Experimental Staphylococcus Food Poisoning

A Study of the Growth of a Food Poisoning Staphylococcus and the Production of an Enterotoxic Substance in Bread and Meat

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THE elaboration of an enterotoxic substance by the growth of staphylococci in foods is of significance as a public health problem. The importance of starch for the production of this food poisoning substance was pointed out by Jordan and Burrows.[1] These investigators found that bacteria of various kinds, *i.e.*, staphylococci, streptococci, *Proteus, Bact. coli, Bact. aerogenes,* and *Salmonella aertrycke,* grown under suitable conditions, especially on starch medium, were capable of producing a substance which caused gastrointestinal reactions in monkeys. By successive transfers on starch media, they further succeeded in restoring the enterotoxic property to strains which originally had produced that substance and had later failed in this capacity. Bacteria which had never possessed this characteristic before could produce the toxic material when grown on starch medium. A list of the foods involved in a number of naturally occurring outbreaks is given in Table I. From the table it is evident that many of the incriminated foods are rich in starch, but they also contain proteins. In Stone's [2] analysis of outbreaks occurring in California, he observed that "protein was the only consistent major ingredient of the food involved in every outbreak."

The purpose of the present study was to discover whether "food poisoning" staphylococci could grow and

TABLE I

STAPHYLOCOCCUS FOOD POISONING OUTBREAKS

Date	*Investigator(s)*	*Incriminated Food*
1914	Barber [4]	Milk
1929	Dack, Cary, Woolpert and Wiggers [5]	Sponge cake, portion of a custard filled cake
1930	Jordan [6]	Cheese
1930	Jordan, Dack [7]	Cream filled cake
1931	Jordan and Hall [8]	Chicken gravy
1931	Ramsey and Tracy [9]	Milk
1932	Buchanan and Ecker [10]	Custard pie
1932	Jordan and Burrows [11]	Cream filled cake
1932	Jordan and Burrows [11]	Cream filled eclairs and tarts
1932	Jordan and Burrows [11]	Custard filled doughnuts
1933	Jordan and Burrows [11]	Chocolate eclairs
1933	McBurney [12]	Chocolate eclairs
1934	Crabtree and Litterer [13]	Milk
1935	Corpening and Foxhall [14]	Custard filled cake
1935	Dack, Bowman and Harger [3]	Tongue sandwiches
1936	Health Department, New York City [15]	Cream puffs
1936	Shaughnessy and Grubb [16]	Milk

elaborate an enterotoxic substance in sandwiches, and to determine whether the bread or the sandwich filler or both were involved. This problem was suggested as a result of finding enormous numbers of yellow, hemolytic staphylococci in bread from meat sandwiches involved in an extensive food poisoning outbreak in Indianapolis in 1935.[3] These organisms were found in the bread and tongue filling of the sandwiches and in the stock tongue, but were absent in cheese, peanut butter, and jelly sandwiches. It was concluded that they had probably grown into the bread from the meat filling. The sandwiches had been made at night and given to workmen the next morning for their mid-day meal. Feeding experiments on rhesus monkeys proved the organism to be capable of producing an enterotoxic substance.

GROWTH IN BREAD AND MEAT

Using these " food poisoning " staphylococci, experiments were performed to test their ability to penetrate and grow in the bread of peanut butter, ham, tongue, and chicken sandwiches when they were introduced in these fillers.* In every instance the yellow, hemolytic staphylococci were recovered from portions of bread not in contact with the fillers after a few hours' incubation. Other staphylococci—stock cultures and several strains which were normally found as contaminants of the meats—were also tested. All these cocci penetrated into and grew in the bread. When different morphological types of bacteria (*Bact. coli, S. aertrycke, Bact. dysenteriae* (Flexner), *Bact. typhosum, B. niger, B. subtilis*) were introduced into ham and tongue sandwiches by inoculation of the meats, they were not recovered from the bread. However, certain of these rod forms grew into bread from chicken filling. Bread proved to be a favorable medium for all the bacteria tested when they were inoculated directly into this food. The " food poisoning " staphylococci did not distinguish themselves by exceeding the growth rates of other organisms.

Assuming that the salt content of the meats might be a factor, the growth of the " food poisoning " and other staphylococci, as well as the bacilli, was studied in salted and unsalted meats, *i.e.,* ham, beef tongue, and chicken. Staphylococci grew in salt meat which prohibited the increase of spore-forming and non-spore-forming rods. Salt seemed to have no more effect on the food poisoning strain than on other staphylococci. In the unsalted chicken all the bacteria increased, and there was no correlation between morphological type and relative growth. Sodium chloride was added to chicken to obtain 5, 10, and 20 per cent salt content, and again estimates of the numbers of organisms before and after incubation were made. The five strains of staphylococci tested (including the " food poisoning " organism) multiplied in 10 per cent sodium chloride, but none of the rod forms grew in this concentration of salt. Whenever salt-cured ham and tongue and canned chicken were analyzed previous to using them as experimental fillers, staphylococci were found to be the most common natural contaminants. They invariably over-grew other organisms in ham and tongue if others were present. The same was true in chicken at refrigeration temperature. The " food poisoning " staphylococci reproduced most rapidly at 37° C., but they were quite active at room temperature and even grew in the icebox after a lag period of more than 1 day. Given a start in warmth, they developed more rapidly

* Details of these experiments are to be found in a thesis by Florene C. Kelly submitted in partial fulfillment of the requirements for the degree of Master of Science in the Department of Hygiene and Bacteriology, University of Chicago, August, 1936.

at 8° C. Their ability to grow in the cold was further substantiated by the fact that agar slants and broth inoculated with these organisms showed scant growth after several days' refrigeration.

An attempt was made to determine whether the penetration of the staphylococci into the bread of sandwiches was accomplished by growth or by mechanical means. India ink and carmine particles, as well as killed, stained staphylococci, were used instead of actively growing staphylococci to compare the migration of living and lifeless particulate matter into the bread. Results of these experiments indicated that, after a distance of 1 cm., the penetration was due to active growth on the part of the bacteria. The influence of water content in bread was also investigated, and it was found that while there was plenty of moisture (37–43 per cent) in the average loaf, an increase to 51 per cent greatly accelerated the growth of the staphylococci. Certain sandwich fillings, for example lettuce, may considerably enhance the water content. The modern practice of wrapping sandwiches in waxed paper to prevent drying allowed more rapid growth, as shown when wrapped and unwrapped sandwiches were stored for more than 4 hours.

PRODUCTION OF ENTEROTOXIC SUBSTANCE IN BREAD AND MEAT

Having established the fact that the "food poisoning" staphylococci multiply in the bread of sandwiches as well as in the meat filling, experiments were undertaken to determine whether the enterotoxic substance resided in the meat, which originally contributed the organisms, or in the bread, a medium naturally rich in starch, or in both these components. Four human volunteers who were not subject to gastrointestinal upsets participated in feeding experiments.

In preparing the foods an attempt was made to imitate the natural conditions, especially that of moisture content. A saline suspension of the food poisoning organism which had been isolated from the bread of the Indianapolis tongue sandwich was swabbed over one side each of two slices of fresh bread. A sandwich was made by placing crisp lettuce between the two inoculated surfaces of the bread. It was then wrapped in waxed paper and incubated at 37° C. The meat used was sliced from a home baked ham. These slices, sufficient for one generous sandwich, were inoculated by swabbing one side of each piece, just as the bread had been treated. Lettuce was placed between the slices of ham, and this "sandwich" was wrapped and incubated as described for the bread and lettuce. After 5 hours' incubation one individual ate the bread sandwich, which contained 125,000,000 yellow staphylococci per gm., and the other volunteer ate the ham, containing 30,000,000 of these organisms per gm., with no ill effects.

Duplicate samples had been made when the first food was prepared. These samples were kept at 37° C. for 5 hours, refrigerated for 16 hours, and then reincubated for another 6 hours. Such time and temperature conditions more nearly simulated those to which the Indianapolis sandwiches had been subjected, although the latter were kept 18 hours at room temperature in warm weather. These foods were fed to the same volunteers, meat to one and bread to another. The person who ate 55 gm. of bread, containing 600,000,000 staphylococci per gm., experienced no reactions. On the other hand, 4¾ hours after eating 48 gm. of ham, containing 1,000,000,000 organisms per gm., the second individual developed a typical case of food poisoning (see Table II). A control sample of ham which was in-

cubated and refrigerated along with the food poisoning sample contained about 5,000,000 organisms per gm. These were staphylococci of various pigmentation, probably from the ham, together with typical soil and water, Gram-negative, non-spore-forming rods which might well have been introduced by the lettuce. Salmonella and dysentery organisms were not present. The test samples of bread and ham were not changed in appearance, odor, or taste by the enormous numbers of staphylococci growing in them.

Because there were more staphylococci in the ham than in the bread in the above experiment, the bread feedings were repeated on two more volunteers (a test and a control), and again on the same individual who ate the bread in the previous tests. This time larger amounts of the bread, containing more staphylococci, were eaten. The method of inoculation and preparation of the lettuce sandwiches and the incubation time and temperatures were the same as those used in the previous experiment. The control sandwich was treated exactly the same as the others, except that no staphylococci were introduced. The volunteer (No. 4) eating the uninoculated bread (control) and the one (No. 2) who had previously eaten the contaminated bread and upon whom this test was repeated, were not made ill, but the volunteer (No. 3) eating contaminated bread for the first time developed typical severe food poisoning symptoms and was confined to bed for 2 days (see Table II).

DISCUSSION

The report of the Indianapolis epidemic describes a situation in which food was prepared for a large number of persons about 18 hours before it was consumed. Foods incriminated in staphylococcus food poisoning outbreaks usually have a history which includes storage at room temperature. Under similar circumstances staphylococci entered and grew to huge numbers in the bread when they were

TABLE II

FEEDING OF HUMAN VOLUNTEERS WITH HAM OR BREAD IN WHICH A FOOD POISONING STRAIN (S2) OF STAPHYLOCOCCUS HAS GROWN

Volunteer No.	*Food Ingested in Grams*	*Staphylococci per Gram in Millions*	*Results*
1	Ham, 48	1,000	4¾ hours after eating, nausea, vomiting and diarrhea. Blood and mucus in stools toward end of acute illness. Acute symptoms lasting 3 hours.
2	Bread, 55	600	No effect
2	Bread, 75	1,000	No effect
3	Bread, 63	1,100	3¾ hours after eating, nausea, vomiting and diarrhea. Severe prostration. No blood or mucus in the stools. Acute symptoms of nausea and vomiting lasting over period of 13 hours.
4	Bread, 63	None *	No effect

* 8,000,000 soil and water bacteria (from lettuce) per gram

introduced in meat fillings of experimental sandwiches. Incidentally, when wrapped minced ham and mayonnaise sandwiches bought in drug stores were analyzed, they commonly showed enormous numbers of staphylococci and very few, if any, other types of bacteria. In no instance did any of the staphylococci cause perceptible changes in the appearance or odor of meats or bread, even when there were millions present. In view of the large numbers of staphylococci present in sandwiches purchased at food counters where they have been exposed to warm temperatures for hours, it would seem that such foods should more often be involved in food poisoning. Apparently these sandwiches are usually eaten with no untoward results. However, isolated cases of acute gastrointestinal upsets may be more common than is generally supposed. There is no way of estimating the incidence of such cases when physicians are not called and health officers are not notified. Only when an epidemic occurs is any investigation made or any record kept. It seems unlikely that the frequency of staphylococcus food poisoning can be fairly judged from such reports.

Dolman [17] suggests that the power to initiate gastrointestinal symptoms may be due to a metabolite produced by only a few strains of staphylococci growing under unusual circumstances. In our experiments, however, the "food poisoning" staphylococci needed no special conditions for the production of the toxic factor, except a long enough incubation period in the meat sandwich. Dolman bases his contention that certain strains of staphylococci produce the enterotoxic substance on experiments in which about 200 strains of pathogenic staphylococci were tested and in which only one, a strain obtained from Dr. Jordan, yielded a filtrate which caused gastrointestinal disturbances in human volunteers.

Our experience justifies the opinion that individual resistence to the enterotoxic substance may be extreme. This is well illustrated in the case of one volunteer who was not affected by eating bread in which the food poisoning strain of staphylococcus had grown, although a similar specimen of bread fed to another volunteer produced severe symptoms.

The material necessary for the production of the enterotoxic substance was supplied by both the bread and the ham. The fact that the staphylococci growing in ham could cause poisoning is in agreement with Stone's epidemiologic studies in California, where he observed that protein was the consistent major ingredient of food involved in every outbreak.[2] However, according to our findings, a food rich in protein is not essential; bread, a food naturally rich in starch and poor in proteins, supported the growth of the food poisoning staphylococci and the elaboration of their enterotoxic substance.

It is noteworthy that this staphyloccus has retained the property of producing such a toxic substance after having been cultivated on laboratory media for over a year.

SUMMARY

Experimental food poisoning has been produced in human volunteers by feeding either bread or meat in which a "food poisoning" strain of staphylococcus has grown. One individual who ate bread containing millions of these staphylococci developed severe food poisoning symptoms, suffering severe prostration lasting 13 hours. The same organism growing in ham caused similar characteristic reactions in a second volunteer. A third individual proved resistant on two occasions after eating bread containing millions of this "food poisoning" strain. Since both the bread and ham

served as favorable media for the production of an enterotoxic substance by the staphylococci, it is evident that the toxic factor in sandwiches which cause food poisoning may reside not only in the meat filling but also in the bread. When these yellow, hemolytic staphylococci were introduced into meat sandwiches by inoculation of the filler, they quickly invaded the bread, penetrating slices 1 cm. thick in a few hours. Bread is not a selective medium for this staphylococcus in the sense that only this type of organism can grow in bread and others cannot; nor is its rate of multiplication in bread greater than that of other bacteria. On the other hand, meat of high salt content proved selective for staphylococci in general. The food poisoning organism, along with other similar morphological types, thrived in a concentration of salt which prevented the growth of rod forms.

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Tribute to Dr. Park

ACCORDING to an ancient myth a mortal beloved of the gods was granted immortality, but eternal youth was not joined to the gift and he was left "to dwell in presence of immortal youth—immortal age beside immortal youth." That may be said to be the fate of Dr. Park, who yesterday retired from his long and remarkable official service in saving lives of others. If the years which he has added to their lives could be added to his own, he would indeed know the full fate of Tithonus. As it is, the people of this city, and not alone they, have reason for a gratitude outlasting generations, for the lengthening of the life of one generation has a cumulative value. It is a pleasure to think of him as toiling on in the seclusion of his private laboratory high above the city whose guarding has been his care for so many years.—Editorial. *New York Times*, Oct. 1, 1936.