APPLIED MICROBIOLOGY, July, 1966
Copyright © 1966 American Society for Microbiology
Vol. 14, No. 4
Printed in U.S.A.

Mice and Monkeys as Assay Animals for *Clostridium perfringens* Food Poisoning[1]

K. F. WEISS, D. H. STRONG, AND R. A. GROOM[2]
Department of Foods and Nutrition, University of Wisconsin, Madison, Wisconsin

Received for publication 29 November 1965

ABSTRACT

WEISS, K. F. (University of Wisconsin, Madison), D H. STRONG, AND R. A. GROOM. Mice and monkeys as assay animals for *Clostridium perfringens* food poisoning. Appl. Microbiol. 14:479–485. 1966.—Spores and vegetative cells of *Clostridium perfringens*, in combination with meat or starch paste, sterile culture filtrates, lecithinase, and phosphorylcholine, were administered to mice and rhesus monkeys in an attempt both to evaluate the animals as test agents and, if possible, to elucidate the active factors producing food-poisoning symptoms caused by this organsim. Some of the preparations were administered to the monkeys by stomach tube; others, in gelatin capsules which were treated with formaldehyde so that the release of their contents was delayed and presumably reached the intestines of the animals. Any changes in intestinal passage times and in consistency of stools of the animals were observed, and the counts of *C. perfringens* in the feces of the monkeys previous and subsequent to treatment were recorded. The results obtained were inconclusive. Diarrhea occurred only relatively infrequently in both species, regardless of the substance fed or the mode of administration. The changes in intestinal passage times were not great, although in the monkeys there appeared to be a slight trend toward reduction as the magnitude of the bacterial load increased. Phosphorylcholine appeared to have little, if any, effect in reducing intestinal passage time of mice or monkeys. No procedures explored in these experiments could be said to be satisfactory as a means of animal assay for food poisoning strains of *C. perfringens* since typical symptoms did not appear with regularity.

Clostridium perfringens has found considerable acceptance as a cause of food poisoning in man. It has not been possible, however, to elucidate fully the cause and effect relationship, because efforts have been hampered by lack of a suitable assay method. The present paper presents the results of an attempt to induce a regular and recognizable response in a test animal when viable cells of *C. perfringens* or possible products produced during the growth of such cells were administered by mouth.

Hobbs et al. (9) reported in 1953 that the typical symptoms of *C. perfringens* food poisoning could be induced in human beings when certain suspensions containing viable cells were swallowed. Dische and Elek (5) quantified the observation of this relationship and indicated 5×10^8 organisms as the minimal effective dose which would produce gastrointestinal disturbance in man. Other investigators who have reported some, but not always consistent, positive results in feeding viable cultures of *C. perfringens* to human beings include Østerling (11) and Dack et al. (4).

The questions of the characteristics of the particular strains of *C. perfringens* which cause food poisoning in man, if, indeed, such a grouping exists, has been much debated. Hobbs in a recent paper (8) reiterates the viewpoint that food-poisoning strains of *C. perfringens* are atypical type A strains which produce small amounts of α toxin, but no θ toxins, and are variable in regard to the production of κ toxin; furthermore, they are heat-resistant and nonhemolytic or α-hemolytic on horse blood-agar. Earlier, Hall et al. (7), after completing extensive studies on 83 strains, concluded that the cause of *C. perfringens* food poisoning in the United States apparently is not limited to strains meeting the criteria for classification suggested by Hobbs.

Reports of attempts to use animals to test for

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station, Madison.

[2] Present address: Digby Stuart College, Roehampton, London, S.W. 15, England.

the food-poisoning factor in *C. perfringens* have not been numerous, nor have they been conclusive. Hobbs et al. (9) described results obtained from observations of two monkeys. It appeared that, whether they were fed contaminated food or had cultures of *C. perfringens* administered directly into the stomach, they produced only an occasional loose stool. These authors also described a variety of tests involving the use of frogs and mice. Yamamoto et al. (12) showed that heat-resistant spores, which survived holding for 1 hr at 100 C, could be isolated from mouse feces but not from chicken feces 24 hr after oral administration of vegetative cells.

There apparently has not been a report of gastrointestinal syndromes associated with sterile filtrates of *C. perfringens*, thus presumably excluding any exotoxin produced by *C. perfringens* as the disease-causing agent. Nygren (10) postulated, however, that phosphorylcholine, the end product of the action of the α toxin (lecithinase C) on lecithin is the causative factor for the intestinal disturbances. He reported that feeding 1 mg of phosphorylcholine to mice reduced the intestinal passage time to one-fourth, and the stools were loose. A similar result was obtained with one monkey. Later, Dack (3) disclaimed the validity of these findings as an explanation for the gastrointestinal upsets caused by *C. perfringens*. He reiterated that, in naturally occurring outbreaks and feeding experiments, large numbers of living organisms must be present in a suitable food to cause food-poisoning symptoms. Dack also noted that the ingestion of 500 mg of phosphorylcholine by one human volunteer did not cause a gastrointestinal upset.

With the exception of one experiment (9) in which live cultures of *C. perfringens* were injected directly into the duodenum of young guinea pigs, no consideration has been given to the possible effect of passage through the stomach on the organisms, the α toxin, or phosphorylcholine. Canada and Strong (2), using gnotobiotic mice, demonstrated that the organism does pass through the stomach; however, the relative number of organisms surviving passage through the stomach was not ascertained. The fact that large numbers of viable cells of *C. perfringens* must be ingested by man to produce food poisoning symptoms suggests the possibility of inactivation or destruction of part of the cells, or other active principle, in the stomach.

In the present study, the effect on intestinal passage time and the appearance of stools after administering total cells or spores of *C. perfringens*, cell-free filtrates, lecithinase, or phosphorylcholine by mouth to mice and to rhesus monkeys was reinvestigated. In some cases, an attempt was made to eliminate the possible action of the secretions of the stomach of the monkeys on the test preparations by using formaldehyde-treated gelatin capsules as carriers of the test material. For certain series of experiments, the counts of total viable cells and spores of *C. perfringens* in the feces of the monkeys were also determined.

Materials and Methods

The following were used for Series I.

Mice. Swiss albino mice (4 to 6 weeks old) weighing from 15 to 20 g were used.

Microorganisms. Five strains of *C. perfringens* were included: ATCC-3624 type A, S-45, IU1168, NCTC-8238-2, NCTC-8799-10.

In preliminary work, the biochemical and toxicological reactions of the organisms were checked. All five strains proved to be type A, although their sporulating characteristics and their heat resistance varied considerably. The cultures were maintained in Noyes' veal broth which was used also to grow the inocula for the starch and starch-meat pastes. For α-toxin production, glucose in a final concentration of 1% was added to the veal broth (1). To obtain the α toxin-containing supernatant fluids, the inoculated veal broth was incubated for 5 hr at 37 C and centrifuged at 30,000 $\times$ g for 20 min; the clear supernatant fluid was then decanted. The amount of α toxin was determined by the lecithovitellin reaction by use of the method suggested by Ellner (6).

Paste. Starch paste consisted of 30 g of cornstarch, 3 g of salt, and 475 ml of tap water. The cornstarch and salt were mixed in a saucepan and the water was added. The mixture was cooked with constant stirring until it became thick and translucent. Portions (150 ml) were placed in 200-ml screw-cap bottles and autoclaved 15 min at 121 C.

Starch-meat paste consisted of 150 g of ground beef, 4.5 g of salt, 62.5 g of wheat flour, and 600 ml of tap water. The water was added to the flour and salt and mixed until smooth. The ground beef was heated in a frying pan only until all pink color disappeared. The flour-water mixture was added, and the whole was brought to a boil with constant stirring. The paste was apportioned and sterilized as described above.

Fatty starch-meat paste with the same composition as the starch-meat paste, except that 90 g of fat was added to the ground beef, was also prepared.

To obtain the test preparations, the pastes were inoculated with 1 ml of 24-hr veal broth cultures of the test organisms and were incubated for 12 hr at 37 C. Plate counts for the test preparations were made at the time the material was fed to the mice. The procedures for carrying out the plate counts were identical with those described in the next section.

Mouse feeding procedure. Initially, the average normal intestinal passage times were established by feeding each mouse about 0.5 g of bread wetted with 1 ml of a 0.4% suspension of carmine after the animals had been starved for 24 hr. Once the passage times,

as indicated by the appearance of carmine in the feces, were determined, the mice were kept on a normal diet of commercial mouse ration for 3 days before the actual test preparations were fed. Each animal was used twice. In the first test, after a 24-hr period in which the mice received no food, they were fed controlled quantities of cell-free supernatant fluids, lecithinase, or phosphorylcholine. After a resting period of 3 days, the mice were again starved for 24 hr and the cell suspension or the inoculated pastes were fed. Bread impregnated with carmine suspension and then dried was used as a carrier for all of the test preparations.

The mice were observed individually at 0.25-hr intervals, and the time periods from consumption of the bread mixtures to the appearance of the first red-colored feces were taken as the intestinal passage time. The consistency of the feces was also noted.

The following were used for Series II.

Monkeys. Twenty-four young Rhesus monkeys ranging from 4 to 6 lb in weight were used in four series of feeding trials. The animals were housed in individual cages and kept on a diet of a commercial monkey biscuit.

Materials used in Series II were aqueous carmine suspension, and starch or meat pastes containing carmine and inoculated with one of two levels of inocula of strain NCTC-8238-2. The meat paste was prepared from strained baby food so modified that it contained, per test dose, 5 g of meat and 2 g of beef fat. The test dose of starch paste included 1.4 g of cornstarch and 0.2 g of table salt. Formaldehyde-treated gelatin capsules containing carmine, phosphorylcholine, α-lecithinase, or lyophilized cultures of strain NCTC-8238-2 were also used. The formaldehyde-treated capsules were used so that the release of the contents was delayed and presumably took place in the intestine rather than in the stomach of the animals. To treat the capsules with formaldehyde, they were charged with the preparations to be fed, the two capsule sections were sealed by slightly moistening one section, and the sealed capsules were immersed in 10% formaldehyde for 5 sec. The capsules were dried and then dipped in edible oil to check for possible leaks.

This procedure was suggested by D. E. Wurster of the Department of Pharmacy at the University of Wisconsin. It had been shown in human studies that gelatin capsules so treated were not dissolved in the stomach, but released their contents in the intestine.

Monkey feeding schedules and bacteriological procedure. In the first of the four feeding trials which were conducted at weekly intervals, the individual intestinal passage time for the 24 monkeys was determined. Half of the animals were given, by means of a catheter tube passed into the stomach, 350 mg of carmine in 20 ml of water. To each of the remaining 12 monkeys, a capsule containing 350 mg of carmine was administered orally; care was taken that the capsules were swallowed intact. The monkeys were observed at 0.5-hr intervals, and the passage time for the carmine, as indicated by the appearance of red feces, was recorded.

In the second trial, feces were collected from each animal before the test preparations were fed. Each fecal sample was placed in a sterile preweighed petri dish. After determining the weight of a sample, a portion of it was aseptically removed and placed in a sterile screw-cap test tube. A 1:10 dilution in 0.1% peptone water was prepared, and total cell and spore counts were made as described below. The remaining portion of the sample was reweighed and held for dry-weight determination. The weight of the sample portion removed for plating was determined by difference. After administering the test preparation to the monkeys, fecal samples were collected at the passage time of carmine and after the next subsequent defecation (passage time plus t). The procedure for manipulation of the sample was identical in all cases.

Each stool sample in the peptone water was emulsified on a Vortex mixer; 3 ml of each 1:10 dilution was transferred to sterile 16- by 20-mm test tubes and pasteurized at 75 C for 20 min. After appropriate dilutions of the original (unpasteurized) and pasteurized samples were made, each was plated on SPS agar (1) to obtain total cell and spore counts. The plates were incubated at 37 C in anaerobic jars under an atmosphere of 90% N and 10% CO_2 (1). The other portions of the fecal samples were dried in a vacuum oven at 75 C to constant weight. The total cell and spore counts were recorded per gram (dry weight) of feces.

Twenty animals were used in the second trial. They were divided into four groups of five animals each. Groups one and two were administered, by stomach tube, 60 ml of meat paste containing 350 mg of carmine and approximately 3×10^3 organisms, or 60 ml of starch paste containing 350 mg of carmine and approximately 5×10^3 organisms. Groups three and four received, by formaldehyde-treated gelatin capsule, 2×10^4 freeze-dried spores plus 350 mg of carmine per capsule, and 20 mg of phosphorylcholine plus 350 mg of carmine, respectively.

In the third trial, uninoculated starch or starch-meat paste was administered to the animals to see if the pastes themselves would affect the intestinal passage time. Only 20 ml of paste was administered, since the first experiment suggested that stress due to large quantities of fluids in the stomach had affected the results. The same amount of solids was contained in the second volume, however. Groups of six monkeys received uninoculated meat paste and starch paste, respectively.

Of the remaining 10 monkeys, 5 were each given one capsule containing 350 mg of carmine and one capsule containing 100 mg of phosphorylcholine. The fourth group of five animals each received one capsule containing 1 mg of α-lecithinase and 350 mg of carmine. The consistency of the stools and the individual passage times were noted.

In the fourth trial, the 23 monkeys were divided into five groups. Only 20 ml of paste was administered, but the number of viable organisms was 2.1×10^7 in the meat paste and 2.5×10^7 in the starch paste. One group of five animals received, by catheter tube, the meat paste, and a second group received the starch paste. The four animals in group three received 350 mg of phosphorylcholine in 20 ml of water. Each preparation contained also 350 mg of carmine. Of the remaining nine monkeys, five were given, by capsule,

TABLE 1. *Intestinal passage time for control monkeys and those to which phosphorylcholine or lecithinase was administered*

Substance	Amt	Mode of administration[a]	No. of monkeys	Passage time[b]
				hr
Carmine	350 mg in 20 ml of distilled water	Stomach tube	12	9
	350 mg	Capsule	12	23
Uninoculated starch paste[c]	20 ml	Stomach tube	6	9.5
Uninoculated meat paste[c]	20 ml	Stomach tube	6	6.75
Phosphorylcholine[c]	300 mg in 20 ml of distilled water	Stomach tube	4	12.5
	20 mg	Capsule	5	26
	100 mg	Capsule	5	23.75
	300 mg	Capsule	5	26.75
Lecithinase[c]	1 mg	Capsule	5	22
	3 mg	Capsule	4	13.75

[a] All capsules were formaldehyde-treated.
[b] Measured by appearance of carmine in feces.
[c] Each preparation contained 350 mg of carmine.

350 mg of carmine and 300 mg of phosphorylcholine; four animals received 3 mg of α-lecithinase and 350 mg of carmine, also by capsule. Sampling and plating procedures were as in the second trial.

RESULTS

For the control mice, the times necessary to complete intestinal passage, as measured by the appearance of carmine in the feces, varied considerably, ranging from 1.5 to 6 hr, with a mean value of 4.5 hr.

All of the test preparations produced average intestinal passage times for the mice which were less than the average time for the control group, but since the values for the ranges always fell within the perimeters established by the control group, little significance could be attached to the observation. If the results have any meaning, one would be forced to conclude that, with the possible exception of the inoculated starch-meat paste, the active principle was almost equally distributed in all preparations. This seems unlikely. The substance which appeared to have least effect on the passage time was starch-meat paste inoculated with *C. perfringens*; the greatest effect resulted from feeding the inoculated starch-meat-fat paste.

Diarrhea, which was judged subjectively by the laboratory worker, occurred only infrequently. The highest observed incidence appeared to be among the mice fed the inoculated starch-meat paste (26%), whereas the lowest (14%) number of cases occurred in the group fed inoculated starch-meat-fat paste. (The term "diarrhea" is used to describe watery consistency of the stools and is not necessarily synonymous with reduced carmine passage time.)

In Table 1 are presented the average intestinal passage times for the monkeys to which were administered the carmine suspensions, the gelatin capsules containing carmine, or the uninoculated pastes. As can be seen, the passage of time observed for substances in the formaldehyde-treated gelatin capsules was consistently longer than that for the liquid preparations. It is possible that the introduction of liquid into the stomach by the catheter tube caused some traumatic reflex resulting in an increased rate of peristalsis. The average passage time of 23 hr, obtained with the gelatin capsules containing carmine, is more in line with previously reported values of approximately 24 hr (13) and 18 hr (Bergdoll, *personal communication*).

The effect of lecithinase and phosphorylcholine on the passage times of the monkeys is also shown in Table 1. None of the three concentrations of phosphorylcholine fed by capsule caused any reduction in the average passage times; rather, they were slightly longer than the control. The consistency of the stools also was unaffected. Only the high concentration of α-lecithinase reduced the time of intestinal passage appreciably, and study of this relationship should perhaps be extended. The feces of the animals in this latter case were softer than was normally observed (before treatment), but no acute diarrhea was present. The phosphorylcholine fed by stomach tube also lengthened the passage time for the animals when compared with their controls. The consistency of the feces was again generally unchanged.

Table 2 presents the results obtained when the monkeys were fed varying levels of viable cells or spores of one strain of *C. perfringens* in com-

TABLE 2. *Intestinal passage times for monkeys and fecal spore counts after administration of test preparations containing Clostridium perfringens, strain NCTC-8238-2*[a]

Substance	Amt	Mode of administration	Pretreatment		Passage time[b]			Passage time + t[c]		
			Spores/g of feces (dry wt)	Total cells-spore ratio	Hr	Spores/g of feces (dry wt)	Total cells per spores ratio	Hr	Spores/g of feces (dry wt)	Total cell-spore ratio
Starch paste	60 ml, containing 5×10^3 total cells, 3×10^3 spores	Stomach tube	7.6×10^2	12:1	10.5	2.7×10^7	1.7:1	20	9.6×10^7	0.18:1
Starch paste	20 ml, containing 2.5×10^7 total cells, 2.5×10^5 spores	Stomach tube	2.8×10^2	13:1	6.5	3.6×10^5	1,100:1	13.5	9.5×10^5	27:1
Meat paste	60 ml, containing 3×10^8 total cells, 1×10^8 spores	Stomach tube	1.3×10^2	11:1	9.75	4.8×10^6	0.31:1	16.25	1.5×10^7	0.73:1
Meat paste	20 ml, containing 2.1×10^7 total cells, 3×10^5 spores	Stomach tube	8.4×10^2	1.3:1	5	8.4×10^5	65:1	12.5	5.5×10^6	0.01:1
Lyophilized culture of *C. perfringens*[d]	2×10^4 spores	Formaldehyde-treated gelatin capsule	1.3×10^2	92:1	29	5.3×10^2	4.2:1	33.25	7.6×10^2	530:1

[a] Values given are for the average of five monkeys.
[b] Measured by appearance of carmine in feces.
[c] Abbreviation: t = time to first defecation after appearance of carmine.
[d] Average from only three monkeys.

bination with differing volumes of two types of food materials. The test samples were administered by stomach tube to insure their ingestion by the monkey. When the results for the starch paste and the meat paste are compared, it is apparent that numbers of organisms present had more bearing on the rate of intestinal passage than the food which served as a carrier. Feces of diarrheal consistency appeared only among the animals administered meat concurrently with the test dose of viable *C. perfringens* cells. These results must be considered in light of the effect on digestive system activity apparently stimulated by use of the stomach tube. Usual feeding procedures might produce quite different results.

Of equal interest is the relationship between cell and spore counts and the consistency of the feces. Contrary to the results obtained for the monkeys examined by Hobbs (9), *C. perfringens* was recovered from the feces of all monkeys used in these experiments previous to the administration of test materials. After feeding the inoculated test pastes, the plate counts for both total cells and spores increased, but diarrhea appeared comparatively infrequently—in 4 of 20 animals. (In feces judged to be diarrheal, the average ratio of liquid to solid was 5.82:1.) Within arbitrarily set groups, a trend toward correlation was observed for after-treatment fecal plate counts and consistency of feces, e.g., 8 of 11 animals having "high" counts produced feces with a high liquid to solid ratio, whereas the feces of 18 of 22 animals with "low" counts showed a lower proportion of liquid to solid.

The samples taken to establish plate counts at the first normal defecation after the appearance of the carmine indicated viable counts at about the same or slightly higher levels than at passage of the carmine. The ratio of spores to cells appears to be higher, supporting the view of Hobbs (8) and others that the organism sporulates rapidly in the intestines. A further observation was that, in the week which elapsed between the two tests (60 and 20 ml of paste), the viable counts returned to approximately the original levels.

The lyophilized cultures (fed by capsule, and the contents presumably released in the intestines of the animals) did not cause any change in consistency of feces, nor did the fecal spore counts increase markedly (Table 2). The bacterial load per capsule was relatively low (approximately 2×10^4 spores), and the organisms may not have had sufficient time to become rehydrated and to begin to proliferate before they were eliminated; however, in no case was a capsule ever passed with the stools.

The only very moderate response of the two animal species to all of the preparations fed makes it impossible to draw any definite conclusions about the nature of the illness-producing agent. Nor does it make possible recommendation of the routine uses of either species as assay animals by any technique tested here. The use of the stomach tube for administering test substances seemed only to add complication and probably should not be used. The definite increase in counts of *C. perfringens* in the monkey fecal plate counts, after ingestion of the inoculated pastes, with no regular occurrence of symptoms would suggest that these animals do not respond vigorously to the presence of *C. perfringens* in the digestive tract. In many cases, however, the fecal counts did not indicate presence in their digestive tract of a magnitude of viable cells, suggested by Dische and Elek (5) as necessary for symptoms to occur. Unexplained is the disagreement between the findings in this investigation with phosphorylcholine and those reported by Nygren (10). The response to phosphorylcholine by the one monkey Nygren used could be dismissed as an exception were it not that the results obtained with the mice were also at variance with Nygren's findings. Since any differences which could be noted between administration of test materials by stomach tube and by formaldehyde-treated gelatin capsule indicated the effectiveness of the stomach tube treatment to be as marked as that of the capsules, possible adverse effects of the stomach secretions on phosphorylcholine or α toxin seem unlikely. It should be mentioned that no attempts were made to trace the gelatin capsules through the monkeys' gastrointestinal tract, and it cannot be said with certainty that the contents of the capsules were actually released in the intestine of the animals, although no capsule was ever recovered in the feces of the monkey.

Also, a formaldehyde-treated capsule containing 350 mg of phosphorylcholine was ingested by a worker in this laboratory (barium tracer studies have been conducted on humans) but no gastrointestinal symptoms occurred.

There are obvious limitations in these experiments; for example, only one strain of the organism was tested. It is evident that more work is required to elucidate the physiological and pharmacodynamic action of *C. perfringens* or its by-products and their possible role in inducing food-poisoning symptoms. Likewise, an adequate animal assay technique remains to be developed.

ACKNOWLEDGMENTS

This investigation was supported by Department of Health, Education, and Welfare research grant

EF 00131-5 from the Bureau of State Services, Division of Environmental Engineering and Food Protection.

We thank Gail Dack and M. S. Bergdoll of the Food Research Institute at the University of Chicago for use of the monkeys and for providing technical assistance in the monkey feeding experiment.

The technical assistance of Mrs. L. W. Higgins and Earnell Sams is gratefully acknowledged.

LITERATURE CITED

1. ANGELOTTI, R., H. E. HALL, M. J. FOTER, AND K. H. LEWIS. 1962. Quantitation of *Clostridium perfringens* in foods. Appl. Microbiol. **10**:193–199.
2. CANADA, J. C., AND D. H. STRONG. 1965. Effect of animal alimentary passage on the heat resistance of *Clostridium perfringens*. Appl. Microbiol. **13**:788–792.
3. DACK, G. M. 1964. Microbial food poisoning. Food Technol. **18**:80–82.
4. DACK, G. M., H. SUGIYAMA, F. J. OWENS, AND J. B. KIRSNER. 1954. Failure to produce illness in human volunteers fed *Bacillus cereus* and *Clostridium perfringens*. J. Infect. Diseases **94**:34–38.
5. DISCHE, F. E., AND S. D. ELEK. 1957. Experimental food poisoning by *Clostridium welchii*. Lancet **2**:71–74.
6. ELLNER, P. D. 1961. Fate of partially purified C^{14}-labeled toxin of *Clostridium perfringens*. J. Bacteriol. **82**:275–283.
7. HALL, H. E., R. ANGELOTTI, K. H. LEWIS, AND M. J. FOTER. 1962. Characteristics of *Clostridium perfringens* strains associated with food and food borne disease. J. Bacteriol. **85**:1094–1103.
8. HOBBS, B. C. 1965. *Clostridium welchii* as a food poisoning organism. J. Appl. Bacteriol. **28**: 74–82.
9. HOBBS, B. C., M. E. SMITH, C. L. OAKLEY, G. H. WARRACK, AND J. C. CRUICKSHANK. 1953. *Clostridium welchii* food poisoning. J. Hyg. **51**: 75–101.
10. NYGREN, B. 1962. Phospholipase C-producing bacteria and food poisoning. Acta Pathol. Microbiol. Scand. Suppl. **160**:1–88.
11. ØSTERLING, S. 1952. Food poisoning caused by *Clostridium perfringens*. Nord. Hyg. Tidskr. **33**:173–180.
12. YAMAMOTO, R., W. W. SADLER, H. E. ADLER, AND G. F. STEWART. 1961. Characterization of *Clostridium perfringens* (*welchii*) isolated from market poultry. Appl. Microbiol. **9**:337–342.