Staphylococcal Growth and Enterotoxin Production in Meat

E. P. CASMAN, D. W. McCOY, AND P. J. BRANDLY

Division of Microbiology, Food and Drug Administration, U.S. Department of Health, Education, and Welfare, and Meat Inspection Division, Agricultural Research Service, U.S. Department of Agriculture, Washington, D.C.

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ABSTRACT

CASMAN, E. P. (Department of Health, Education, and Welfare, Washington, D. C.), D. W. McCOY, AND P. J. BRANDLY. Staphylococcal growth and enterotoxin production in meat. Appl. Microbiol. **11**:498–500. 1963.—Preliminary attempts were made to explain the association of staphylococcal food poisoning with cooked rather than uncooked meats. The abilities of various meats to support the growth of an enterotoxigenic staphylococcus, and the production of enterotoxin A, were determined. The production of enterotoxin was detected by means of serological procedures. Little or no growth was obtained when the inoculum was mixed with raw ground beef. When the surfaces of raw and cooked meats were inoculated, however, good growth was obtained with the production of enterotoxin.

Meat products that have been incriminated in cases of staphylococcal food poisoning are usually those which had been heated before being subjected to careless handling and inadequate refrigeration. Meats which are consumed shortly after heating or are consumed without having been cooked are rarely responsible for staphylococcal food poisoning. Involvement of ham, for example, has occurred with the development of the "tenderized" product (heating the ham to an internal temperature of 60 C in the smoke house).

This paper describes a preliminary attempt to explain the association of cooked, as opposed to raw, meat with staphylococcal food poisoning. It was suspected that the association with food poisonings could be attributed to differences in the abilities of meats to support growth or the production of enterotoxin, or both. The growth-supporting properties of the meats were determined by the Meat Inspection Division, Agricultural Research Service of the Department of Agriculture. Studies of the production of enterotoxin were carried out in the Division of Microbiology, Food and Drug Administration of the Department of Health, Education, and Welfare, by use of serological methods under development for this purpose. The blended meats were examined for staphylococcal growth and then kept frozen until examined for their enterotoxin content. The development of simplified serological procedures for the detection of enterotoxins (Casman, 1958, 1960) facilitated their identification. The production of enterotoxin A (Casman, Bergdoll, and Robinson, 1963), the predominant cause of staphylococcal food poisoning in the United States was studied.

MATERIALS AND METHODS

Test organism. Staphylococcus strain 234 was used in this study. The senior author isolated it in 1947 from ham which was epidemiologically responsible for an outbreak of food poisoning. It produces enterotoxin A.

Preparation and inoculation of meat samples. In preliminary experiments, it was found that to obtain good growth of staphylococci in raw meat it was necessary to use surface inoculation. For example, when inocula were distributed uniformly in ground raw beef, growth was poor. When the surfaces of cuts of raw beef were inoculated, however, excellent growth was obtained. The surfaces of various meats were, therefore, uniformly inoculated with an enterotoxigenic staphylococcus, and the meats were examined for their ability to support growth and enterotoxin A production.

Five different types of meat were included in the study: raw beef, raw pork, cooked beef, cooked pork, and canned ham. Portions of the beef and pork were cooked for approximately 3 hr (the beef to an internal temperature of 63 C and the pork to an internal temperature of 77 C).

Discs (5 cm in diameter and 1-cm thick) of raw and cooked meat were prepared in the following manner. An alcohol-dipped and flamed knife was used to cut slices of meat 1-cm thick. A cooky cutter, dipped in alcohol and flamed, was used to cut a disc from the freshly exposed slice, and precautions were taken not to include meat from the outer edges of the slice. The discs were placed in sterile petri dishes and, after inoculation with an enterotoxin A-producing staphylococcus, incubated at 30 C for 72 hr in a high-humidity incubator. The inoculum was 0.1 ml of a dilution of an overnight Trypticase Soy Broth (BBL) culture of strain 234 and was spread uniformly over the surface of each disc to give approximately 250 organisms per cm^2.

Culture methods. After 72 hr of incubation, 20% slurries of the meats were prepared in sterile distilled water by treatment at high speed for 3 min in a Waring Blendor. The numbers of staphylococci present in the suspensions were determined by spreading 0.1 ml of tenfold dilutions

of the slurries over the surfaces of tellurite glycine agar plates (Difco). The typical black colonies were counted after overnight incubation at 35 C.

Separation and concentration of enterotoxin. A 50-ml amount of the 20% aqueous suspension of the meat sample was adjusted to pH 6.8 with 1 N NaOH and centrifuged at 32,800 × *g* for 5 to 10 min. The supernatant was decanted and diluted with 19 volumes of 0.01 M sodium phosphate (pH 6.8). The pH was readjusted to 6.8. The extract was then passed through a column of carboxymethylcellulose (CM-cellulose; Whatman, Powder, CM70, manufactured by W. and R. Balston, Ltd., 0.7 meq/g capacity).

A 2.5-g amount of CM-cellulose was suspended in approximately 100 ml of 0.01 M sodium phosphate (pH 6.8). The suspension was adjusted to pH 6.8 with NaOH. The column was packed by pouring the suspended CM-cellulose into a chromatographic tube 2 cm in diameter (fitted with a coarse sintered-glass disc). The particles were allowed to settle under flow conditions until the absorbent reached a height of 8 cm. The column was washed with 50 ml of 0.01 M sodium phosphate (pH 6.8).

The column was then placed in a cold room, and the diluted meat extract was allowed to pass through the column at a flow rate of approximately 1 ml/min, mainly because it was convenient to do this step overnight. When the last of the extract just entered the adsorbent bed, the column was washed with 100 ml of 0.01 M sodium phosphate (pH 6.8). (Occasionally, passage of the extract through the column was completed prematurely; when this happened, the column was hydrated by passing 25 to 50 ml of distilled water through it.) The toxin was then eluted from the column with 150 ml of 0.2 M sodium phosphate (pH 6.8).

The eluted toxin was concentrated to 3.0 to 5.0 ml at 7 to 10 C with a LKB ultrafilter. Further concentration to approximately 0.1 ml was achieved by dialyzing the concentrate against 35% polyvinylpyrrolidinone (Matheson, Coleman, and Bell, East Rutherford, N.J.).

Serological detection and assay of enterotoxin. Serial dilutions of the concentrate were tested by use of Wadsworth's (1957) slide modification of the gel double diffusion test of Ouchterlony as described in detail by Crowle (1958). Measurement consisted of the determination of the highest dilution giving a line of precipitation which could be identified through its coalescence with a reference line of precipitation produced by a known sample of enterotoxin A (Casman, 1958, 1960). The product of the volume of the concentrate and the reciprocal of the dilution giving such a line of precipitation was used to indicate the amount (in μg) of enterotoxin, since it is estimated that a concentration of 1 μg/ml represents the limit of sensitivity of the test.

TABLE 1. *Growth and enterotoxigenicity of staphylococcal strain 234 on meat*

Meat	Staphylococcal count (per g)	Enterotoxin A
		μg/10 g
Raw beef	32×10^7	0.6
Cooked beef	220×10^7	0.64
Raw pork	19×10^7	1.28
Cooked pork	150×10^7	0.4
Canned ham	150×10^7	0.96

RESULTS

The numbers of coagulase-positive staphylococci per g of meat after inoculation with approximately 250 organisms per cm^2 of surface and incubation at 30 C for 72 hr are presented in Table 1. The amounts of enterotoxin A recovered from 10-g portions of these meats are also included.

It is evident (Table 1) that enterotoxin A production and good growth were obtained in all the meat samples. Although growth was better in the cooked meat, there was no significant increase in enterotoxin production.

DISCUSSION

The results of this preliminary attempt to explain the association of staphylococcal food poisoning with cooked meats indicate that the explanation may lie in the growth-supporting properties of various meats. Evidence is presented indicating that cooked meats support somewhat better growth than raw meats. It is conceivable that this difference would be more marked if the attempts to attain asepsis were less efficient, because of the recognized inability of the staphylococcus to compete with other organisms present in foods (Miller, 1955; Newman, 1943; Peterson, Black, and Gunderson, 1962*a*, *b*; Straka and Combs, 1952; Troller and Frazier, 1963*a*, *b*). The inability to obtain good growth in ground raw beef may have been due to competition which could be more effective under anaerobic conditions than under the aerobic conditions preferred by the staphylococcus.

Conclusions as to the relative abilities of beef and pork and of raw and cooked meats to support the production of enterotoxin A must be deferred until differences are consistently obtained in experiments scheduled for the future. It is evident, however, that when good growth is obtained on meat enterotoxin A is produced.

Further studies are contemplated in which smaller inocula will be employed, and in which an attempt will be made to approximate the environmental conditions to which meats are normally exposed.

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