety and his colleagues at the University of Michigan made important contributions in the early years doing work that, in retrospect, was very similar to the work from our laboratory in Boston. Recent work by multiple groups has provided a better definition of the pathophysiology of PMC, has shown the importance of C. difficile as a nosocomial pathogen, and has improved diagnostic testing with alternatives to the cumbersome cytotoxic assay (although no test has matched tissue culture for sensitivity). Metronidazole has become the preferred antibiotic for treatment of C. difficile infection, and multiple relapses are now recognized as one of the major challenges to treatment. Vaccines are now being explored as a method of prevention.

At the present time, C. difficile is recognized as one of the most important enteric bacterial pathogens in the developed world. Clinical expression is tightly linked to exposure to antibiotics, diagnostic testing is readily available and reasonably sensitive, and metronidazole therapy is generally highly effective and inexpensive. In retrospect, it is important to acknowledge that most of the important clinical observations were initially made in a hamster model, including detection of the etiologic agent, discovery of the implicated antibiotics, development of the diagnostic test, and treatment with oral vancomycin. This hamster model proved to be exceptionally valuable in providing insights into human disease.

References

Clindamycin-Associated Colitis Due to a Toxin-Producing Species of Clostridium in Hamsters

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Clindamycin-associated enterocolitis in hamsters was studied to detect and characterize a transmissible agent. It was found that the disease could be transferred by cecal contents and filtrates of cecal contents (pore size of filter, 0.92 μm) obtained from animals after administration of clindamycin. Subsequent work showed that enterocolitis could be produced with broth cultures of a species of Clostridium recovered from cecal contents of animals with clindamycin-induced disease. The cell-free supernatant of this strain also caused enterocolitis. Cecal contents from animals with clindamycin-induced disease incubated with gas gangrene antitoxin failed to cause intestinal lesions. These experiments indicate that clindamycin-associated colitis in hamsters is due to a clindamycin-resistant, toxin-producing strain of Clostridium.

Previous studies [1, 2] have shown that administration of lincomycin or clindamycin to hamsters almost invariably produces lethal enterocolitis. It is currently uncertain whether this represents a model of clindamycin-associated pseudomembranous colitis as it is encountered in clinical practice. Nevertheless, the disease in hamsters is being investigated widely in the hopes that the information obtained may prove applicable to patients. The experiments reported here were designed to detect a transferrable agent in clindamycin-associated enterocolitis in hamsters.

Materials and Methods

Syrian hamsters were challenged with various inocula to define the causative agent of clindamycin-associated enterocolitis in hamsters.

Method of challenge. Male Syrian hamsters (Charles River, Wilmington, Mass.) weighing 90–110 g and maintained on Agway Chow (Charles River) were used in all experiments. Animals were challenged by intracecal injection. The animals were anesthetized with sodium pentobarbital, a midline abdominal incision was made, and 1 ml volumes of various inocula were injected into the cecum using a no. 25 gauge needle. The abdominal incision was then closed with sutures, and the animals were returned to their cages.

Assessment of results. Animals were observed daily, and survivors were sacrificed six or seven days after intracecal challenge. The criterion for enterocolitis was a dilated and hemorrhagic cecum at autopsy. Histologic studies were performed on the ceca of animals that were sacrificed. Absence of disease was defined as a normal intestinal tract on gross inspection and no abnormality on histologic studies of the cecum.

Inocula. Serial passage experiments. Clindamycin-associated enterocolitis was produced by three daily 2 mg im doses of clindamycin phosphate (The Upjohn Company, Kalamazoo, Mich.). The animals were sacrificed when they appeared moribund, and cecal contents were aspirated with a no. 15 gauge needle. A 1 ml aliquot of cecal contents from each hamster was promptly inoculated intracecally into a second hamster that had not previously received clindamycin. The recipient animals were observed daily, and when they expired cecal contents were obtained for a second passage. This procedure was repeated for a total of five serial passages. Controls for these experiments were 10 hamsters that received intracecal injections of cecal contents from healthy hamsters.

The amount of clindamycin transferred in the serial passage experiments was determined by an
agar diffusion assay using a clindamycin-sensitive strain of Clostridium perfringens [3]. The intracecal dosage of clindamycin required to produce enterocolitis was estimated by intracecal injections of 500, 50, 5, or 0.5 µg of clindamycin phosphate into four groups of four animals.

**Broth cultures of cecal contents.** An aliquot of 0.05 ml of cecal contents from hamsters not given clindamycin was placed in 50 ml of brain-heart infusion (BHI) broth. Trinitated clindamycin phosphate with a specific activity of 3.61 µCi/µg was added to a final concentration of 50 µg/ml. After anaerobic incubation for 72 hr, the broth was dialyzed within the anaerobic chamber against 3 liters of phosphate-buffered saline with three changes over 24 hr. Hamsters were challenged intracally with 1-ml aliquots of the dialysate. An additional aliquot was assayed to determine the amount of labeled clindamycin contained in the transferred material. Controls for this experiment included hamster cecal contents treated in an identical fashion without addition of clindamycin.

**Filtrates of cecal contents from hamsters with enterocolitis.** Cecal contents were obtained from 10 hamsters with colitis following the second passage as previously described. This material was pooled and then centrifuged at 15,000 g for 30 min. The supernatant was removed, and aliquots were pressure-filtered through filters with pore sizes of 0.45 µm and 0.02 µm (Millipore Corp., Bedford, Mass.) and through a PM-10 membrane filter (Amicon Corp., Lexington, Mass). Inocula for intracecal injection in these experiments were 1-ml aliquots of each of the three filtrates.

**Organisms recovered from cecal contents of hamsters with enterocolitis.** Cecal contents were obtained for culture from moribund animals with enterocolitis following three daily i.m. injections of clindamycin. The specimens were diluted 10-fold with prereduced VS1 dilution salts [2] and mixed thoroughly on a vortex apparatus, and serial 10-fold dilutions were prepared. Volumes of 0.1 ml of the 10⁻¹ and 10⁻² dilutions were plated onto prereduced brucella-base blood agar and Clostridial media (Scott Laboratories, Fiskeville, R.I.) for incubation in the anaerobic chamber. The numerically dominant colony types were isolated and then inoculated into separate BHI broth solutions for incubation in the anaerobic chamber at 37 C for 72 hr. A volume of 10 ml of each broth was centrifuged, and the pellet was resuspended in 1 ml of prereduced BHI broth for intracecal inoculation.

**Cell-free supernant of Clostridium BVA 17 HF 1-9.** The previous experiment yielded one organism that produced enterocolitis. It was a clostridial species that could not be speciated according to current taxonomic schema [4] and will be subsequently referred to as Clostridium BVA 17 HF 1-9. A 72-hr broth culture of this organism was centrifuged at 15,000 g for 30 min, and the supernatant was filtered through a filter with a pore size of 0.45 µm. (Aerobic and anaerobic cultures of the filtrate showed no bacterial growth.) Hamsters were challenged intracally with 1-ml aliquots of the cell-free supernatant. Controls for this experiment included five hamsters inoculated with sterile BHI broth.

**Cecal contents of a hamster with colitis following incubation with gas gangrenous antitoxin.** Cecal contents were obtained from five moribund animals with enterocolitis following three daily i.m. injections of clindamycin. The cecal contents were pooled, and a volume of 3 ml was incubated with an equal volume of gas gangrenous polyvalent antitoxin (Lederle Laboratories, Pearl River, N.Y.) at 37 C for 1 hr in an anaerobic chamber. Hamsters were challenged intracally with 1-ml aliquots of the incubated mixture. Controls for this experiment were animals inoculated intracally with the same pooled cecal contents following incubation with an equal volume of equine serum instead of gas gangrenous antitoxin.

**Results**

**Passage experiments.** All five animals challenged i.m. with clindamycin appeared moribund on the third day and were sacrificed at that time. Cecal contents were aspirated and injected intracally into five previously healthy hamsters. There were four subsequent sequential passages with a combined total of 25 recipient animals (table 1). All recipients expired within three days and had enterocolitis at autopsy. Control animals, which received intracoral inoculations of cecal contents from healthy hamsters, survived and had
Table 1. Results of intracecal injections in hamsters to detect a transferable agent causing enterocolitis.

<table>
<thead>
<tr>
<th>Intracecal inocula</th>
<th>No. tested</th>
<th>% with enterocolitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serial passage of cecal contents from hamsters with clindamycin-associated colitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First passage</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Second passage</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Third passage</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Fourth passage</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Fifth passage</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Cecal contents from hamsters with clindamycin-associated colitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filtrate of 0.45-μm filter</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Filtrate of 0.02-μm filter</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Filtrate of PM-10 membrane filter</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Cecal contents from normal hamsters incubated with clindamycin and then dialyzed</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Pure cultures of bacteria recovered from cecal contents of hamsters with clindamycin-associated colitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>One strain of <em>Clostridium</em></td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>BVA 17 HF 1-9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other bacteria†</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Cell-free supernatant of <em>Clostridium</em></td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>BVA 17 HF 1-9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cecal contents from hamsters with enterocolitis after incubation with gas gangrene polyanal antigen</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

*Values indicate pore size.
†Five strains of *Clostridium*, two strains of anaerobic gram-negative bacilli, one strain of an anaerobic gram-positive coccus.

No gross or microscopic evidence of intestinal disease when sacrificed six days after inoculation.

Assays of transferred material showed decreasing levels of clindamycin in cecal contents with serial passages. Levels in animals sacrificed for the initial passage were 61–81 μg/ml; for the second passage they were 0.2–2 μg/ml, and for the third passage they were uniformly < 0.2 μg/ml, which is the lowest level detectable with our bioassay technique. Intracecal inoculations of clindamycin phosphate showed that induction of colitis was dose-dependent. With a 500-μg dose all four recipients acquired disease; the 50-μg dose produced lesions in two of four animals; the 5-μg dose caused colitis in just one animal; and all four recipients of the 0.5-μg dose survived and had no intestinal lesions at autopsy.

**Broth cultures of cecal contents.** The inocula in these experiments were cecal contents (from normal hamsters) grown in BHI broth containing clindamycin. After dialysis of the broth culture, there was no detectable labeled clindamycin. Intracecal administration of the dialysate produced enterocolitis in both of the two hamsters tested. Cecal contents treated in an identical fashion but without addition of clindamycin failed to cause detectable lesions in each of two control animals.

**Filtrates of cecal contents.** Filtrates of the supernatant of cecal contents that was pooled from hamsters with enterocolitis following the second passage were inoculated intracereally into three groups of two animals. Enterocolitis was produced in recipients of the 0.45-μm filtrate and recipients of the 0.02-μm filtrate. Animals challenged with supernatant filtered through a PM-10 membrane filter showed no intestinal lesions when sacrificed seven days after challenge.

**Inocula of bacteria in pure culture.** The predominant isolates from cecal contents of hamsters with colitis following intrinjection of clindamycin included two strains of anaerobic gram-negative bacilli, an anaerobic gram-positive coccus, and six strains of *Clostridium*. Each of these strains was inoculated intracereally into at least two animals in inocula of ~10⁸ cfu/ml. Only one strain of *Clostridium* (BVA 17 HF 1-9) produced enterocolitis, and this strain was subsequently retested in 14 additional hamsters. All 16 animals challenged with this organism developed enterocolitis. Intracecal injections of the cell-free supernatant of this organism also produced enterocolitis in each of seven recipients. Inocula of sterile BHI broth failed to produce detectable lesions.

The *Clostridium* species responsible for inducing enterocolitis was recovered from the cecal contents of each of five hamsters with clindamycin-induced disease in concentrations of 10⁶-10⁹ cfu/g of dry weight. Phenotypic characteristics of this isolate include fermentation of cellobiose, fructose, glucose, mannitol, mannose, melizitose, salicin, and trehalose; hydrolysis of gelatin and esculin; and production of acetic, butyric, and isovaleric acids from glucose fermentation.

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17 HF 1-9 is similar in many respects to *Clostridium difficile*. Antimicrobial susceptibility testing of this strain by the broth dilution technique [3] showed resistance to 128 µg of clindamycin/ml and susceptibility to 4 µg of vancomycin/ml. cecal contents of a hamster with colitis after incubation with gas gangrene antitoxin. This inoculum failed to produce disease in any of the five recipients. Three animals challenged with cecal contents from the same pooled batch of cecal contents, but with equine serum instead of gas gangrene antitoxin, developed enterocolitis.

**Discussion**

The hamster model used in these experiments was originally described by Small in 1968 [1]. Our previous studies of the model confirmed these earlier observations in that administration of clindamycin or lincomycin produced enterocolitis in 65 of 67 hamsters [2]. Autopsies showed extremely large distended and hemorrhagic ceca with involvement of the terminal ileum and ascending colon. Histologic studies revealed disruption of the mucosal epithelium, capillary congestion, and a polymorphonuclear infiltration of the lamina propria. In many sections there were luminal collections of inflammatory cells, mucus, and sloughed epithelial cells with a focus of attachment to the mucosal surface suggesting pseudomembrane formation. It was also noted that vancomycin treatment prevented clindamycin-associated enterocolitis in this model. Because of the limited spectrum of activity of vancomycin, we postulated that gram-positive bacteria played a role in the pathogenesis of this disease.

The present report represents an extension of these previous observations. It was shown initially that enterocolitis could be transferred with five sequential passages starting with cecal contents from clindamycin-treated hamsters. There was initial concern that the passage of the disease might result from clindamycin contained in cecal contents from the original animals. However, in vitro antibiotic assays showed that concentrations of biologically active antibiotic were reduced to levels below detectable limits after the second passage. Intracecal injections of clindamycin indicated that doses of 5 µg were required to produce enterocolitis; even at this dosage, only one of four animals developed detectable disease. Subsequent experiments involving the possible transfer of clindamycin employed either dialysis or cecal contents after second passage to ensure that there was an insufficient amount of antimicrobial agent to affect results.

In the next experiment it was shown that clindamycin could "induce" the responsible agent when incubated with cecal contents from normal hamsters. In addition, it was noted that the active fraction could be passed through filters with pore sizes of 0.13 µm and 0.02 µm. These filtrates would be expected to exclude bacteria and viruses larger than 20 nm. However, the PM-10 filtrate failed to produce disease, a result suggesting a toxin with a molecular weight of >10,000 daltons.

The next experiments concerned attempts to produce the disease using inocula of bacteria that were recovered from animals with enterocolitis. The results showed that enterocolitis with anatomical changes identical to those in clindamycin colitis could be reproduced with a clindamycin-resistant strain of *Clostridium*. This strain proved sensitive to vancomycin, a finding possibly explaining the protection afforded when this antimicrobial agent was given prior to challenge with clindamycin in previously reported studies [2]. The cell-free supernatant of this clostridial strain also produced enterocolitis, a result that provides a necessary link to explain transferability with a 0.15-µm filtrate of cecal contents from hamsters with enterocolitis.

The final experiment showed that gas gangrene antitoxin protected against enterocolitis when it was added to cecal contents of animals with the disease prior to transfer. It should be noted that this antitoxin is produced by injection of horses with the five species of *Clostridium* that cause gas gangrene. These five species produce a multiplicity of toxins so that the antitoxin preparation is active against numerous clostridial toxins in addition to those that cause myonecrosis [6].

Our conclusion from these experiments is that a clindamycin-resistant toxin-producing *Clostridium* species causes an enterocolitis in hamsters that is identical to the disease produced by clindamycin administration to these animals. It is uncertain at present whether this is the only organism...
that is responsible. Conceivably, other bacteria, particularly other strains of *Clostridium* that were not tested, could also produce this disease.

The implication of these observations as they relate to patients is uncertain since it is not known whether the hamster represents a model of clindamycin-associated colitis as it is encountered in clinical practice. Compared with humans, hamsters have anatomically distinctive ceca, a unique colonic flora, and a poor tolerance for several antimicrobial agents [7–9]. Nevertheless, it is of interest that similar shifts in the flora, including the emergence of clostridial species in high concentrations, have been noted with administration of clindamycin to both humans and hamsters [2, 10].

The histologic changes found in the hamsters are also similar to those noted with colonic biopsies from patients with this disease. Finally, a recent report by Larson et al. showed that stools from three patients with clindamycin-associated colitis were cytotoxic, and the authors postulated that a bacterial toxin was responsible [11].

The experiments reported here clarify the probable pathogenic mechanism of clindamycin-associated enterocolitis in hamsters. Further work will be required to know whether the hamster is a suitable model of human disease before these results can be applied to the clinical setting.

References