CLOSTRIDIUM PERFRINGENS FOOD POISONING[1]

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THE anaerobic bacteria are among the most versatile of the microbes. Probably the most versatile of the anaerobes, however, is *Clostridium perfringens.* This organism grows rapidly and ferments a variety of compounds. It produces many enzymes, including those that hydrolyze starch, collagen, hyaluronic acid, deoxyribonucleic acid, ribonucleic acid and blood group substances, as well as four different lethal toxins. It is responsible for a variety of diseases in man and animals, including enterotoxemia of sheep and goats, fatal infections of calves, lambs, and colts and necrotic enteritis in many animals as well as in man. In man, it is also responsible for most cases of gas gangrene, a rapidly fatal wound infection.

It also causes a very common but seldom fatal kind of poisoning. Most of us have probably suffered from it at one time or another. I, myself, have had it several times, at least once from food I prepared myself. It has undoubtedly been a nuisance and a minor menace and source of discomfort to man ever since he developed intelligence enough to try to save food from one day to the next.

For some reason, this particular disease has been singularly attractive to microbiologists. It was a microbiologist who described the disease and clearly pointed out its cause in 1945, microbiologists who worked out the details of the epidemiology, and microbiologists who finally uncovered the mechanisms of pathogenesis.

The organism that causes this disease, *Clostridium perfringens*, or *Clostridium welchii* as the British microbiologists term it, is a short, fat Gram positive rod. It is not motile, and forms spores very rarely, indeed, on the usual laboratory media. Although it is considered an anaerobe, it is just barely an anaerobe, for it can grow with only a little protection from the oxygen of the air.

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The first outbreak of perfringens food poisoning to be described may have been that of Klein (1895) who reported on an outbreak occurring in St. Bartholomews Hospital in London. This had the salient features of perfringens food poisoning — nausea but no vomiting, abdominal cramps, diarrhea, but no fever, but differed in that many cases had blood in the feces. The causative organism does not sound like *C. perfringens*, for Klein described it as a motile, spore-forming rod, and stained the flagella with Loeffler's flagella stain. *C. perfringens*, of course, is not motile and forms spores only rarely. Later, he reported that he was dealing with a mixture of organisms, one of which he called *Bacillus enteritidis sporogenes,* which possibly was *C. perfringens,* and another, *Bacillus cadaveris sporogenes,* a proteolytic, motile anaerobic rod with terminal, oval spores (possibly *Clostridium cadaveris* or *Clostridium putrificum).* Three more outbreaks in the wards of the same hospital were described by Andrews four years later. One of these he ascribed to rice pudding made with milk that contained *Bacillus enteritidis sporogenes,* the other to milk containing this organism. Whether his cultures were *Clostridium perfringens* is possible but open to question, for he described his organism as having oval, terminal spores, which *C. perfringens* does not have.

The views of Klein and Andrews were not generally accepted. Several investigators concluded that *C. perfringens,* had nothing to do with the outbreak from which Klein first isolated it, or those described by Andrews because, first, it was found in milk that could be consumed with impunity; second, it was found in considerable number in the stools of healthy humans, children as well as adults; third, there was no appreciable difference in virulence for laboratory animals between the strains isolated from normal and those isolated from diarrheal stools; and fourth, the ingestion of a considerable number of living *C. perfringens,* cells did not result in diarrhea.

The possibility of *C. perfringens* causing food poisoning was very largely ignored for fifty years after Klein's first description of the disease. In 1945, perfringens food poisoning was recognized by McClung at the University of Indiana. He described four outbreaks occurring in students, faculty, and others who ate at the University cafeteria. The food involved was, in each case, chicken that had been cooked the day before and that had stood overnight without refrigeration. On laboratory investigation, the food was found to contain very large numbers of *Clostridium perfringens.* The incubation period for most cases was 10 to 12 hours; nausea, diarrhea and sharp abdominal pain were common; much intestinal gas was produced; there was no vomiting, in spite of the nausea, and there was no fever. The discomfort lasted for about eight hours and recovery was complete within one day. This outbreak was typical in all respects. When McClung fed human volunteers food that had been neavily inoculated with *C. perfringens* the disease was reproduced without difficulty. He did not determine how many cells of *C. perfringens* a person

must ingest, however, to suffer an attack of food poisoning. Table I shows a comparison of perfringens, salmonella, and staphylococcus food poisoning. As can be seen, the incubation period for perfringens food poisoning is longer than that for staphylococcal food poisoning but shorter than that for salmonella. The most characteristic feature, however, is the absence of vomiting in spite of the marked nausea and abdominal pain.

Table I

COMPARISON OF DIFFERENT TYPES OF FOOD POISONING

	C. perfringens	*Salmonella*	*Staphylococcus*
Incubation	8-20 hrs.	12-24 hrs.	2-6 hrs.
Duration	12-24 hrs.	1-14 days	6-24 hrs.
Diarrhea	Extremely common	Very common	Common
Abdominal pain	Present	Present	Present
Vomiting	Very rare	Common	Very common
Fever	None	Very common	None

Seven years after McClung described this disease, Osterling reported on it from Sweden where he investigated 33 outbreaks of food poisoning. From 15 of these outbreaks, he isolated *C. perfringens,* inoculated the organism into the same type of food, and fed the inoculated food to human volunteers. Those who had eaten food inoculated with 7 of the strains developed typical perfringens food poisoning. Food inoculated with the other eight strains did not produce the disease. This was the first indication that some strains of *C. perfringens* could induce food poisoning in man and that others could not.

The next year — 1953 — Betty Hobbs and her colleagues in London reported on 18 outbreaks that had occurred there. Sixteen of the strains responsible had heat-resistant spores, withstanding one hour at the temperature of boiling water. In each outbreak, the food responsible was meat, or some dish containing meat. Other workers — Dische and Elek in London, and Hauschild and Thatcher in Canada — later showed that the ingestion of very large numbers of perfringens cells was necessary to induce food poisoning, about 10^9 cells, about one billion. Smaller doses produced very mild attacks or none at all. Filtrates of cultures were inactive; cells killed by heat were inactive; only 10^9 living cells of *C. perfringens* were effective.

This was an informative point. If a person is to eat 10^9 perfringens cells in one meal, the food that he is eating must contain 10^6 to 10^7 — one to ten million — cells per gram. When Hobbs and her colleagues examined food involved in two outbreaks, this is just what they found — one food contained 4.5 million *C. perfringens* per gram; the other contained 18 million per gram. Such food can be of very nearly normal taste and odor, surprisingly enough.

These findings raised much interest in *C. perfringens.* It had long been known as the most frequent cause of gas gangrene in war wounds, and as the cause of various diseases of domestic animals, but the discovery of *C. perfringens* food poisoning emphasized the need for more knowledge concerning this organism. Although *C. perfringens* is an anaerobe, it is just barely an anaerobe and will withstand considerable exposure to oxygen. It is highly active metabolically, fermenting most of the simple sugars, starch, glycogen, and several amino acids. It does not form spores in the usual laboratory media, but there are special media on which it does not form spores. There are five types of this organism, A, B, C, D, and E, determined by the toxins that they produce. Today, we are interested only in type A which is the type involved in perfringens food poisoning and which is by far the most widespread of all the types.

Some strains of type A perfringens form spores that are very resistant to heat; others do not. At one time, some microbiologists thought that only the strains forming heat-resistant spores caused perfringens food poisoning; now we know that either heat susceptible or heat-resistant strains may be involved.

C. perfringens has two principal habitats in nature, the soil and the intestinal tract of various animals and man. In the intestinal tract, it occurs in number from 100 to 100 million per gram; in soil, from 1000 to 50,000 per gram. Indeed, *C. perfringens* is more widely spread over the face of the earth than any other potential pathogen. Because it forms spores in the intestinal tract and in soil, it is resistant to drying and to exposure. It is not surprising, then, to find every dustcovered object contaminated with *C. perfringens.* Many items of food are also contaminated with it, but with usually fewer than 10 cells per gram.

It is obvious, then, that we do not, and cannot keep this organism out of our food without using inconvenient and excessive precautions. It is equally obvious that we can ingest thousands of *C. perfringens* cells without harm. We can ingest several million without harm. It is only when we ingest a hundred million or more that we have difficulty. The prevention of *C. perfringens* food poisoning consists, then of keeping the numbers of this organism in the food relatively low.

Because many items of our food will be contaminated with *C. perfringens,* a circumstance we cannot easily prevent, the real problem in preventing perfringens food poisoning is to inhibit, or slow the rate of growth of this organism in the food. The question then comes up — what factors govern the growth of this organism? First, let us consider the effect of temperature. Table II shows the generation time of *C. perfringens* growing at different temperatures. The lower the generation time, the more rapidly the culture is growing, because the generation time is time required for the cells to double in number. From these data, it is evident that temperatures from 40 to 50 C are most favorable for the growth of *C. perfringens,* but that temperatures as low as 20 may result in considerable growth if enough time is allowed.

Table II

THE EFFECT OF TEMPERATURE ON THE RATE OF GROWTH OF *CLOSTRIDIUM PERFRINGENS*

Temperature	*Generation time, pH 7.0*
25C	100 minutes
30	50
35	35
40	12.5
45	10
50	15*

* Variable.

Table III shows the effect of hydrogen ion concentration on the rapidity of growth. In this experiment, a temperature of 37 C was used. Growth is most rapid from pH 6 to 7, slightly on the acid side of neutrality.

Table III

EFFECT OF HYDROGEN ION CONCENTRATION ON THE RATE OF GROWTH OF *CLOSTRIDIUM PERFRINGENS*

pH	*Generation time, 37 C*
5.0	100 minutes
5.5	30
6.0	22
6.5	21
7.0	23
7.5	25
8.0	48

C. perfringens has rather complex growth requirements. Table IV shows the seven vitamins required by most strains. However, some strains do not require riboflavin; some strains do not require uracil. All of them do require adenine, biotin, nicotinamide, pyridoxal, and pantothenate. In addition to these vitamins, thirteen to fifteen amino acids are needed. Twenty-one to twenty-five organic compounds, then, are required by this organism.

The effect of salt is shown in Table V. It does not reduce the rate of growth if the concentration is lower than 3 per cent, so the amount of salt used for seasoning food would have no effect. Where salt is used for preservation at 10 to 20% or higher, however, it would be effective in inhibiting growth.

Table IV

GROWTH FACTOR REQUIREMENTS OF *CLOSTRIDIUM PERFRINGENS*

Biotin
Pantothenate
Pyridoxal
Adenine
Nicotinamide
(Riboflavin)
(Uracil)

Table V

THE EFFECT OF SODIUM CHLORIDE ON THE RATE OF GROWTH OF *CLOSTRIDIUM PERFRINGENS*

% NaCl	*Generation time pH 7.0, 45 C*
0	10 minutes
1	11
2	11
3	27
4	30
5	*

* Too long to be accurately measured.

These data indicate that *C. perfringens* grows best at neutrality or slightly below, in an environment of low salt concentration, at temperatures from 20 to 50 C, and also that it requires a plentiful array of amino acids and vitamins. Foods that have these characteristics would provide the principal hazard in outbreaks of food poisoning. Now, let us see how rapidly this organism grows in common foods.

Table VI shows the pH, the mean generation time, and the final count of *C. perfringens* growing in various meat and meat products. In each case, a 1% inoculum was used, and incubation was at 45 C for 1-1/2 hours. In beef liver, which had pH of 5.9, the mean generation time was 9.6 minutes, growing somewhat faster in this material than it did in bacteriological media. The count, at the end of the log period of growth, was 8.2×10^8, or 800,000,000 cells per gram. A single gram of this material would be enough to cause food poisoning in an adult human. The rate of growth in pork sausage was very nearly the same, and the final count was in the same range. The pork sausage differed from ground pork in having an appreciable amount of cereal and spices mixed with it. The

amount of growth in both beef and pork was lower than in the beef liver or the pork sausage, yet two grams would be enough to cause food poisoning. The data for growth in chicken and turkey was not obtained in our laboratory, but came from Mead's laboratory in England. As you can see, *C. perfringens* grew rapidly in these meats, also. From these generation times, it is obvious that a few hours under favorable conditions can markedly increase the extent of *C. perfringens* contamination. If, for example, food containing 10 perfringens spores per gram were kept under conditions where the mean generation time was 10 minutes, the number of perfringens cells would increase from 10 per gram to 2,600,000 per gram in three hours, and a wholesome food would be changed to one that was definitely hazardous.

Table VI
GROWTH OF *CLOSTRIDIUM PERFRINGENS* IN FOOD

Meat	*pH*	*MGT* [1]	*Viable count* × 10^8
Beef liver	5.9	9.6	8.3
Pork sausage	6.0	9.8	9.3
Pork	6.1	14	5.3
Beef	5.9	15	3.5
Chicken	6.5	11 [2]	
Turkey	6.4	10 [2]	

1 = Mean generation time in minutes.
2 = from Mead, 1969.

The growth of *C. perfringens* in fish showed some interesting differences. There was fairly rapid growth in cod (Table VII), although not as rapid as with meats, and a lower final count. The rate of growth in shrimp was lower than that of cod. But when we tried salmon, we found *C. perfringens* to be growing so rapidly that we could not be sure of the generation time, for we could not calculate it accurately. We didn't quite believe it, so we used another sample of salmon with very much the same results.

Table VII
GROWTH OF *CLOSTRIDIUM PERFRINGENS* IN FOOD

Fish	*pH*	*MGT* [1]	*Viable count* × 10^8
Cod	6.7	15	1.3
Shrimp	7.4	20	5.8
Salmon	6.7	(7.5)	4.9
Salmon	6.7	(7.2)	4.8

1 = Mean generation time in minutes.

In vegetables, growth was considerably slower (Table VIII). The dried beans and peas were soaked, and then autoclaved in water, as was the potato. Only corn, and this was corn that had been canned with milk, gave a short generation time and a high final count. Vegetables, by themselves, probably offer poor substrate for *C. perfringens.*

Table VIII

GROWTH OF *CLOSTRIDIUM PERFRINGENS* IN FOOD

Vegetable	*pH*	*MGT* [1]	*Viable count* × 10^8
Peas, dried	6.3	20	1.0
Beans, dried	6.0	30	0.15
Corn, canned	6.3	11	5.1
Potato	5.7	43	2.1

1 = Mean generation time in minutes.

The growth of *C. perfringens* in milk was also determined (Table IX). This shows the rate and extent of growth in canned milk and in whole milk. There was a considerable difference between the two samples of market milk, with a difference of more than one hundred per cent in mean generation time, but very much the same total count in the end.

Table IX

GROWTH OF *CLOSTRIDIUM PERFRINGENS* IN FOOD

Milk	*pH*	*MGT* [1]	*Viable count* × 10^8
Canned	6.0	12	2.8
Fresh	6.7	9.4	2.4
Fresh	6.3	20	1.7

1 = Mean generation time in minutes.

If we can reason from these laboratory findings to actual foods that people eat, we would say that meat and fish dishes can be a potential hazard, as could milk, but that vegetables probably would not be involved unless they had been prepared with meat or milk.

So much for the organism that causes this disease. Now, what has been the situation so far as actual outbreaks in man are concerned?

First, the public health authorities in the United States, at least, have been slow in recognizing that perfringens food poisoning exists. It was not considered a reportable disease in the United States until 1959, fifteen years after its description by McClung. Table X shows the increase in

the number of *reported* outbreaks and cases in the United States. The number of food poisoning outbreaks reported in the U.S. is usually about one-tenth of the actual number. So, if we multiplied these figures by ten, we would have an approximation of the actual incidence. The real number of *C. perfringens* food poisoning outbreaks each year in the United States is probably between 500 and 600, not counting those occurring in homes. The number of cases each year is probably between 60,000 and 100,000.

Table X

REPORTED CASES OF PERFRINGENS FOOD POISONING
(U.S., 1959-1970)

Year	*Outbreaks*	*Cases*
1959	4	200 [1]
1960	2	90
1961	6	400
1962	3	1000
1963	1	250
1964	1	200
1965	6	500
1966	12	1700
1967	29	3400
1968	56	5800
1969	65	18000
1970	53	7000

1 = Approximate number of cases.

The majority of the outbreaks are associated with establishments that feed many people at one time. The distribution is shown in Table XI for 99 outbreaks of this disease. This relation between food poisoning and meals served at restaurants, at schools, at hospitals, etc. is probably a function of the difficulty of preparing large masses of food for service in a short time without violating the rules of good food handling. If food can be kept really hot, over 60 C, or really cold, below 10 C, it can be stored for hours, but if it is merely kept warm, or cool, there is a definite hazard and this often happens in mass feeding establishments.

Table XI

PLACE OF ACQUISITION OF PERFRINGENS FOOD POISONING *

	Home	Restaurants	Colleges or schools	Health institutions	Other
N.° of outbreaks	10	57	25	3	14

* U.S., 1959 - 1968.

The foods associated with these outbreaks were primarily meat and meat dishes such as thick soups, gravies, stews, roasts, and dressings. These are the types of food often served in the mass feeding establishments such as schools, hospitals, or factory cafeterias. They generally require low temperature cooking, and are often prepared one day, kept over without refrigeration, and served the next day, after being warmed only to serving temperature. Table XII shows the kinds of meat that were involved in various outbreaks. The large number of outbreaks of perfringens food poisoning attributable to beef probably represents the frequency with which it is served, as well as the fact that beef roasts are often cooked one day and served the next, not that beef is particularly hazardous. The same reasoning may hold for turkey. Chicken, pork and fish are more apt to be served on the day that they are cooked.

Table XII

FOODS ASSOCIATED WITH PERFRINGENS FOOD POISONING *

	Beef	Turkey	Chicken	Other meat	Other or unknown
N.° of outbreaks	107	58	18	14	53

The epidemiology of perfringens food poisoning is then, fairly simple. Largely a problem of restaurants, cafeterias, and institutional canteens where food is prepared in large quantity, it is also largely a problem of meat or meat dishes that are cooked and then held for some hours, or for a day, without adequate refrigeration. These relatively simple factors have been found to apply wherever perfringens food poisoning has been a problem. Thus, the main points of the epidemiology seem clear.

The means by which the disease is brought about in the human body have been elucidated only recently. The mechanism of pathogenesis remained unknown for some time. We were unable to answer the questions: Just how does this organism produce the illness that it does? Why are so many bacterial cells needed to cause the disease? Why is it of such short duration?

It was difficult to get answers to these simple questions so long as the human volunteer was the only experimental animal. While the use of human volunteers gives reliable information, the supply of volunteers is limited. Consequently, there was a very great need for some experimental animal if perfringens food poisoning was to be investigated. Early work indicated that feeding cultures

of type A *C. perfringens* to cats, rats, guinea pigs, mice, rabbits, and monkeys did not result in the symptoms of food poisoning in these animals.

Two laboratories started intensive work on this problem, and we owe our knowledge of the pathogenesis of perfringens food poisoning very largely to these two groups of people. One was the laboratory of the Food and Drug Directorate of the government of Canada and the other laboratory was at the Food Research Institute of the University of Wisconsin.

The first real advance in solving this problem was made by Hauschild and his colleagues in Canada. Because it was well known that *C. perfringens* of other types — B, C, and D — were capable of multiplying and producing toxin in the alimentary tract of sheep, these Canadian microbiologists considered that sheep might also provide a suitable environment for type A strains of *C. perfringens*.

They used lambs weighing about 16 kilos for most of their work and administered cultures of food-poisoning strains orally or by means of a fistula to the duodenum. They found that, when a suitable dose was employed, one-half or more of the experimental lambs showed symptoms of perfringens food poisoning. The intraduodenal route of administration provided better results than did the oral route. Symptoms appeared within 6 to 12 hours after either the oral or the intraduodenal administration of whole cultures, and subsided within the next 12 hours. No effect was produced by the administration of the supernatant fluid of cultures. Two lambs were killed shortly after developing diarrhea. Necropsy showed only slight congestion of the duodenum, jejunum, and small intestine. Histological examination of these portions of the intestine revealed no significant lesions, and no invasion of the intestinal wall

Immunization of the lambs did not protect them against the disease, although appreciable immunity to *C. perfringens* alpha toxin — the only toxin known then to be produced in quantity by type A strains — was attained.

These same workers showed that ligated intestinal loops of lambs could also be used, and several cultures tested at the same time. Strains of *C. perfringens*, suspended in fresh medium and injected into the loops, caused the fluid volume of the ligated loops to increase sevenfold. Injection of a fully grown culture was without effect. Uninoculated medium had no effect. These findings were quite important, because the inability of a fully grown culture to cause a response, while the same strain growing in the intestine did so, indicated that some substance was being produced when *C. perfringens* was growing in the intestine, but not when it was growing in the laboratory. It seemed very unlikely that this substance was alpha toxin.

While the Canadian workers were experimenting with sheep as the experimental animal, Strong and Duncan in Wisconsin were going through much the same steps, but with rabbits. Early experiments had shown that diarrhea could not be induced in rabbits by giving *C. perfringens* by

mouth. The Wisconsin group did find, however, that fairly typical perfringens food poisoning could be induced in rabbits if the bacterial cells were injected directly into the ileum. They also found that the injection of some but not all cultures of *C. perfringens* into the ligated intestinal loops of rabbits caused the accumulation of fluid, just as the Canadian workers had found for sheep. Moreover, there was good correlation between the ability of strains to produce fluid accumulation in the ileal loops, and their ability to induce diarrhea in human volunteers. Of 26 strains of *C. perfringens* isolated from food poisoning outbreaks, 12 were unable to produce diarrhea in the rabbits, no matter in what medium they were grown, nor how many cells were administered. These results were very much like those of Osterling with human volunteers — he had found 46 per cent of his strains of *C. perfringens* to induce diarrhea in humans; the Wisconsin workers found 54 per cent of their strains to be active in rabbits.

One other essential piece of information came out of these experiments at the Food Research Institute of the University of Wisconsin. Although *C. perfringens* ordinarily does not produce spores when grown in laboratory media, there are a few media on which spores are produced by this organism. Strong and Duncan found that only when cultures were grown on these sporogenic media would they produce diarrhea in the rabbits. It seemed likely, then, that there was some relation between sporulation and the ability to produce diarrhea. This fitted in with the Canadian workers' finding that a substance inducing diarrhea was formed when *C. perfringens* was growing within the intestine but not when it grew in ordinary media. Sporulation and the production of the enteropathogenic factor then seemed to be related.

Further work, both with rabbits and with lambs, showed that food poisoning strains of *C. perfringens*, when undergoing sporulation, produce a substance that causes the accumulation of fluid in ligated intestinal loops, and that also causes diarrhea in human volunteers. This enteropathogenic factor is heat labile, not dialyzable, inactivated by Pronase, but not by trypsin, by lipase, or by amylase. It apparently is a protein. It is inactivated when exposed to an acid environment, pH 5.0 or below and one would not expect it to withstand the action of gastric juice. It is stable under neutral or slightly alkaline conditions, not being inactivated until the pH reaches 12. And most significantly, it is formed by the cells only when they are sporulating.

The Wisconsin workers found that non-sporing mutants of a sporing, enterotoxic strain did not produce enterotoxin. Mutants with a low spore-producing capacity produced but little enterotoxin. It seems clear, then, that the ability of *C. perfringens* strains to form spores and their ability to produce enterotoxin and cause diarrhea are related, quantitatively and qualitatively.

The production of the enteropathogenic factor of *C. perfringens* in vitro allowed the investigation of its

toxic properties by the Canadian investigators. When moderate doses were given intravenously to lambs, transitory diarrhea, lacrimation, salivation, nasal discharge, and dyspnea became evident in one to five hours. Large doses given intravenously caused death. Examination of the dead animals revealed intensely hyperemic small intestinal mucosa and some congestion in the liver, lungs, spleen and kidneys. Similar symptoms were produced in rabbits and in guinea pigs. The primary actions of the enterotoxin are vasodilation, increase of capillary permeability, and action as a parasympathomimetic agent. In this respect, perfringens enterotoxin is much like cholera enterotoxin, but acts much more quickly. It is noteworthy that perfringens enterotoxin given intravenously causes passage of fluid into the intestinal lumen, and results in increased intestinal motility.

Atropine and epinephrine alleviate the clinical signs induced by the enterotoxin. Atropine has a greater effect than epinephrine, probably because of its ability to act as an antagonist of parasympathomimetic agents. The enterotoxin was found to be antigenic and could be neutralized by antitoxin prepared against it, but only if the enterotoxin and its antitoxin were mixed and allowed to react *in vitro* before injection. When the enterotoxin and its antitoxin were mixed and injected immediately, the enterotoxin was still active. Apparently, there is slow binding of the enterotoxin to its antitoxin but rapid binding of the enterotoxin to the specific tissue sites. Once it is bound to a tissue site, the enterotoxin cannot be neutralized by the antitoxin.

Lambs that had been actively immunized to the enterotoxin were still susceptible to the administration of whole cell suspension, or to the injection of the enterotoxin into ligated intestinal loops. Even lambs that had been exposed to the enterotoxin nine times failed to develop a protective immunity. Apparently, the antitoxin does not pass into the lumen of the intestine in sufficient concentration to inactivate the enterotoxin before it attaches to a tissue site. The same lack of clinical immunity was found for human volunteers who had been exposed several times to experimental food poisoning by Dische and Elek, and for rabbits that had been repeatedly challenged by Duncan and Strong.

In summary, perfringens food poisoning is not a simple intoxication like staphylococcal food poisoning, nor a simple infection, like salmonella food poisoning. Instead, it is a toxic-infection resulting from the growth and sporulation in the small intestine of a very large number of cells of *C. perfringens* with the release of a specific enterotoxin. This enterotoxin is a protein that is not digested by trypsin. It is antigenic but active immunity does not protect against its action. Consequently, even a number of attacks of *C. perfringens* food poisoning does not provide protection against future attacks.

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