Staphylococci and Salmonellae
in Commercial Wrapped Sandwiches

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PUBLIC HEALTH officials have long considered refrigeration essential to the safe handling of perishable foods. In recent years the application of this time-honored procedure has been challenged by a rapidly expanding food industry specializing in the production of wrapped sandwiches. Sandwiches are prepared almost entirely by hand from a variety of perishable foods known to support bacterial growth. Following fabrication they are transported in panel trucks and displayed for sale on the shelves of filling stations, grocery stores, fruit stands, and similar establishments at ambient air temperatures. Previous investigation has shown that exposure frequently ranges up to 48 hours and may sometimes approach 96 hours from the time that the sandwich was made.

Although one would expect numerous food poisoning outbreaks as a result of the industry's apparent disregard for acceptable food handling practices, very few are definitively reported in the literature (*1–3*). In addition, reported incidents have not increased in proportion to expanded production. In Georgia, only 1 of more than 60 manufacturers has ever come under suspicion in a reported foodborne outbreak. This recent episode involved a box lunch which contained two kinds of wrapped sandwiches in addition to several other items. Circumstances were such that neither the exact food nor the etiological agent could be specifically identified.

However, if one chooses to review the food poisoning history of sandwiches in general, an entirely different impression is gained. According to the *Morbidity and Mortality Weekly Report* published by the Public Health Service, between the years of 1951 and 1963 sandwiches of various kinds served as vehicles in 133 foodborne outbreaks involving approximately 5,947 persons. Almost without exception incriminated sandwiches were prepared by caterers or restaurateurs using ingredients of uncertain age and condition—ingredients which if consumed prior to being incorporated into sandwiches could have engendered food poisoning, as has been demonstrated on occasion (*4, 5*).

Closer examination of the situation will reveal that practically all data adduced as favoring refrigeration of wrapped sandwiches are based upon experiments with various sandwich ingredients. Uniformly these ingredients have been divorced from an actual ecological situation, sterilized by autoclaving, inoculated with a million or more food-poisoning organisms per gram, and held at incubation temperatures for several hours. Although such an approach unquestionably demonstrates the ability of these organisms to multiply rapidly in certain perishable foods under ideal growth conditions, it would seem somewhat unrealistic.

In the absence of a body of epidemiologic evidence condemnatory of wrapped sandwiches as causes of food poisoning despite a continuing effort to find such evidence, considerable doubt

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arises as to the necessity for refrigeration of wrapped sandwiches between the time of manufacture and sale. The purpose of this study was to get some measure of the actual food-poisoning hazards incident to commercially prepared, wrapped sandwiches under present conditions of fabrication, transportation, and sale.

The summary prepared by Dauer (*6*) and our own experience in Georgia indicate that staphylococci and salmonellae continue to be the organisms most often incriminated in food-borne outbreaks in this country. Therefore, this investigation was directed primarily toward isolation and identification of these two genera. Near the conclusion of the study several groups of sandwiches were inoculated with a strain of coagulase-positive *Staphylococcus* previously isolated from a sandwich to get some idea of what might be expected if unusually large numbers of such organisms found their way into the sandwich system.

Materials and Methods

Sandwiches originally selected for study included ham and cheese, deviled egg, and chicken salad, since these foods are known to support the growth of coagulase-positive staphylococci or salmonellae or both. After sampling had begun it was discovered that a number of manufacturers were substituting for ham a less expensive canned spiced ham containing chopped pork, pork hearts, pork tongue, salt water, sodium nitrate, sodium nitrite, and spices. In addition, two manufacturers from whom samples were collected were using an imitation chicken product consisting of chopped pork, pork skins, pork stomachs, vinegar, and spices as a replacement for chicken in their chicken salad. There was no attempt to persuade the manufacturers using these materials to change or to control the environmental conditions to which sandwiches were subjected. Most of the sampling was done from August through October 1961 and April through July 1962. During these periods ambient temperatures ranged between 60° F. and 95° F.

Twenty sandwiches (one sampling) were collected from the line at a given manufacturing site. All samples were coded. Ten were placed in a wire basket which was locked and taken to a retail outlet by a route man along with regular delivery. The other 10 were quick frozen on dry ice and transported to the laboratory. At the laboratory the samples were transferred from dry ice to a mechanical freezer set at −20° F.

On several occasions, spot checks were made to insure that sandwiches in the basket did not get preferential treatment. Forty-eight hours after these sandwiches had been made, they were picked up from the retail outlet, also frozen on dry ice, taken to the laboratory, and transferred to a mechanical freezer. No sandwiches were held in a frozen state more than 6 days prior to bacteriological examination.

Sandwiches to be examined were removed from the freezer and allowed to thaw at room temperature for approximately 20 minutes.

Using an aseptic technique, one-half of each sandwich was taken from its cellophane wrapping, weighed to the nearest 0.1 gm., transferred to a sterile Waring Blendor jar, and triturated with sufficient sterile phosphate buffered diluent (pH 7.0) to obtain a 1:10 dilution. Operating the blendor motor for 1 minute on low speed and 1 minute on high speed gave an homogenous mixture devoid of large particles.

Standard plate counts were obtained from serial dilution pour plates using the standard method agar (BBL). Colonies were counted at the end of 48 hours incubation at 32° C. Coliform counts were made from violet red bile agar (Difco) pour plates incubated at 32° C. for 24 hours.

In an effort to give salmonellae the maximum advantage for differential growth, approximately 1-ml. quantities of the original 1:10 homogenate were inoculated into tetrathionate broth which was subcultured after 18 to 24 hours incubation at 37° C. to brilliant green agar plates (Difco). *Salmonella*-like colonies appearing on the plates following 24-hours incubation at 37° C. were picked to triple sugar iron agar (Difco) slants for presumptive identification. Final confirmation or rejection was based on urease production and slide agglutination tests.

One-tenth ml. aliquots from serial dilutions were pipetted onto the surface of mannitol salt

Table 1. Average findings for various types of sandwiches (combined results of 10 sandwiches in each sample)

Kind of sandwich and sample number	Average plate count		Average coliform count		Coagulase-positive staphylococci per gm.		Average pH	
	Fresh	48 hours	Fresh	48 hours	Fresh	48 hours	Fresh	48 hours
Chicken salad:								
1	9, 000	270, 000	90	<10		[2] (1) 1, 000	4. 79	4. 74
2	340, 000	520, 000, 000	2, 000	[1] 170, 000		[2] (1) 1, 000	5. 40	4. 33
3	340, 000	290, 000, 000	55, 000	180			5. 27	4. 79
4	490, 000	130, 000, 000	68, 000	6, 900			5. 39	4. 90
5	560	4, 800	<10	<10			5. 09	5. 09
6	180, 000	590, 000, 000	65, 000	2, 600			5. 34	4. 51
7	180, 000	28, 000, 000	1, 300	300			5. 04	5. 00
8	380, 000	310, 000, 000	6, 500	1, 300			5. 30	4. 24
Imitation chicken salad:								
1	210, 000	460, 000	140	<10			5. 11	5. 07
2	74, 000	110, 000	140	30			5. 02	
Deviled egg:								
1	65, 000	4, 900, 000, 000	15, 000	620, 000, 000			6. 18	5. 28
2	95, 000	770, 000, 000	30	330	[2] (1) 2, 000		5. 78	4. 83
3	31, 000	46, 000	20	<10			4. 93	5. 01
4	92, 000	77, 000, 000	9, 300	1, 300			5. 03	4. 99
Spiced ham and cheese:								
1	36, 000, 000	620, 000, 000	10	10			5. 46	5. 35
2	17, 000	20, 000, 000	100	120			5. 97	5. 94
3	370, 000	350, 000, 000	440	130			5. 86	5. 80
4	3, 000	1, 100, 000	10	10			5. 89	5. 88
5	3, 000	980, 000	10	250			5. 93	5. 80
6	3, 000	15, 000, 000	10	10			5. 83	5. 87
Ham and cheese:								
1	570, 000	5, 300, 000	60	10			5. 60	5. 51
2	19, 000	5, 100, 000	10	10			6. 21	6. 16
3	8, 800	100, 000, 000	30	150			5. 86	5. 62
4	3, 000	9, 700, 000	10	10		[2] (1) 3, 000; [2] (1) 2, 000; [2] (1) 1, 000		

[1] Contained leaf of lettuce.
[2] Numbers in parentheses indicate isolations of coagulase-positive staphylococci made from a sampling of 10 sandwiches. Accompanying numbers indicate counts per gram of sandwich.

agar plates (Difco). The 0.1 ml. drop was then spread with a sterile glass rod having a 45° angle bend near one end. After 48-hours incubation at 37° C., representative *Staphylococcus*-like colonies were picked to infusion broth, which was held at 37° C. overnight. Two drops of the overnight infusion broth culture were added to 0.5 ml. reconstituted coagulase plasma (Difco). Any sign of coagulation within 3-hours incubation at 37° C. was considered a positive test. When coagulation occurred, the coagulase-positive staphylococci per gram of sandwich were determined by counting the number of similar colonies appearing on the corresponding mannitol salt agar plates.

Following inoculation of all media, the pH of blendorized sandwiches was determined with a Leeds-Northrup Model 7664–A1 pH meter equipped with miniature electrodes and a device for temperature compensation.

One group of imitation chicken salad, three groups of chicken salad, four groups of spiced ham and cheese, and two groups of deviled egg

sandwiches were inoculated with coagulase-positive staphylococci by placing one drop of a 1:10 dilution of an overnight broth culture directly onto the fill. Coagulase-positive staphylococcus counts and pH determinations were obtained on all inoculated samples as described previously. Where uninoculated controls were tested, plate counts, coliform counts, coagulase-positive staphylococcus counts, salmonellae determinations, and pH determinations were done. Sandwiches to be examined at the end of 48 hours were held at room temperature (23°–26° C.). Samples were not incubated because it was felt that a constant favorable temperature situation would seldom be encountered under field conditions.

Table 2. Average findings for fresh chicken salad sandwiches (combined results of 10 sandwiches in each sample)

Time of preparation and sample number	Average plate count	Average coliform count
Day:		
1	9, 000	90
2	560	10
3	180, 000	65, 000
4	180, 000	1, 300
5	[1] 210, 000	140
6	[1] 74, 000	140
Night:		
1	340, 000	2, 000
2	340, 000	55, 000
3	490, 000	68, 000
4	380, 000	6, 300

[1] Made from imitation chicken salad containing chopped pork, pork stomach, water, pork skin, vinegar, and spices.

Results

Eight hundred and twenty wrapped sandwiches from 15 different manufacturers were examined by the procedures outlined. The results are summarized in tables 1 through 3.

Fresh sandwiches usually contained a relatively large number of bacteria per gram, and the count tended to increase after 48-hours exposure to actual conditions of transportation and display. As would be expected from the amount of handling required in their preparation, fresh salad-type sandwiches generally con-

Table 3. Average findings for commercially prepared wrapped sandwiches inoculated with coagulase-positive staphylococci (combined results of 10 sandwiches in each sample)

Kind of sandwich and sample number	Average plate count		Average coliform count		Average coagulase-positive staphylococci per gm.		Average pH	
	Fresh	48 hours	Fresh	48 hours	Fresh	48 hours	Fresh	48 hours
Imitation chicken salad:								
1	94, 000	110, 000	140	30	110, 000	100, 000	5. 02	[1]
Chicken salad:								
1	410, 000	310, 000, 000	6, 300	1, 300	21, 000	210, 000	5. 30	4. 25
2	[1]	[1]	[1]	[1]	38, 000	68, 000	5. 27	4. 64
3	[1]	[1]	[1]	[1]	26, 000	17, 000	5. 40	4. 53
Spiced ham and cheese:								
1	7, 600	3, 000, 000	20	10	[2] 300, 000	6, 200, 000	5. 78	5. 74
2	6, 500	2, 900, 000	90	30	[2] 170, 000	4, 300, 000	5. 80	5. 80
3	[1]	[1]	[1]	[1]	[3] 59, 000	62, 000, 000	6. 78	6. 12
4	[1]	[1]	[1]	[1]	[3] 53, 000	63, 000, 000	6. 34	6. 06
Deviled egg:								
1	[4] 290, 000, 000	850, 000, 000	[4] 350, 000	94, 000	35, 000	14, 000	5. 20	4. 51
2	58, 000	1, 100, 000	10	10	21, 000	15, 000	5. 11	5. 10

[1] Not done.
[2] Inoculum placed on the slice of spiced ham in contact with the bread and mayonnaise.
[3] Inoculum placed between the slice of spiced ham and the slice of cheese.
[4] Counts are based on 2 sandwiches rather than 10.

tained more micro-organisms than either ham and cheese or spiced ham and cheese sandwiches of equal age. Surprisingly, fresh chicken salad sandwiches made at night consistently gave higher counts than those made during the day (table 2).

Coliform organisms. Coliform counts on fresh samples were generally proportioned to the plate counts. With one exception, a marked reduction in numbers of coliform organisms was observed at the end of the 48-hour exposure period in both chicken salad and imitation chicken salad sandwiches (table 1). No definite trend toward an increase or decrease in coliform bacteria could be established for deviled egg, ham and cheese, or spiced ham and cheese sandwiches (table 1).

Staphylococci. Although large staphylococcal populations were often observed, coagulase-positive strains were present much less frequently and in far smaller numbers than had been anticipated at the beginning of this study. Recoveries were made from 6 of 820 (0.73 percent) of the sandwiches examined (table 1). Of these six sandwiches, one was fresh and the remaining five were 48 hours old. In no case were coagulase-positive staphylococci found in both fresh and 48-hour samples. Even though the latter group yielded these organisms more frequently, none of the counts obtained were of the magnitude usually associated with staphylococcal food poisoning. The differences observed were not statistically significant, and counts ranged between 1,000 and 3,000 per gram in both fresh and 48-hour samples.

Coagulase-positive staphylococci inoculated into commercially prepared chicken salad sandwiches increased slightly after 48 hours (table 3). When imitation chicken salad and deviled egg sandwiches were cultured following similar exposure conditions, growth of staphylococci could not be demonstrated. Not only did the inoculated organisms fail to multiply in imitation chicken salad sandwiches, but the growth of the natural flora was equally unimpressive (table 3). Since large saprophytic populations developed in regular chicken salad sandwiches and in the deviled egg sandwiches held under identical conditions, the imitation chicken product seems to be a very poor medium for bacterial growth.

By contrast, a substantial increase of coagulase-positive staphylococci occurred in spiced ham and cheese sandwiches, and initial placement of the inoculum appeared to have a dramatic influence upon the final numbers attained. Staphylococci seemingly grew far better if placed between the slice of spiced ham and the slice of cheese than if dropped onto the surface of a slice of spiced ham which, when the sandwich was completed, had contact with the mayonnaise and bread (table 3).

Salmonellae. Although the methods used were those routinely employed with success in the recovery of salmonellae from foods involved in outbreaks of disease, no salmonellae were isolated from sandwiches during the entire study. Since all results were negative, information concerning search for them has been omitted from the tables and will be included in a report dealing with *Salmonella* inoculation studies now in progress.

pH. The pH of fresh sandwich samples was below 7 and tended to become more acid, within the error of measurement, after 48 hours at ambient temperatures.

Discussion

The presence of food-poisoning organisms at some time prior to serving is requisite to food-borne bacterial gastroenteritis. From the results of this study and the work of Adame and associates (*7*), it appears that commercially prepared, wrapped sandwiches, even though handmade, are seldom contaminated with potentially enterotoxigenic staphylococci. The occurrence of salmonellae in this packaged food item would seem a rarity indeed. Thus one factor responsible for the paucity of reported outbreaks attributable to unrefrigerated, wrapped sandwiches is the general absence of an etiological agent.

However, since coagulase-positive staphylococci can be found occasionally, this explanation will not suffice entirely. Conditions within the sandwich itself undoubtedly influence the ability of food-poisoning organisms to increase to dangerous proportions. Competition is a factor deserving considerable attention in this regard.

The tables contain evidence that few coagulase-positive staphylococci chanced to

gain entrance into fresh sandwiches, and in every instance they appeared to be overgrown by the truly enormous saprophytic population which developed during the 48-hour holding period. Such competition or antagonism is even more apparent in salad sandwiches inoculated with coagulase-positive staphylococci (table 3). When the bacterial count of fresh samples was in the tens and hundreds of thousands and the inoculum was considerably less, little or no growth of staphylococci occurred during the holding period. Even when the inoculum was slightly more than the initial total bacterial count, as in the imitation chicken salad sandwiches, staphylococci failed to grow. In this instance, however, lack of substantial increase probably was partially attributable to the medium alone (table 1).

In the spiced ham and cheese sandwiches, where at the outset the inoculum was considerably greater than the natural bacterial population, a sizable increase in staphylococci occurred (table 3).

Similar findings have caused other workers to stress the importance of competition in preventing food poisoning. Peterson and associates (*8*) reported that staphylococci occurring naturally in frozen chicken potpies did not reach significant numbers under any condition of defrost; whereas large psychrophilic and other mesophilic populations developed under the same conditions. Staphylococci were able to reach large numbers in macaroni and cheese dinners upon extended incubation, but only after the development of a tremendous saprophytic population had caused advanced spoilage rendering the product inedible. Obvious deterioration due to saprophytic bacterial action most likely prevents the consumption, on numerous occasions, of potentially hazardous wrapped sandwiches.

In further studies (*9*), these investigators found that staphylococci were suppressed when grown on artificial media at room temperature in competition with psychrophiles originally isolated from a frozen chicken potpie. Additionally, the repressive effect appeared to be more pronounced when staphylococci comprised a relatively small portion of the total bacterial population. Such findings led these workers to conclude that large saprophytic populations often provide a built-in safety factor against food poisoning.

Competition and antagonism have been emphasized even further by the recent work of Dack and Lippitz (*10*). These investigators found that the natural flora of frozen chicken, turkey, and beef potpies has an inhibitory effect upon the growth of staphylococci, *Salmonella typhimurium*, and *Escherichia coli* inoculated into a slurry made from the pies and incubated for 18 hours at 35° C. Also, low pH was thought to account for some of the inhibitory effect observed.

In unrefrigerated wrapped sandwiches, low pH appears to be equally as significant in the prevention of foodborne illness as bacterial competition, if not more so. Salad dressing and mayonnaise are among the most commonly used sandwich spreads. Our limited data on inoculated spiced ham and cheese sandwiches (table 3) suggest that small numbers of potentially enterotoxigenic staphylococci on the surface of a slice of meat or cheese subsequently coated with one of these acid spreads would likely be inhibited.

No sandwiches were inoculated with *Salmonella* during the initial study. However, the work of other investigators and results obtained in our laboratory in 1964 imply a similar inhibitory effect on these organisms. Wethington and Fabian (*11*) reported that food-poisoning staphylococci and salmonellae failed to survive in commercial mayonnaise and salad dressing because of the acetic acid content. Earlier, Nunheimer and Fabian (*12*) found acetic acid more inhibitory to food-poisoning staphylococci at a given pH than other organic acids.

In view of the apparent effects of these acid products, it is entirely conceivable that the food-poisoning history of commercially prepared, wrapped sandwiches might be quite different were oleomargarine or some other less acid spread customarily used. Additional studies concerned with this aspect are also now in progress.

As previously pointed out in the results section, coagulase-positive staphylococci inoculated into chicken salad and egg salad sandwiches showed little or no increase after the

48-hour holding period. In addition to salad dressing and mayonnaise, these sandwiches often include ingredients such as lemon juice, dill pickles, and sweet pickle relish which, according to Longree and associates (*13*), result in an acid end product more acceptable to the consumer but less favorable for the growth of food-poisoning staphylococci. Unless contamination of the meat and a chance for incubation occur prior to the addition of various acid ingredients, it would seem that meats such as chicken, ham, and tuna in salad form are less likely to cause *Salmonella* or staphylococcal food poisoning than in other forms. To lend further support to this theory, a search of the literature revealed that in almost every well-documented outbreak incriminating chicken salad or ham salad, ample opportunity for contamination and incubation occurred before the salad was mixed. Blending in of acid salad ingredients without delay is apparently a very important food poisoning deterrent which heretofore has been overlooked.

Adame and associates (*7*) in their work on wrapped sandwiches suggested that bacteria probably increase more readily in wet or salad-type sandwiches than in dry-type sandwiches when exposed to ambient air temperatures for several hours and that the salad types constitute a potential food-poisoning hazard. In addition to receiving greater initial bacterial contamination, salad or wet sandwiches contain more mayonnaise and are usually more acid than the dry type. We have also noted that general saprophytic micro-organisms grow luxuriantly in the wet type regardless of acidity. In our experience, however, the coagulase-positive staphylococci have reproduced more readily in the less acid, less bacterially competitive dry type. Thus it would be amiss to say that one kind of sandwich is potentially more hazardous from the standpoint of food poisoning than another kind purely on the basis of its ability to support general bacterial growth. A simple ham and cheese sandwich with mayonnaise on the bread only may be potentially more likely to support the growth of pathogens than sandwiches constructed from mixtures hitherto regarded as being figuratively tailormade for the purpose of causing gastroenteritis.

The larger bacterial populations in fresh chicken salad sandwiches made by night operators than in those made by day operators (table 2) are thought to have been caused by the night operators holding salads in bulk for a longer period prior to sandwich making. Night operators customarily mix salads during the day for use at night; whereas day operators routinely mix salads early in the morning before sandwich making begins. Although salads were refrigerated, storage in large quantities results in slow cooling and undoubtedly provides opportunity for bacterial increase on numerous occasions.

The only group of chicken salad sandwiches with increased coliform organisms after 48 hours contained a leaf of lettuce (table 1). The presence of lettuce would somehow seem to enhance the growth of coliform organisms.

Whether enterotoxin may have been released in inoculated sandwiches is not known. It is very doubtful that this occurred in the chicken salad, imitation chicken salad, or deviled egg sandwiches since growth in 48 hours was so slight. Enterotoxin may well have formed in spiced ham and cheese sandwiches, particularly where staphylococci were deposited between the slices of ham and cheese. Yet, epidemiologic evidence suggests otherwise. One possible explanation may be low pH. Some investigators believe that for maximum release of enterotoxin, the pH must go to 8.5 (personal communication, Elizabeth C. McCoy, department of bacteriology, University of Wisconsin College of Agriculture). Spiced ham and cheese sandwiches were considerably lower on the scale than this. However, the pH at a given focus of growth may have approached 8.5 without being reflected in a measurement of the total sandwich.

As a further check on the validity of reporting of food poisoning from wrapped sandwiches and in an effort to correlate our bacteriological findings with the truly enormous number of "feeding experiments" performed daily, we sent a letter to reporting physicians in Georgia asking them to notify the Georgia Department of Public Health of any case of gastroenteritis which might be attributable to wrapped sandwiches. To date, no physician on our list of

general practitioners, pediatricians, and internists has indicated that he has had occasion to suspect wrapped sandwiches as the source of either an outbreak or sporadic case. During the study period, a number of food poisoning outbreaks of various etiologies have been reported and investigated in detail.

Summary and Conclusions

A total of 820 commercially prepared, wrapped sandwiches of various kinds were evaluated bacteriologically when fresh and after 48 hours' exposure to conditions of ambient temperature encountered routinely. The pH of samples was also determined at these intervals. Coliform counts tended to decrease in salad-type sandwiches during holding. No salmonellae were found.

Staphylococci were sometimes very numerous, but few were coagulase-positive. No significant increase in these could be demonstrated with aging of sandwiches. Coagulase-positive staphylococci inoculated into egg salad and chicken salad sandwiches failed to increase appreciably when held at room temperature for 48 hours, but large saprophytic populations developed under these conditions. Competition from these saprophytic organisms was thought to have been partially responsible for suppressing the growth of inoculated staphylococci. Acidity also appeared to be an important factor.

Spiced ham and cheese sandwiches seem to be more hazardous. However, growth of coagulase-positive staphylococci was affected by placing the inoculum on the side of the spiced ham in contact with the mayonnaise.

The customary use of commercial mayonnaise and other acid ingredients produces a low pH which with time tends to become more acid, often creating an unfavorable environment for the growth or even survival of the limited numbers of coagulase-positive staphylococci which occur. Results of this study offer some insight into the epidemiologic observation that these products, although seemingly mishandled, are rarely incriminated in food-poisoning outbreaks.

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