Salmonellae and Salmonellosis Associated with Milk and Milk Products. A Review.

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Abstract
There has been a renewed interest in the occurrence of salmonellae in food products, prompted mainly by recent disclosures that the organisms were recovered from a wide variety of foods. The 1,200 or more serotypes of these Gram-negative, facultative, asporogenous, rod-shaped bacteria are all considered to be human pathogens. In man they can cause enteric fevers (i.e., typhoid fever and related ailments), gastroenteritis, and septicemias. Treatment of salmonellosis is often difficult, and a limited number of patients continue to shed the organisms for extended periods even though they appear to be recovered.

During 1966 there were approximately 17,000 reported cases of salmonellosis in the United States. This is an increase from 6,693 in 1957. Cases of typhoid fever decreased by nearly 75% during this same period. It is believed by some that the number of reported salmonellosis cases represents from 1 to 5% of the actual cases in the United States. Reported deaths in the United States from all types of salmonellosis averaged slightly less than 100 annually during the 1957-1966 period. Although most outbreaks of human salmonellosis are attributable to such animal products as eggs and meat (mainly poultry), dairy products, on occasion, have been reported as vehicles for the spread of this ailment.

Salmonellae grow at temperatures of 5.5 to 45 C, at $a_w$ values of 0.945 to 0.999, are retarded by acids and are inactivated if the pH is low enough, are destroyed by conventional pasteurization treatments, are easily destroyed by chlorine and quaternary ammonium compounds, can survive but not grow in media with 15 to 30% sodium chloride, and may be inhibited by sorbic acid.

Salmonellae are known to occur in raw milk, but the frequency and level are unknown. Consumption of contaminated raw milk has led to outbreaks of salmonellosis. Pasteurized milk, too, has been responsible for disseminating these organisms which occurred in the product through contamination after heating. Occurrence of salmonellae in certain dairy products has been demonstrated in the United States and elsewhere. This review of the literature further discusses the circumstances under which these organisms may occur in dairy products or ingredients used in combination with dairy products in the preparation of various foods.

Interest in the occurrence of salmonellae in milk and milk products has been greatest during two periods of time. One of these occurred some years ago, when pasteurization was not a widespread practice and outbreaks of typhoid fever were associated with consumption of the untreated products. Another period of great interest began in 1966, when outbreaks of salmonellosis were associated with ingestion of contaminated nonfat dried milk. As a consequence of this renewed interest in the relationships between salmonellae and dairy products, it was felt that a review of the literature should be prepared so that concerned persons might have available a ready source of information on this subject. The present paper is the result of that belief.

It is the purpose of this review to discuss: a) the bacteria responsible for salmonellosis, b) the disease itself, c) how frequently salmonellosis occurs in the United States, d) some characteristics of salmonellae of particular importance to the processor of dairy products, and e) occurrence of salmonellae in milk and milk products. Major emphasis in this review will be given to characteristics of salmonellae with which the processor should be familiar and to the occurrence of these organisms in dairy products. The discussion of the bacteria, the disease, and the frequency with which salmonellae occur in milk and milk products was featured in this review.

1 Publication of this paper was financed by a grant from the Dairy Industry Committee, 1105 Barr Building, Washington, D.C. 20006. Members of this committee include the American Butter Institute, American Dry Milk Institute, Dairy and Food Industries Supply Association, Evaporated Milk Association, International Association of Ice Cream Manufacturers, Milk Industry Foundation, and the National Cheese Institute.
Salmonellae

Typhoid fever was studied by William Budd, in 1856, and he concluded the disease was infectious, the causative agent was excreted in the feces of patients, and contaminated milk and water were important in its dissemination (135). It was not until 1880 that Eberth observed the typhoid bacillus in tissues of dead patients. Four years later, in 1884, Gaffky isolated and cultivated this organism. Another year later, Salmon and Smith isolated an organism from cases of swine fever which they considered to be the causative agent and which they named Bacillus cholerae suis (192). It is now known that swine fever is caused by a virus, and the bacillus which Salmon and Smith isolated was probably present as a secondary invader. Nevertheless, the bacteria in the typhoid-paratyphoid-enteritis group were given the generic name Salmonella, on the recommendation of Lignieres, in honor of the American bacteriologist, D. E. Salmon, first chief of the U.S. Bureau of Animal Industry (231).

In 1888 Gaertner isolated Salmonella enteritidis from a patient who died after consuming contaminated meat, and soon afterward Durham and de Noeble described Salmonella typhimurium which was also recovered from patients ill with gastroenteritis following ingestion of infected meat (135). Loeffler, in 1892, identified the causative agent of mouse typhoid as a member of this group, and soon many additional related organisms were described by other investigators (231).

Bacteria in the genus Salmonella are Gram-negative, asporogenous, facultative short rods which are usually motile by means of peritrichous flagella, although nonmotile forms may occur (35a). They are easily cultivated on ordinary media and are able to produce acid (and usually gas) from glucose, mannitol, maltose, and sorbitol (35a). Generally lactose, sucrose, salicin, and adonitol are not attacked. Fermentation of carbohydrates other than those listed is variable. Salmonellae generally fail to produce acetoin or hydrolyze urea. They do, however, produce nitrite from nitrate.

Salmonellae average about 2 to 3 μ in length and about 0.6 μ in width, but may vary in size under different environmental conditions (135). Young cultures on agar may form a predominance of coco-bacillary cells, whereas filamentous forms are occasionally seen in cultures grown in liquid media (135). Capsules are normally not formed by salmonellae grown at 37 C, but most species give rise to mucoid colonies consisting of encapsulated cells, especially when grown at 20 C.

On ordinary agar media, salmonellae produce colonies, averaging 2 to 3 mm in diameter, difficult to distinguish from those of coliform bacteria (135). Freshly isolated strains almost invariably produce circular, smooth, glossy colonies that are more translucent and have a more delicate texture than those of Escherichia coli (198a). Colonies formed by strains which have frequently been subcultured on artificial media tend to be rough, with a granular surface and irregular edge (198a). This variation, which also occurs in some other bacteria, is associated with a loss of virulence and of the somatic O antigen.

Cells of salmonellae possess antigens which fall into three main categories. The K (from the German word Kapsel) or envelope antigens are thought to surround the cell and chemically are similar to 0 antigens (21a). Generally, the K antigens are heat-labile and tend to mask the somatic antigens of the cells, thus making live cells agglutinable by O antisera (21a). The Vi antigen of Salmonella typhi is an example of the K antigen. Cells of S. typhi may undergo a loss of the Vi antigen, at which time they can be agglutinated by both O and Vi antisera (198a).

The O (from the German ohne Hauch) or somatic antigens are located in the body of the cell (presumably near the surface), are phospholipid protein polysaccharide complexes, and are heat stable (21a). Chemically, the polysaccharide moiety of the antigen is extremely complex and consists of a variety of sugars including: hexosamines, heptoses, pentoses, hexoses, and desoxyhexoses (198a). About 45 serologically distinct O antigens have been recognized. Most species possess more than one O antigen; thus, many species share the same O antigens. Species can be divided into a limited number of groups on the basis of their O antigen composition, with each group characterized by possession of an O antigen not found in the other groups. In this way the majority of salmonellae can be divided into nine O antigen groups, with most of those commonly encountered falling into the first four of these, designated as A, B, C, and D (198a). Cells can lose their normal O anti-
gens and then are not agglutinable by O antiserum.

The H (from the German Hauch) or flagellar antigens are located in the flagella, are proteinaceous in nature, and are heat-labile (21a). Many *Salmonella* species may have one or the other of two sets of flagellar antigens and are designated as diphasic in regard to this characteristic. These sets of antigens are known by the terms Phase 1 and Phase 2. When a diphasic species is grown, one of the two phases predominates and more often than not it is Phase 1 (198a). Phase 1 antigens are more and Phase 2 antigens less specific than are O antigens. Loss of flagella by a cell is accompanied by loss of the H antigens (198a).

The presence of antigens in salmonellae has led to the development of the Kauffmann-White schema for identification of the different serotypes. In this schema *Salmonella* serotypes are arranged in subgroups on the basis of their O antigens, while the H antigens represent the type (21a). Use of these serological procedures has led to the identification of more than 1,200 serotypes, all considered pathogenic to humans. The Kauffmann-White schema is detailed in the book, *The Bacteriology of Enterobacteriaceae* by Kauffmann (96).

The genus *Salmonella* is grouped together with the genus *Shigella* in the tribe *Salmonellae*. This tribe, together with the tribes *Proteae*, *Serratiae*, *Erwiniae*, and *Escherichiae*, comprise the family *Enterobacteriaceae* (35a).

**Salmonellosis**

According to Morgan (135), there are three main types of salmonellosis. They are: enteric (typhoid) fever, gastroenteritis, and a localizing type with foci in one or more organs accompanied by septicemia. Every *Salmonella* strain is potentially able to produce any of these three clinical types of infection. Each of these types will be described below.

**Enteric fevers** (135). Typhoid fever is the classic example among the enteric fevers. The incubation period is seven to 14 days and onset of the disease is insidious, usually beginning with malaise, anorexia, and a headache. This is usually followed by a fever which, in a step-wise manner, rises to an average of 40 C. The pulse rate tends to be slow in relation to the degree of fever, and nosebleeds may occur at this stage of the disease.

During the first week, the patient usually is prostrate and may have diarrhea, although constipation is even more common. Either condition is accompanied by abdominal tender-

ness and distention. A cough and bronchitis also may be present at this time. Rose spots frequently appear during the first or second week. Splenomegaly is common and the temperature remains elevated. In severe cases, the patient may become delirious and show the so-called typhoid state for which the disease was named (typhoid fever is derived from the Greek and originally designated a state of irrationality and coma). After the third week, the temperature curve shows morning remissions, and returns to a normal level by a gradual lysis.

Blood cultures taken during the first and second weeks often yield the typhoid bacilli, but less frequently when taken during the third week. The organisms may appear in stool cultures from the beginning and continue to do so until convalescence is completed. Organisms may also be recovered from urine (during second and third weeks), bone marrow, and rose spots.

Relapses occur in about 10% of the cases and probably represent a reinvasion of the blood stream by organisms multiplying in areas such as lymphoid tissue, bone marrow, the spleen, and biliary system. The mortality rate in untreated patients is about 10%, and death generally results from intestinal hemorrhage or perforation. In some instances, salmonellae may establish themselves in the tissue of the host to produce a carrier state after recovery. This is most likely to occur after typhoid fever, where about 3% of the cases are found to excrete *S. typhi* in their stools for over a year after recovery from the disease. The carrier state is observed less frequently with *Salmonella paratyphi* and *Salmonella schottmuelleri* than with *S. typhi*, and its duration is much shorter.

Other enteric fevers usually have a shorter incubation period (one to ten days) than typhoid fever and are not as severe. Fever and malaise are the dominant symptoms and they usually last from one to three weeks.

**Gastroenteritis** (69). This form of salmonellosis has an incubation period of from 3 to 72 hr, with most outbreaks occurring within 12 to 24 hr after the organisms have been ingested. The principal symptoms of a *Salmonella* gastrointestinal infection are nausea, vomiting, abdominal pain, and diarrhea that usually appear suddenly. Their occurrence may be preceded by a headache and chills. Additional symptoms often associated with the disease include watery, greenish, foul-smelling stools; prostration; muscular weakness; faintness; a moderate fever; restlessness; twitching; and drowsiness.
Severity and duration of the disease vary with the amount of food (and hence salmonellae) consumed, the kind of Salmonella, and the resistance of the individual. Intensity varies from slight discomfort and diarrhea to death in two to six days. Usually, symptoms persist for two to three days, followed by an uncomplicated recovery. In some instances, however, symptoms may linger for weeks or months. Some patients (0.2 to 5%) become carriers of the Salmonella organism which caused their infection. The mortality rate is generally less than 1%.

Septicemias (135). Septicemias caused by salmonellae are characterized by a high remittent fever and blood cultures which yield the causative organism. Intestinal involvement is usually absent in adults, but in children the septicemia may occur as a complication of gastroenteritis. Organisms may localize in any tissue of the body and may produce local abscesses in the perineal and pelvic regions, cholecystitis, pylonephritis, endocarditis, pericarditis, meningitis, arthritis, or pneumonia. Salmonella choleraesuis is one of the most common organisms found in this type of infection. The mortality in Salmonella septicemia ranges from 5 to 20%.

Treatment of salmonellosis (135). Formerly, treatment for typhoid fever and other Salmonella infections was largely supportive and consisted of maintaining the fluid balance and nutritional state of the patient. More recently, sulfonamide drugs have been found beneficial in the treatment of certain Salmonella infections, but their use to treat typhoid fever has been disappointing. A combination of sulfonamides with larger than ordinary doses of penicillin has been found to be of limited therapeutic value.

Streptomycin, although active against salmonellae in vitro, has not produced beneficial results when used to treat typhoid fever. Oral administration is accompanied by a marked reduction in number of typhoid bacilli in stools, but the bacteria reappear when streptomycin is discontinued.

Chloramphenicol is effective in treatment of typhoid fever, but patients do not become afebrile until about the fourth day after administration of the antibiotic is started. Patients may become carriers in spite of adequate therapy with this antibiotic. Treatment of other Salmonella infections with chloramphenicol has been even less satisfactory. It is thought that the intracellular location of the typhoid organisms accounts for the slow response of this infection to antibiotic therapy.

Incidence of Salmonellosis

The number of reported salmonellosis and typhoid fever cases in the United States during 1957 to 1966 is summarized in Table 1. An inspection of the data reveals: a) the number of typhoid fever cases declined from 1,231 in 1957 to 378 in 1966, b) salmonellosis cases increased from 6,693 in 1957 to 16,841 in 1966, c) salmonellosis cases was nearly constant (approximately 17,000 per year) since 1964, d) the reported incidence in 1966 of seven diseases was greater and that of 22 diseases lesser than the incidence of salmonellosis plus typhoid fever.

Table 2 records the number of deaths in the United States attributable to salmonellosis and typhoid fever during 1956 to 1965. From the data it is evident that: a) the number of deaths attributable to typhoid fever decreased gradually from 54 in 1956 to six in 1965, b) a slight increase in number of deaths from salmonellosis was noted during 1963 to 1965, when compared to data from 1956 to 1958, c) more deaths than from salmonellosis plus typhoid fever were...
TABLE 3. Reported cases of salmonellosis and typhoid fever in the United States during 1966 by month (217).

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<th></th>
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</thead>
<tbody>
<tr>
<td>Salmonellosis (excluding typhoid fever)</td>
<td>1,144</td>
<td>951</td>
<td>1,013</td>
<td>1,115</td>
<td>1,462</td>
<td>1,369</td>
<td>1,361</td>
<td>1,800</td>
<td>1,723</td>
<td>1,613</td>
<td>1,446</td>
<td>1,818</td>
</tr>
<tr>
<td>Typhoid fever</td>
<td>19</td>
<td>19</td>
<td>23</td>
<td>28</td>
<td>27</td>
<td>30</td>
<td>53</td>
<td>46</td>
<td>46</td>
<td>37</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>1,163</td>
<td>970</td>
<td>1,036</td>
<td>1,143</td>
<td>1,489</td>
<td>1,339</td>
<td>1,414</td>
<td>1,846</td>
<td>1,769</td>
<td>1,650</td>
<td>1,469</td>
<td>1,841</td>
</tr>
</tbody>
</table>

Characteristics of Salmonellae Important to the Dairy Foods Processor

The processing of dairy products utilizes a variety of heat treatments, low temperatures, drying, and fermentation. Frequently, substances such as salt, fruits, acids, eggs, or other products of animal origin may be added to dairy ingredients in the manufacture of certain products. These additives may contribute to the Salmonella problem by adding organisms or by creating conditions either favorable or unfavorable for the growth and survival of these bacteria. Sanitizers and cleaning compounds, hopefully, will aid in the control of salmonellae by inactivating the cells. The relationships between salmonellae and the processing treatments listed above, as well as others, will be described. Data on destruction of salmonellae by heat or other agents cited in this review are those reported by the investigators who did the work. It must be recognized at the outset that many of these investigators failed to give adequate recognition to the logarithmic nature of death of organisms. Furthermore, some of these workers did not have the benefit of present-day experience in recovery of salmonellae. Nevertheless,
TABLE 4. Reported cases of salmonellosis and typhoid fever in the United States during 1966 by geographic region (217).

<table>
<thead>
<tr>
<th>Area</th>
<th>Salmonellosis</th>
<th>Typhoid fever</th>
<th>Total</th>
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<tbody>
<tr>
<td>New England</td>
<td>1,488</td>
<td>13</td>
<td>1,501</td>
</tr>
<tr>
<td>Middle Atlantic</td>
<td>3,362</td>
<td>62</td>
<td>3,424</td>
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<tr>
<td>East North Central</td>
<td>2,548</td>
<td>47</td>
<td>2,595</td>
</tr>
<tr>
<td>West North Central</td>
<td>836</td>
<td>37</td>
<td>873</td>
</tr>
<tr>
<td>South Atlantic</td>
<td>3,345</td>
<td>65</td>
<td>3,410</td>
</tr>
<tr>
<td>East South Central</td>
<td>574</td>
<td>43</td>
<td>617</td>
</tr>
<tr>
<td>West South Central</td>
<td>1,049</td>
<td>38</td>
<td>1,087</td>
</tr>
<tr>
<td>Mountain</td>
<td>872</td>
<td>18</td>
<td>890</td>
</tr>
<tr>
<td>Pacific</td>
<td>2,767</td>
<td>55</td>
<td>2,822</td>
</tr>
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</table>

the data can provide valuable guidance on the behavior of salmonellae under a variety of conditions.

**Temperatures for growth.** Although under laboratory conditions most salmonellae grow best at 37 C, some of these bacteria can grow at temperatures of 5.5 to 45 C. Matches and Liston (117) determined the minimum growth temperatures of ten serotypes by means of a temperature gradient block. *Salmonella heidelberg* and *S. typhimurium* were found able to grow in a liquid medium at 6.6 C but not at 6 C. The pH of the substrate influenced the minimum temperature at which growth was observed. Lowest growth temperatures were associated with pH values of 7.0 to 8.0. Length of incubation is another factor which must be considered when the organisms are held at low temperatures. These same investigators found that the minimum temperature for growth of *S. heidelberg* was between 6.0 and 7.0 C when observations were made after seven days and that it dropped to between 4.0 and 5.7 C after 15 days. Comparable results obtained for *S. typhimurium* and *Salmonella derby* at seven days were 8.5 to 9.0 C and 8.3 to 9.0 C and at 15 days, 7.8 to 8.2 C and 6.6 to 7.0 C, respectively. Initiation of growth by salmonellae at low temperatures can be important when foods are stored for long periods at temperatures of 4 C or above. Deotto (54) observed that cells of *S. schottmuelleri*, after exposure to a temperature of -2 to -3 C for 20 to 40 min consumed 200 to 500% more oxygen in their respiratory process than did similar cells held constantly at 38 C.

Growth of *S. enteritidis* in a variety of foods at 22 and 37 C was studied by Segalove and Dack (180). They inoculated cans of asparagus, spinach, string beans, tomato juice, peaches, shrimp, salmon, corn, and peas. Duplicate cans of each food were incubated at 22 and 37 C. Growth was observed at 22 C in all foods except peaches and at 37 C in all but peaches and asparagus. In nearly all instances, growth was greater at 22 than at 37 C. Recently Subramanian and Marth (202) compared the growth of *S. typhimurium* in skim milk at 22 and 37 C. They observed numbers approaching $1 \times 10^6$ per milliliter after 12 hr at 37, with little additional growth during the next 4 hr at the same temperature. In contrast to this, at 22 C numbers slightly in excess of $1.0 \times 10^6$ per milliliter were attained after 16 hr of incubation. Undoubtedly, higher numbers would have been obtained at 22 C if the incubation period had been extended beyond 16 hr.

Growth of *S. typhi* at 45 C has been reported by several investigators. Spencer and McRoy (193) noted that this organism grew well for 36 transfers at 45 C and for at least 148 transfers with alternating temperatures of 45 and 37 C. According to Ware (229), growth of *S. typhi* at high temperatures was enhanced by adding certain amino acids to the culture medium. A simple glucose-salts medium supported the growth of *S. typhi* at 37 but not at 40 C. Growth occurred at 40 C when the medium was fortified with L-arginine, L-glutamic acid, thymine, or L-lysine. Addition of L-glutamic acid plus L-arginine and thiamine or L-lysine permitted cell production at 43 C in 24 hr. Elimination of L-arginine was accompanied by cell multiplication in 48 but not 24 hr at 43 C.

**Moisture requirements for growth.** The moisture requirements for growth of salmonellae can best be expressed in terms of water activity ($a_w$). Christian and Scott (46) studied the $a_w$ requirements of 16 *Salmonella* serotypes at 30 C. Growth of 15 motile strains occurred in liquid media at $a_w$ values between 0.945 and 0.999. In foods, the lower limit for growth was slightly less than in culture media. Anaerobic growth was only slightly less than aerobic growth at each $a_w$ value. The single nonmotile strain grew more slowly over a smaller range of $a_w$ values.

The $a_w$ requirement for growth of *Salmonella oranienburg* in liquid media was investigated by Christian (44). He adjusted the $a_w$ of 0.25-strength brain heart infusion broth, nutrient broth, and a casamino acids-yeast extract-casitone broth with a salts mixture (NaCl, KCl, and Na$_2$SO$_4$) or sucrose. In all instances growth was observed at an $a_w$ value of 0.95 but not at 0.94. Substitution of a glucose-inorganic salts broth for the media listed was accompanied by multiplication at an $a_w$ value of 0.97 but not at 0.96, regardless of
the substance used to control the water activity. Christian (44) further noted that the minimum $a_w$ for growth in the glucose-inorganic salts medium could be reduced to 0.96 by addition of five amino acids including methionine, histidine, proline, serine, and glutamic acid. Addition of eight vitamins (thiamin, riboflavin, biotin, folic acid, pyridoxine, calcium pantothenate, nicotinic acid, p-aminobenzoic acid) plus the five amino acids was accompanied by a further reduction in the minimum $a_w$ permitting growth at 0.95. Addition of the vitamins without the amino acids had no effect in reducing the $a_w$ required for growth.

In other experiments Christian (45) used sucrose, glucose, glycerol, NaCl, and KCl to control the $a_w$ in the glucose-inorganic salts medium and then studied the behavior of S. oranienburg in these media. Use of glycerol to adjust the $a_w$ of the medium permitted growth of the organism at 0.96 in comparison to the 0.97 required when other compounds were added. Respiration of cells was inhibited less by glycerol than by the salts or sugars used to control the $a_w$. Control of $a_w$ by addition of glucose or sucrose was accompanied by accumulation of potassium (but not sodium) in S. oranienburg cells. Accumulation of potassium was greatest at an $a_w$ value of 0.975. When NaCl served to adjust the $a_w$, accumulation of potassium was small and none was concentrated when glycerol replaced NaCl.

The influence of amino acids on $a_w$ requirements of S. oranienburg was examined further by Christian and Waltho (47). They observed that a reduction in the $a_w$ value by adding NaCl or sucrose induced a lag and then decreased the rate of glucose oxidation by the organism. At a relatively low value ($0.970 a_w$) addition of amino acids such as proline, aspartic acid, asparagine, glutamic acid, glutamine, and cysteine caused an appreciable synergistic increase in respiration rate. Proline was the most stimulatory of the amino acids tested and at an $a_w$ value of 0.960 only this amino acid and its analogue azetidine-2-carboxylic acid gave appreciable stimulation. Proline was also stimulatory when glucose was replaced by pyruvate or succinate. These authors believe that proline is stimulatory by increasing the amino acid pool of the organism and, hence, decreasing internal water activity.

Effects of acids on growth and survival. Most investigations on the relationship between salmonellae and acids have dealt with destruction of the organisms. Only relatively few experiments have considered the effect of different acid concentrations on the growth of these organisms in a variety of substrates.

Stearn and Stearn (194) conducted tests to determine the effect of pH on colonial characteristics, morphology, motility, and staining behavior of S. enteritidis. When grown on nutrient agar adjusted to pH values in the range of 5.16 to 6.0, colonies appeared opaque, discrete, granular, iridescent, and had raised centers which flattened and appeared lysed and in which were formed secondary colonies with toothed margins. At pH values of 6.1 to 6.3, colonies appeared circular, glistening, white, moist, smooth, and entire. Normal translucent colonies developed at pH values in the range of 6.3 to 7.6, whereas at pH values between 7.8 and 8.4 colonies appeared very thin, spreading, and translucent. Changes in the pH of a broth medium were accompanied by morphological changes in the cells of S. enteritidis. During the first 24-hr incubation period at pH 5.15 to 5.8, chains and clumps of cells were evident and individual cells varied greatly in size and shape, ranging from coccoid forms to long, curved rods. At pH values of 5.6 to 7.0, cells were generally short and plump and exhibited marked bipolar staining. An increase in pH to 8.4 was accompanied by a greater tendency toward evenly stained, slender rods, which appeared Gram-variable. This organism lost its motility when cultivated at pH values below 6.0, but regained it after subculturing in a neutral medium. At acid pH values, cells stained a deep pink with safranin. The color was lighter at a neutral pH value and cells became Gram-variable when the pH entered the alkaline range.

The effects of lactic, acetic, and hydrochloric acids on Salmonella aertrycke were studied by Levine and Fellers (110). When hydrochloric acid was added to nutrient broth, growth was not inhibited until a pH value of 4.0 was attained. Inhibition with lactic acid also occurred at pH 4.0, whereas with acetic acid it was observed at pH 4.9. The organism was destroyed during a 48-hr incubation period when the medium was adjusted to pH 3.1 with hydrochloric acid, pH 4.0 with lactic acid, and pH 4.5 with acetic acid. Subramanian and Marth (202) determined the effect of citric, lactic, and hydrochloric acids, when added to milk in increments over a 16-hr period, on the growth of S. typhimurium. During incubation at 37 C, a slight reduction in growth became evident after 8 hr, regardless of acid added, when a pH value in the range of 5.05 to 5.35 was attained in the milk through gradual addition of acid. Additional incuba-
tion was accompanied by further inhibition, citric acid being most inhibitory, followed in order by lactic and hydrochloric acids. Inhibitory effects of all acids were greater at 22 than 37 C, with citric being most active, followed in order by lactic and hydrochloric acids.

In addition to the work on destruction of salmonellae, cited above, additional studies with lactic acid were conducted by Schillinglaw and Levine (175). They suspended centrifuged cells of *S. typhi* in a 0.02 N solution of lactic acid at 30 C. Less than 1 hr was required to destroy 99.9% of the cells. Violle (225) also studied the effect of lactic acid on *S. typhi* and noted that the cells were killed in less than 1 hr by addition of 1% lactic acid to the culture and in less than 24 hr by adding 0.5% of the acid. The combined effect of lactic acid and NaCl on *Salmonella breslau* was determined by Vizir (226). According to this investigator, five to ten days of exposure to 0.90% lactic acid was required to destroy the organism. Addition of up to 15% NaCl did not enhance destruction of the organism by the acid.

Limited trials on the destruction of *S. typhi* by citric acid were conducted by Schillinglaw and Levine (175). Cells of the organism were centrifuged from their growth medium and resuspended in a 0.02 N solution of citric acid. Ten hours of exposure were required to kill 99.9% of the added *S. typhi* cells; whereas, 32 hr were needed before a similar *Escherichia coli* population was destroyed.

Since acetic acid is commonly used in the preparation of mayonnaise and salad dressings, and since these products can become contaminated with salmonellae from eggs, numerous experiments have been conducted on the survival of these bacteria in acetic acid at various concentrations. Wethington and Fabian (233) added 1 ml of 24-hr cultures of various salmonellae to salad dressing and mayonnaise and held the inoculated products at room temperature and 37 C. At both temperatures, the survival in mayonnaise (0.48% acid, pH 3.80) was 12, 12, 6, 6, 1, and 6 hr, respectively, for *S. schottmuelleri*, *S. typhimurium*, *S. paratyphi*, *S. enteritidis*, *S. choleraesuis*, and *Salmonella pullorum*. In salad dressing (1.10% acid, pH 3.20) survival was 6, 1, 6, 1, 1, and 1 hr, respectively, for the organisms in the sequence listed above.

When mayonnaise was made to contain 0.15% acetic acid (pH 5.0), survival was 144, 144, 156, 156, 156, and 132 hr, respectively, for the organisms listed above. Tests using salad dressings with 0.4% acetic acid (pH 4.4) yielded survival periods of 144, 120, 24, 96, 96, and 96 hr, respectively, for the salmonellae in the above sequence. Comparable observations to those just summarized were also made by Kintner and Mangel (100), who studied survival of *S. typhimurium*, *S. enteritidis*, *S. pullorum*, *Salmonella gallinarum*, and *S. choleraesuis* in salad dressing.

Jandl (94) found a freshly isolated culture of *S. typhi* to be more acid-resistant than old laboratory cultures. In the presence of milk higher concentrations of hydrochloric acid than occur in the stomach were required to kill the organism. This was not true when broth or water served as the substrate. This author concluded that viable salmonellae ingested with milk will pass through the stomach and into the intestines.

Since carbon dioxide forms carbonic acid when in solution, its effect on salmonellae will be considered at this time. Schillinglaw and Levine (175) exposed *S. typhi* cells to carbon dioxide (42 psi) and observed that 90% of the bacteria were destroyed in 6 hr at 30 C. For comparative purposes, 28 hr were required to achieve a similar reduction in number of *E. coli* cells. A pigmented, nonpathogenic strain of *S. typhi* was isolated by Sokolov (190). He exposed cells of this organism to an atmosphere of CO₂ and found that they lost their ability to form pigment and also became pathogenic, with virulence which approached that of conventional *S. typhi* strains.

The effect of high pH values on survival of *S. typhi* and *Salmonella monticola* was studied by Riehl et al. (158). They reported that at a pH of 11.0 to 11.5 and a temperature of 15 C most cells were destroyed in 4 hr. Additional tests indicated prolonged periods of exposure to alkaline water, regardless of other components, killed many of the bacteria.

**Thermal destruction of salmonellae.** Read et al. (154) isolated *Salmonella anatum*, *Salmonella binza*, *Salmonella cubana*, *Salmonella meleagridis*, *Salmonella nebraskensis*, and *Salmonella senftenberg* from dry milk. They then studied these organisms and *Salmonella senftenberg* 775W for their heat resistance, to determine if they would survive pasteurization of milk as recommended by the 1965 U.S. Public Health Service Pasteurized Milk Ordinance. Thermal inactivation tests were made on washed cells of test organisms which were resuspended in sterile whole milk. Excluding *S. senftenberg*, D values ranged from 3.6 to 5.7 sec at 62.8 C, from 1.1 to 1.8 sec at 65.0 C, and from 0.28 to 0.52 sec at 68.3 C. Similar
values for *S. senftenberg* were 34.0, 10.0, 1.2, and 0.6 sec, respectively, for exposures of 65.6, 68.3, 71.7, and 74.0 C. Results of these tests suggest that the present recommended pasteurization processes are adequate to inactivate all seven strains of salmonellae studied, provided the initial concentration does not exceed a calculated $3 \times 10^{12}$ salmonellae per milliliter of milk.

Tests on the destruction of *S. typhi* in milk were conducted recently by Evans and Litsky (62). They inoculated milk to provide an initial concentration of $10^6$ cells per milliliter and then processed it at varying temperatures, using a commercial plate-type pasteurizer which had a heating time of 14.5 sec above 37.8 C, to produce the desired temperature, a calculated holding time of 0.6 sec, and a cooling time of 5.0 sec from process temperature to 37.8 C. Data obtained indicate that strains of *S. typhi* studied showed a thermal extinction temperature of 73.9 C. These results suggest that milk can be freed from salmonellae by the use of rapid heating and cooling rates, with no intended holding time at process temperatures readily attainable in present continuous flow pasteurizers.

The z values (degrees F required for passage of a decimal reduction time curve through one log cycle) for rough and smooth variants of *S. senftenberg* 775 W in various media were determined by Thomas et al. (207). They found the rough variant had z values of 11.494, 10.989, 10.638, and 10.204 in 0.5% NaCl, skim-milk, beef bouillon, and green pea soup, respectively. The smooth variant yielded values of 10.753, 10.417, and 10.753 in 0.5% NaCl, skim-milk, and green pea soup, respectively. The rough variant had a holding time of 0.6 sec, and the smooth variant required a higher treatment temperature, a longer exposure time, or both. Data on destruction of these organisms in dry dairy products seem to be missing, but such information is available for other materials. Rasnussen et al. (153) utilized modified thermal death time tubes to determine the heat resistance of salmonellae in naturally contaminated meat meals containing 8 to 10% moisture. A temperature of 82.2 C for 7 min was sufficient to consistently destroy all salmonellae in these meals. A third meal containing 13% fat required an exposure of 7 min at 90.6 C to consistently free it from viable salmonellae.

Destruction of salmonellae in dry products requires a higher treatment temperature, a longer exposure time, or both. Data on the thermal resistance of salmonellae in various poultry products. No attempt will be made to review the literature in this area. However, a few recent and pertinent references will be discussed below. Bayne et al. (25) determined the heat resistance of *S. typhimurium* and *S. senftenberg* 775 W in ground chicken muscle heated to four temperatures in the range of 55 to 75 C. Multiple 1-g samples of meat containing $3 \times 10^6$ cells of *S. typhimurium*, after exposure for 5 min at 60 C, contained no viable cells. The more heat-resistant *S. senftenberg* 775 W required an exposure of 10 to 15 min at 65 C to kill an equal number of cells.

Destruction of salmonellae on eggshells during washing was investigated by Bierer and Barnett (30). They found that *S. pullorum*, *S. gallinarum*, and *S. typhimurium* on eggshells were killed by washing the eggs at 65.6 C for 3 min. This procedure, however, resulted in slight albumen coagulation on the inner surface of the shell. Coagulation did not occur when eggs were washed at 65.6 C for 1 min, although salmonellae were recovered from one egg out of 600 that were washed. The salmonellae used in this study were destroyed in wash water at 53.3 C. Data just cited may be applicable to destruction of salmonellae on equipment surfaces by use of wash water.

The times required to free hard-boiled eggs from salmonellae was calculated by Lieceardiello et al. (113). When raw eggs were placed directly into boiling water, they indicated that 5.6, 8.4, 8.7, and 9.4 min of exposure are required to free small-, medium-, large-, and jumbo-sized eggs, respectively, from *S. senftenberg*. Values for *S. typhimurium* were found to be 4.5, 7.2, 7.3, and 7.8 min, respectively, for egg sizes as listed above. Exposure periods of 14.1, 16.0, and 20.6 min are required to destroy *S. senftenberg* in medium, large, and jumbo eggs, respectively, when they are placed into water at 20 C which is then brought to a boil and simmered. Values of *S. typhimurium* under the same conditions are 12.6, 14.8, and 18.8 min, respectively.

Destruction of salmonellae in naturally contaminated dry egg. In dried egg white the resistance of salmonellae was 600 to 700 times higher than in liquid egg white, according to data cited by Prost and Riemann (150). They also reported that seven days of heating at 49 to 50 C was required to eliminate salmonellae from naturally contaminated dry egg.

Stokes et al. (199) were able to recover viable cells of *S. paratyphi* B from sludge...
required for 100% destruction at 20-25°C was
0.74 ppm chlorine. The concentration of chlorine
dropped by 0.74 ppm and after 5 min by 1.0
0.15 ppm, after 20 min by 0.40 ppm, and after 5 min by 1.0
ppm chlorine. The concentration of chlorine
required for 100% destruction at 20-25°C was
somewhat less at each time period than noted
above.

Effect of quaternary ammonium compounds.
The phenol coefficients of several quaternary
ammonium compounds when acting against S.
typhi were determined by Lane (103) and
Croxall and Melamed (52). They reported phenol
coefficients of 335, 75, 310, and 345 for N,
N-bis (trimethylphenylpentenyl)-N, N-dimethyl-
aminomonomium chloride, (4-hydroxy-2-butyl)-di-
methyl (octybenzyl) ammonium chloride, (4-
hydroxy-2-butyl) (dodecyl-methylbenzyl) am-
nonium chloride, and (4-hydroxy-2-butyl) di-
methyl (pentadecylbenzyl) ammonium chloride, respectively.

Goetelius and Grinsfelder (76) exposed S.
typhi for 10 min to various concentrations of
alkyl tolyl methyl trimethyl ammonium chloride.
Their results indicated 50, 80, 90, 95, 98, and
99.5% destruction by concentrations of 6.25 × 10⁻⁷, 7.6 × 10⁻⁷, 8.4 × 10⁻⁷, 9.2 × 10⁻⁷, 10.2 × 10⁻⁷, 10.8 × 10⁻⁷, and 11.5 × 10⁻⁷%, respectively.

Stedman et al. (196, 197), working with
S. schottmuelleri, found that a 1:2,000 concen-
tration of di-isobutyl phenoxy ethoxy ethyl
dimethyl benzyl ammonium chloride destroyed
99.99% of the organisms in 10 min and that
a 1:1,000 concentration was necessary to do
the same job when organic matter (serum) was
present. It was further observed that concen-
trations of 1:500, 1:100, and less than 1:25
were required to destroy the organism on the
surfaces of stainless steel, asphalt tile, and li-
noleum, respectively. Similar tests on a mix-
ture of alkyl dimethyl 3,4-dichloro benzyl am-
nonium chloride and alkenyl dimethyl ethyl
ammonium bromide revealed that slightly high-
er concentrations were needed to accomplish
the same task. In other experiments Stedman
et al. (195) observed that quaternary ammonium
compounds brought about flocculation of bac-
terial cells, but no germicidal activity was
associated with this phenomenon. Flocculation
of S. schottmuelleri was attributed to altera-
tions in the charge at the cell surface produced
by adsorption of the chemical. The relative
adsorption of different quaternary ammonium
compounds varied, depending on differences in
hydrophobic, polar, and other properties.

According to Ross et al. (162), quaternary
ammonium compounds with 12 to 14 carbon
atoms in the alkyl group showed maximum
antibacterial activity against S. typhi. Incor-
poration of more polar substituents in the
benzyl group increased the germicidal action,
provided the alkyl group contained less than
12 carbon atoms. If the alkyl group contained
14 or more carbon atoms, antibacterial action

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decreased. These authors postulated the following relation between structure and antibacterial action. The compound is more strongly attracted to the bacterial cell as the chemical becomes hydrophobic with an increase in carbon atoms in the alkyl group. When the number of carbon atoms exceeds 14, the critical micelle concentration becomes so low that the number of free molecules available for adsorption on the bacterial cell is too low for strong activity. Polar groups increase the critical micelle concentration and the concentration of available compound and, therefore, increase the biological activity except when the number of carbon atoms is high. If the latter is true, then the extra bulk of the polar groups prevents normal packing on the bacterial surface.

**Effect of hydrogen peroxide.** Satta (170) added *S. typhi* (30,000 cells per milliliter) to milk and treated it with hydrogen peroxide in an attempt to destroy the bacteria. Use of 0.25 to 0.30% peroxide destroyed the salmonellae in 4 to 5 hr at temperatures of 17 to 32 C, whereas, a 0.2% concentration of the chemical required 8 to 9 hr to kill the bacteria. Raw milks were inoculated with *S. typhi*, treated with 0.2% hydrogen peroxide, and incubated for 14 to 24 hr at 20-22 C and 28-30 C, in experiments by Monaci (133). When compared to untreated controls, peroxide caused a reduction of 74 to 96% in number of bacteria present in incubated milks. *S. typhi* was recovered from one sample.

In contrast to the results cited above, Roushdy (164) was able to destroy *S. typhimurium* in milk by exposing it to 0.03 and 0.075% hydrogen peroxide for 4 and 1 hr, respectively. Sadilek and Stepanek (167) inoculated milk with *S. enteritidis*, warmed it to 57 C, added 150 or 200 mg hydrogen peroxide per 100 ml, and treated the milk with catalase. Neither level of peroxide destroyed the organisms under the conditions just described.

**Effects of salts.** The behavior of freesly isolated salmonellae in the presence of sodium chloride was described by Bergmann and Seidel (29). They cultured the salmonellae on agar fortified with 5 to 10, 15, 20, and 30% sodium chloride and in broth containing 5 to 10, and 15% of the chemical at 37 C for one and at room temperature for three days. Visual inspection showed that 7 to 10% sodium chloride in agar and 8 to 10% in broth caused impaired viability, or killed salmonellae. The authors also observed that a few strains of salmonellae recovered after they were inhibited by 15 to 30% sodium chloride in broth. Somewhat comparable results were reported by Severens and Tanner (183), who isolated strains of *S. pullorum*, *S. typhi*, and *S. schottmuelleri* able to grow in the presence of 8, 6, and 8% sodium chloride, respectively. Vizir (226) observed that *S. breslau* was resistant to the effects of high sodium chloride concentrations. At 12 to 16 C the organism remained viable for at least 45 days in milks with 5, 10, and 15% sodium chloride and in broth with 5 and 10% of the chemical. The organism was inactivated after 24 days in broth containing 15% sodium chloride. Addition of lactic acid failed to increase the sensitivity of this bacterium to sodium chloride. Recent experiments by Goepfert, Olson, and Marth (74, 75) demonstrated that *S. typhimurium* was able to initiate growth in skim milk adjusted to pH 4.9 with lactic acid and fortified with 3% sodium chloride. Raising the salt content to 4.5% in the same medium was associated with slow death of *S. typhimurium* when held at 7.5 or 13 C for five weeks. A further addition of 0.1% acetic acid to the medium enhanced death of the organism.

Tarr (204) demonstrated that addition of 0.02% sodium nitrite to fish digest broth at an acid pH caused inhibition of *S. typhi*. According to data by Severens and Tanner (183), selected strains of *S. pullorum*, *S. typhi*, and *S. schottmuelleri* were able to grow in the presence of 1:800, 1:800, and 1:600 solutions of copper sulfate, respectively, and 1:25,000, 1:50,000, and 1:25,000 solutions of mercuric chloride, respectively. Normal strains of these organisms were inhibited by a 1:4000 solution of copper sulfate and a 1:300,000 solution of mercuric chloride, respectively. Normal strains of these organisms were inhibited by a 1:4000 solution of copper sulfate and a 1:300,000 solution of mercuric chloride.

**Effect of streptomycin in milk.** Nagel (139), in studies on the use of antibiotics to preserve milk, found that concentrations of streptomycin up to 800 mg/100 ml failed to completely inactivate *S. paratyphi* B and *S. enteritidis* in market milk held at 37 C for 10 hr. In other experiments, 3 mg streptomycin per 100 ml failed to destroy as few as 30 cells of *S. typhi*, *S. paratyphi* B, or *S. enteritidis* per milliliter of raw milk held at 4 or 22 C for 24 hr.

**Effect of sorbic acid.** Doell (56) demonstrated that at pH 5.0, 0.1% potassium sorbate provided a concentration of sorbic acid both bacteriostatic and bactericidal for salmonellae (as well as staphylococci and pseudomonads). When the pH was increased to 7 to 8, a 10% concentration of the chemical was ineffective against the same organisms.

**Effect of lysozyme.** Complete lysis of *S. typhi* and partial lysis of *S. enteritidis* were obtained by Huerta (91) when he treated the
bacteria with a 1:64 concentration of lysozyme recovered from donkey milk and egg white. The optimal pH for enzyme activity was 4.6. Lysozyme appeared to be inactive against S. paratyphi, S. schottmuelleri, S. typhimurium, Salmonella anatis, S. derby, Salmonella thompson, Salmonella newport, Salmonella pensacola, and Salmonella adelaidae.

Effect of ultraviolet radiation and ozone. Catheart et al. (43) found that an exposure of 8 to 45 min was required to inactivate airborne cells of S. enteritidis when they were no more than 24 in. from an ultraviolet light. These authors also noted that exposure of the same organism to an atmosphere of 4 ppm ozone for 60 min had little effect on its survival.

Effect of antioxidants. The antibacterial properties of propyl gallate, ethyl protocatechuate, and nordihydroguaiaretic acid were studied by Hirose et al. (88). The minimum concentrations of antioxidant with inhibitory activity against S. typhi and S. typhimurium were 6.0 and 0.8%, respectively, for propyl gallate; 6.0 and more than 10.0%, respectively, for protocatechuate; and 0.006 and 0.4%, respectively, for nordihydroguaiaretic acid.

Effect of ethylene oxide. Winkle and Adam (235) were able to fumigate 50-kg packages of dried egg or shredded coconut with ethylene oxide without changes in product quality or appearance. A 4,000-liter kettle was loaded with bags or cases of infected food, evacuated to 40 mm, 1.0 g ethylene oxide per liter admitted and circulated for 10 to 15 min by means of a pump, and the container again evacuated after 2 to 4 hr, if bags were treated, or after 6 hr when the foods were in cases. Tests on the treated products revealed the absence of salmonellae and a reduction in total number of bacteria.

Effect of beta-propiolactone. Bruch and Koester (37) treated liquid whole egg or egg white previously inoculated with S. senftenberg or S. typhimurium, using from 0.05 to 0.3% beta-propiolactone. Egg white containing 10^9 cells of S. senftenberg per milliliter was sterile after 12 hr at 10 C when 0.1% lactone was used, and after 2 to 3 hr when 0.3% was added. Liquid whole egg inoculated with 10^8 cells per milliliter of either species of Salmonella was sterilized in 4 to 5 hr at 10 C with 0.2% lactone or in 2 to 3 hr when 0.3% was employed. A mild heat treatment (15 min at 37 C or 1 min at 55 C) reduced the exposure time required for sterilization.

Effect of lecithin. According to results obtained by Levin (108) and Levin and Olitzki (109), addition of 0.2% lecithin to broth was responsible for the loss of virulence by S. typhimurium after 250 daily transfers in the medium under aerobic and after 230 daily transfers under anaerobic conditions. When control cultures were transferred 400 times in broth without lecithin but under anaerobic conditions, no loss in virulence was noted.

**Effect of carotene and riboflavin.** Vasileva (220, 221) investigated the effect of carotene and riboflavin on S. typhi. He observed that the presence of 50 mg carotene in 100 ml of the substrate suppressed the growth of the organism. Low concentrations (10^-5 to 10^-8 mg/100 ml) had a stimulatory effect and the optimum dose (1.5 × 10^-7 mg/100 ml) produced a 75% increase in cell numbers after 24 hr. Riboflavin at concentrations of 4 × 10^-5 to 2 × 10^-5 mg/100 ml suppressed the growth of S. typhi, whereas stimulation of growth was noted when the level was reduced to 5 × 10^-11 mg/100 ml. Maximum stimulation was observed with 2.5 × 10^-9 mg/100 ml, at which concentration an 87% increase in growth was noted after 5 hr, a 1,140% increase after 24 hr, and a 2,642% after 48 hr of incubation.

**Effect of spices and essential oils.** Dold and Knapp (57) tested the inhibitory properties of different spices by sprinkling them on plates previously inoculated with S. typhi. Maximum inhibition of the test organism was associated with garlic, moderate inhibition with onion, and slight inhibition with cinnamon, radish, horseradish, and nutmeg. No inhibition was observed with celery, caraway, fennel, coriander, parsley, thyme, marjoram, chives, paprika, and pepper.

Experiments to determine the effect of essential oils on growth of S. typhi were conducted by Kellner and Koher (99). They applied the test substance to filter paper discs which were inserted in petri dishes inoculated with the test organism and incubated in an inverted position for 24 hours. Growth of S. typhi was inhibited by oils of hyacinth, chenopodium, cassis, camphor, cinnamon, caraway, synthetic rose, carnation, coriander, synthetic wintergreen, hops, spike, thyme, and thymol. The effect of spices and essential oils on microorganisms is discussed more completely in a review by Marth (116).

**Effect of sulphydryl compounds.** Thomas and Cook (208) demonstrated that the germicidal action of basic phenylmercuric nitrate on S. typhi could be neutralized by the sulphydryl-containing compounds cysteine, homocysteine, and glutathione but not by cystine and methionine.

**Effect of pressure.** Cells of S. paratyphi C
were exposed to a pressure of 2,800 kg per cm² for 5 min by Vignais et al. (224). This treatment was accompanied by cessation of multiplication. Reduction of the pressure to atmospheric was accompanied by resumption of growth after a lag period. Respiration generally followed changes in cell multiplication, ceasing when a pressure of 3,700 kg/cm² was attained and being noticeably affected at a pressure of 3,300 kg/cm².

### Occurrence of Salmonellae in Raw Milk

Occurrence of salmonellae in raw milk has been verified repeatedly by investigators who studied certain outbreaks of salmonellosis in humans. Some of these incidents will be described in the sections dealing with sources of salmonellae in raw milk. A comparison of two surveys on food-borne illnesses in England and Wales shows that the incidence of salmonellosis attributable to raw milk has not changed significantly during the interval between 1951 and 1965. Cockburn and Simpson (49) found that four of 235 and four of 274 food-poisoning incidents in 1951 and 1952 were caused by salmonellae. In 1965, according to Vernon (223), the number of outbreaks had increased to 4,091 and of these six resulted from consumption of raw milk contaminated with *S. typhimurium*.

Information on the concentration of salmonellae in raw milk supplies seems to be lacking in the literature and only a few surveys have been made on the kind of salmonellae present. Varela and Olarte (219), in 1952, examined Mexican certified milks and recovered 11 different salmonellae from 25 out of 520 samples. Organisms identified included: *Salmonella esen* (occurred six times); *S. paratyphi* (occurred five times); *S. derby* (occurred three times); *S. typhi*, *Salmonella bredeny*, and *S. newport* (each occurred twice), and *Salmonella abony*, *Salmonella muenchen*, *Salmonella bovis-mordicans*, and *S. senftenberg*, (each occurred once). Ritchie and Clayton (159) examined 762 milks from England, Scotland, and Ireland for *Salmonella dublin* and were able to recover the organism from one sample. A more recent (1966) survey to determine the incidence of salmonellae in Northern Ireland milk supplies was conducted by Murray (138). He was unable to recover these organisms from bulk-collected samples, but did find *S. dublin* in the milk from two individual producers.

Raw milk is most frequently, although not exclusively, contaminated by cows. Other sources include human carriers, water supplies, and equipment. Outbreaks of salmonellosis attributable to raw milk contaminated from the various sources will be described below.

### Infected cows and calves as sources of contamination

Salmonella infections in dairy cattle have been observed and reported by numerous investigators. Some of the reports have resulted from surveys conducted on cattle, whereas others had their origin in outbreaks of human illness attributable to consumption of raw milk. Reports on surveys will be considered first and details of food-borne illnesses associated with raw milk contaminated by the cow will be given later.

**Surveys.** Field (64) conducted a survey in Wales during 1946 and 1947 and found evidence of salmonellosis on 70 farms. Calves were affected on three of the farms and cattle from one to 14 years of age on the other 67 farms. The infective organism on 64 farms was *S. typhimurium*. Eight outbreaks of bovine salmonellosis were investigated by McFarlane and Rennie (121). Three of the outbreaks occurred in adult cattle and five in calves. Organisms isolated from the diseased cattle include: *S. typhimurium*, *Salmonella orientalis*, *Salmonella newington*, *S. anatum*, *S. muenchen*, and *Salmonella londo*.

Murdock and Gordon (137) examined fecal samples from apparently healthy cows in an attempt to learn about the incidence of *S. dublin*. Results of tests on 272 fecal samples revealed the presence of *S. dublin* in 7.4% of the specimens. A similar study on 1,250 cows was carried out by Smith and Buxton (159), who found *S. dublin* in fecal samples from six cows. Post-mortem examinations on 17 cows culled because of chronic mastitis were conducted by Zagaevskii (240), who recovered *S. dublin* from the udders of five of the animals. Nyström et al. (142), working in Norway, noted *S. dublin* infections in 69 dairy calves tested during 1962 and 1963.

A rather extensive survey on dairy cattle in Germany was carried out by Rasch (152) during 1948 to 1956. He examined 1,427 dairy herds and found that 325 cows in 277 herds were carriers of salmonellae. Guinee et al. (81), working in the Netherlands, conducted a recent survey on the incidence of salmonellae in certain tissues obtained from cows and calves. Salmonellae were recovered from the mesenteric lymph nodes of two out of 600 normal cows and 11 out of 265 normal calves. A somewhat more detailed study was made on 1,504 normal calves at the time of slaughter. Tests on the mesenteric and portal lymph nodes, gall bladder, and feces revealed the presence of...
Salmonellae were found in one or more samples from each of 216 calves. When an even more intensive study was made on 63 calves, salmonellae were isolated from the musculature and organs of infected calves. Salmonellae from a variety of sources caused infections, including feed and water. The role of water will be considered later. Hutchinson (92), in 1964, described an outbreak of human illness which may have had its origin in a feed material. Examination of feces from 20 persons who consumed raw milk from a common source revealed S. heidelberg. Upon further investigation the organism was recovered from milk samples and also from the feces of several cows suffering from enteritis. The cows were on the farm that furnished the raw milk in question. Farm personnel were also found to excrete S. heidelberg. The original source of infection is believed to have been barley, which was fed to cows and which contained the pathogen. The barley probably became infected from rats, since their fecal material was evident in the grain.

Cows may become infected (or contaminated) with salmonellae from a variety of sources, including feed and water. The role of water will be considered later. Hutchinson (92), in 1964, described an outbreak of human illness which may have had its origin in a feed material. Examination of feces from 20 persons who consumed raw milk from a common source revealed S. heidelberg. Upon further investigation the organism was recovered from milk samples and also from the feces of several cows suffering from enteritis. The cows were on the farm that furnished the raw milk in question. Farm personnel were also found to excrete S. heidelberg. The original source of infection is believed to have been barley, which was fed to cows and which contained the pathogen. The barley probably became infected from rats, since their fecal material was evident in the grain.

Valles on a dairy farm can serve as sources of salmonellae which contaminate milk. Two such incidents have been described in recent literature. Dobrier (55), in 1963, reported on an outbreak of human illness caused by S. enteritidis present in raw milk. Inspection of the farm revealed that calves were suffering from an illness caused by this organism. Since hygiene practices on the farm were substandard, it was believed that the organisms from the calves entered the milk and, hence, caused illness in the consumers.

An outbreak of gastroenteritis in which diseased calves may have been important occurred in the United States in January and February of 1966 and was described by Holtermann and Mather (90). Eleven persons in five families were affected and all of them had consumed milk produced on a single farm. The farmer responsible for the milk was not in the business of selling raw milk but gave his surplus to relatives and friends. Although the exact route of contamination remained unknown, several possibilities were apparent to the authors. They found that the farmer was busy throughout the winter making trips to a beef-cattle feeding lot several miles from his farm. Apparently, 16 calves were in the feeding lot and three died early in January from diarrheal disease. Fecal samples were taken from six of the calves five weeks after recovering from the disease and S. typhimurium was recovered from one sample. The farmer admitted that he often worked late in the evening with the calves while they were ill and, after returning home, had neglected to wash his hands prior to milking. Milk was collected in open buckets and then poured into large wide-
mouthed jars. In addition to possible contamination from the hands, manure and other barnyard materials could have easily fallen into the bucket during the milking process. The milk was cooled slowly after collection; more than 2 hr were required to bring the temperature to 40 F, thus allowing considerable time for bacterial growth.

Water as a source of contamination. Water can contribute salmonellae directly if it is added to milk, as well as indirectly if it contaminates the cow and she then adds the organisms to milk. Wallace and Mackenzie (228) conducted epidemiological studies on a milk-borne outbreak of paratyphoid fever. Their investigations suggested that a small stream was contaminated with salmonellae by an inefficient cesspool. Dairy cattle waded in the stream, became contaminated, and carried the organisms on their flanks. When the cows were milked, salmonellae entered the milk and then proliferated, since cooling facilities were inadequate.

Run-off water may serve to contaminate streams in a fashion similar to the cesspool incident just described. Miner et al. (129) examined such water collected from two feedlots near the Kansas State University campus and recovered Salmonella infantis.

An incident of enzootic salmonellosis in a herd of cattle, that was caused by an infected stream which furnished drinking water, is described by Schaal (173). He indicates further that fencing the stream and removal of carrier animals served to control the disease in this herd.

An unusual outbreak of gastroenteritis which involved both raw milk and water occurred in Yakima County, Washington, in 1967 and is reported by Francis and Allard (68). At least 40 persons were involved and S. typhimurium was recovered from nearly all of them. More than 50% of those initially infected consumed raw milk supplied by a single dairy farm. Tests on samples taken at the farm showed S. typhimurium to be present in the milk from three cows, in the feces from one calf, and in water from the stream which supplied the cattle. Extensive sampling of the stream revealed it to be contaminated for 15 to 18 miles. A further search was made and a gunny sack containing a dead calf was found in the water just above the highest point at which S. typhimurium was recovered. The pathogen was found in a stool sample taken from the calf. It was postulated that the calf died of the S. typhimurium infection, was thrown into the stream, and served to contaminate the water. The dairy herd downstream then consumed the water, became infected, and produced contaminated milk.

An outbreak of typhoid fever in St. Louis caused by raw milk contaminated by water from a cistern was described by Meyer et al. (127), and an outbreak of paratyphoid fever resulting from raw milk contaminated with water supplied by a hydraulic ram was reported by Parry (145).

Water also can serve to contaminate equipment which, in turn, can add salmonellae to milk. In at least one instance S. dublin was recovered from a washed milking machine cluster (138).

Some years ago it was common practice in Egypt to dilute buffalo milk with water and urine. Mihaeloff (128) examined 200 samples of milk treated in this manner and found that 50% contained S. typhi.

Addition of sewage to pastures has been found to give rise to salmonellae in raw milk. Bederke and Lunt (26) reported that S. paratyphi entered milk from this source and Poppe (148) made a similar observation with regard to Salmonella manchester.

Humans as a source of contamination. Typhoid fever transmitted by raw milk appears to be the most common form of salmonellosis which can be traced to human contamination. More often than not a carrier is responsible for infecting the milk. Such incidents have been reported by Flammer and Fleisch (65), Watt (230), Landau (102), Merrilles (125), Smith (187), Steele (198), the Baltimore City Health Department (23), and Bekenn and Edwards (27). Other salmonellae reported as having been added to milk by farmers who were ill include S. typhimurium (8) and S. paratyphi (77).

Occurrence in milk of antibodies against salmonellae. Porterfield (149) exposed dry and lactating cows to high doses of inactivated S. pullorum cells, in attempts to develop antibodies in the milk against the organism. He found that intramammary infusion during the dry period resulted in a higher antibody content in colostrum than in blood. Intramuscular and subcutaneous injections of the organism were accompanied by lower antibody contents in both colostrum and blood. Intramammary infusions during lactation were associated with the appearance of antibodies in 2 hr, which persisted for 246 to 288 days.

While Porterfield was working with S. pullorum, similar experiments with S. enteritidis were being conducted by Sarantsev (169). He
concluded that milk from cows hyperimmunized with *S. enteritidis* possessed therapeutic and prophylactic properties against paratyphoid infections in calves.

Senft and Porter (182), working with *S. pullorum*, studied antibody formation in goats. They noted that a significant antibody response occurred after the third treatment, when the antigen was infused into the mammary gland at four-day intervals. A second goat developed antibodies after the second infusion which, in this instance, was given at ten-day intervals. Both goats produced abnormal milk for 36 hr following the first two or three infusions. Experiments by Dvorak (58) and Lassila et al. (105) indicated that agglutinins were noted in milk within 12 to 24 hr after inactivated cells of *S. pullorum*, *S. gallinarum*, or *S. anatum* were infused into the udder. The agglutinins produced as a response to *S. pullorum* were found by Greene et al. (78) able to resist normal pasteurization of milk.

**Occurrence of Salmonellae in Milk Products**

*Pasteurized milk.* Although the heat treatment given milk during pasteurization is adequate to destroy salmonellae, pasteurized milk can become contaminated and, hence, has been associated with a number of salmonellosis outbreaks.

Riddell (156) reported an outbreak of food poisoning involving 300 cases, caused by consumption of raw and pasteurized milk contaminated with *S. typhimurium*. It was postulated that raw milk infected pasteurized milk at the bottling machine. The contaminated raw milk was produced on a farm where several cattle had been infected with *S. typhimurium* and where the farmer was suffering from diarrhea at the time of the outbreak.

An incident in which 34 persons became infected with *S. dublin* from raw and pasteurized milk was described by Nyström et al. (142). Investigations at the dairy which supplied the contaminated milk revealed that pasteurized milk was pumped through unclean pipelines used previously for raw milk.

Fifty-five cases of gastroenteritis caused by *S. paratyphi* occurred in two Welsh counties. According to Thomas et al. (209) nearly all patients regularly consumed pasteurized milk processed by a single plant. No evidence of inadequate heating could be obtained, but occasional samples of water taken from the jets of bottle-washing machines gave positive presumptive coliform test results and a residual chlorine content of 0.3 to 1.0 ppm or lower. River water, which after filtration and chlorination, served as the water supply for the plant was found to be heavily contaminated with *S. paratyphi*. It was believed that occasional infection of milk bottles through use of inadequately treated river water caused this outbreak.

Werner and Zöckler (232) described an outbreak of paratyphoid fever which involved 635 persons and resulted from consumption of contaminated pasteurized milk. A woman suffering from paratyphoid fever was living on the dairy premises and it was thought that the infection was transferred from her to pasteurized milk by workers in the dairy.

A somewhat similar situation was reported by Ruys (165). She indicated that an outbreak of typhoid fever involving 175 primary and 283 secondary cases resulted from consumption of pasteurized milk. The milk was contaminated after pasteurization by a carrier working at the plant where it was processed.

The behavior of *S. typhimurium* in refrigerated pasteurized milk was ascertained by Wundt and Schnittenhelm (238). These investigators inoculated the milk, after pasteurization, with *S. typhimurium* and then held it at 7, 4, 2, and −10 C. After six days the number of viable salmonellae had declined by 68, 58, 80, and 95%, at the respective temperatures indicated above.

Mathur (118) recorded an outbreak of gastroenteritis caused by *Salmonella weltevreden* in boiled milk. The milk, boiled at the farm, was transferred to another container which, apparently, was responsible for its contamination.

*Fermented milks.* The literature on the behavior of salmonellae in cultured milks presents a confusing picture. Wilson and Tanner (234) reviewed the literature covering the period from 1886 to 1944 and found more than 25 reports which suggested rapid (within three to four days or less) death of salmonellae in a variety of cultured milks. *S. typhi* was most frequently used in these tests, although a few other salmonellae were examined. Most often the test organism was introduced into already sour milk and the duration of viability (frequently at room temperature or above) was determined. In contrast to the above, more than ten reports were cited which suggested that salmonellae survived for extended periods (often several weeks or longer) in cultured milks.

In some of their own experiments Wilson and Tanner (234) noted that growth in milk of *S. typhi*, *S. paratyphi*, and *S. schottmuelleri* was inhibited by pH values of 4.5 to 4.8, 4.3 to 4.8, and 4.8 to 4.9, respectively. They observed that the three organisms, in some instances,
survived for more than 63 days in buttermilks containing up to 0.73% titratable acid. Survival in acidophilus milks (maximum acidity 1.3%) ranged from one to 58 days.

Probably more tests have been conducted on the survival of salmonellae in yogurt than on their behavior in other cultured milks. Schmidt and Hannemann (176) compared the bactericidal effects of biogurt, a special yogurt containing up to 0.73% titratable acid. Survivors were viable in all products after 15 days at 4°C. In contrast to these observations, Todorov (213) reported that S. typhimurium and S. enteritidis remained viable in yogurt for periods ranging from a few hours to a few days, depending on the activity of the lactic acid bacteria and on storage conditions. In another report Todorov (212) suggested that Lactobacillus bulgaricus and Streptococcus thermophilus produced proteaceous substances which together with lactic acid were responsible for inhibiting salmonellae in yogurt. Gürsel and Fisek (82) had concluded earlier that inhibitory activity of yogurt was associated with the lactic acid present.

The behavior of S. typhi in kefir was studied by Habaj and Michalewicz (83). When milk was inoculated with S. typhi before the starter culture was added, the pathogen survived for eight days in low-acid kefir made from the mixture and for 72 hr in a high-acid product.

Kazberyuk (98) inoculated mare's milk with S. paratyphi and an hour later added a kumiss culture at the rate of 25% of the total milk used. Six hours later he was unable to recover sufficient viable S. typhi cells to induce illness in mice. A fermented milk product known as curds is made from buffalo's milk in the southern province of Ceylon. The fermentation is probably similar to that encountered in kumiss and kefir, since yeasts, S. lactis, and a lactobacillus are involved. Nicholls et al. (140) added S. typhi to the whey from curds and were unable to recover viable cells after 3 hr.

Panja and Ghosh (144) found that the pH values of 27 samples of dahi (an Indian fermented milk) ranged from 4.2 to 4.7. Upon adding S. typhi to these samples, they found the pathogen destroyed in all 27 products after 60 min, in 20 samples after 30 min, and in 16 fermented milks after 15 min. Solutions of hydrochloric, acetic, and citric acids at pH 4.4 were less bactericidal than either a solution of lactic acid at pH 4.4 or whey from dahi. More recent investigations on dahi were completed by Tiwari and Singh (211). They added S. paratyphi to milk and stored the fermented product at 3 to 5°C and 22 to 25°C. Destruction of S. paratyphi was accomplished in 144 hr at the former and in 72 hr at the latter temperature.

The behavior of salmonellae in mazun (a sour milk preparation resembling acidophilus milk) was investigated by Kazaryan (97). He observed that simultaneous cultivation of the lactic acid bacteria and salmonellae (S. typhi and paratyphi) did not hinder growth of the latter. Additional studies with white mice showed that the virulence of S. typhi and S. paratyphi was unchanged after 18 to 24 hr in an acidic milk product. In later studies Polonskaya et al. (147) prepared sterile filtrates from cultures of Lactobacillus acidophilus and found them able to inhibit growth of Salmonella sp.

Abd-El-Malek and Demerdask (1) added S. typhi and S. paratyphi to sterile milk, which was then made into Zahady (another fermented milk product). They noted that the salmonellae survived the manufacturing process which involved incubation at 43 to 45°C and then remained viable for up to 51 hr in the finished product held at 4 to 5°C or 25°C.

Meyer (126) studied the fate of S. paratyphi added to raw milk which was then allowed to sour naturally. In some samples the pathogen could not be recovered after 24 hr, whereas others contained an almost pure culture of S. paratyphi at this time. Tests on additional sour milk samples demonstrated that the paratyphoid organisms remained viable for over one year.

Cultured buttermilk is the most popular fermented milk in the United States and is often made with the aid of Streptococcus lactis. Certain strains of S. lactis produce the antibiotic nisin; therefore, its effect on salmonellae might be questioned. Marth (116) has reviewed the literature dealing with nisin and does not list a report which deals with its action on these organisms. Inhibitory substances are also produced by Leuconostoc citrusvorum, one of the flavor- and aroma-producing bacteria used in the manufacture of cultured buttermilk. Again, according to Marth (116), the literature provides no data on how these agents affected salmonellae. Recently, Vedamuthu et al. (222) observed that S. lactis var. ðicetilactis, another organism able to produce flavor and aroma substances, inhibited S. typhi on agar plate cultures.

and 1967. This, however, was not the first time these organisms were reported as present in dried milk. The British Ministry of Health (130) reported that 12 cases of salmonellosis in 1950 were associated with the consumption of dried milk contaminated with S. typhimurium. In 1954, according to Cozkurn and Vernon (50), another outbreak of salmonellosis resulting from S. typhimurium was caused by contaminated dried milk. Similar outbreaks in Bulgaria during 1945-1950 were recorded by Jordanov et al. (93).

The recent United States experience with salmonellae in dried milk began in January, 1966, when the Division of Epidemiology of the Michigan State Department of Public Health investigated two cases of gastroenteritis resulting from S. new-brunswick (10, 11). The cases occurred in males less than six months old, residing in different areas of the state; each had received a formula made from instant nonfat dry milk. Following this outbreak, it was noted that S. new-brunswick had been reported only rarely in the United States between 1947 and 1964 (0.02% of all isolations). Between April, 1965, and January, 1966, there were 29 reported isolations of this serotype (as compared to 13 from 1947 to 1964) from humans, a situation which suggested a common source of infection.

As a consequence, state health departments that reported isolation of this organism were asked to submit epidemiologic information about the cases. Of the 29 persons from whom S. new-brunswick was isolated, 25 were available for further studies. All of these persons had symptoms characteristic of salmonellosis, but the cases were distributed throughout the United States. Although dietary histories were impossible to obtain, it was determined that dried milk was the only item consumed with greater frequency than was expected. Twenty of the 25 persons had ingested this food within 30 days of their illness.

After dried milk was implicated, bacteriological examinations were made on hundreds of shelf samples of many brands of dried milk. The same rare serotype, S. new-brunswick, was subsequently isolated from many samples of instant nonfat dry milk produced by a single plant in the midwestern United States. The organism was also isolated within the plant and from other milk products on the premises. Schroeder's (178) study on the occurrence of salmonellae in dried milk led him to the following conclusions: a) Since a large number of producers supply a processing plant, it is difficult to identify the original source of infection, but it is important to guard against such contamination, b) temperature requirements for pasteurization should be followed carefully, c) equipment used to dry and to instantize milk is very difficult to clean and, hence, once salmonellae are introduced into a plant great efforts are required to eradicate them, and d) dried milk is distributed extensively at the wholesale level and, hence, contaminated product can received widespread distribution.

The product from this plant was recalled
from the market in April, 1966, and a careful cleanup and remodeling of the plant instituted. Additional recalls of instant dry milk produced at other locations occurred during 1966 and 1967.

A large-scale testing program was instituted by the USDA during 1966. During the initial phase of the program, April through August, 1966, 2,741 samples from 156 plants in 23 states were tested. Thirty-four samples of nonfat dried milk and 27 environmental samples were found to contain salmonellae. Dried milks contaminated with salmonellae were recovered from plants in Idaho, Iowa, Minnesota, South Dakota, Washington, and Wisconsin. Additionally, environmental samples from plants in the following states were found to contain salmonellae: Iowa, Minnesota, Missouri, North Dakota, Oklahoma, South Dakota, Vermont, Washington, and Wisconsin (10, 11).

Salmonellae recovered from the dried products include: *S. montevideo, S. oranienburg, S. heidelberg, S. tennessee, S. senftenberg, Salmonella alachua, S. cubana, and Salmonella orion* (10, 11, 15, 16). Pickett and Agate (146) also isolated a lactose-fermenting strain of *S. newington* from instant nonfat milk powder after the product had given rise to nine cases of salmonellosis.

Tests on environmental samples from dried milk plants revealed the presence of the following serotypes: *Salmonella schwarzengrund, S. tennessee, S. newport, S. cubana, S. typhimurium, S. anatum, Salmonella worthington, Salmonella kentucky, S. infantis, S. oranienburg, S. heidelberg, and S. orion* (10, 11).

Meister (11) reported results obtained in the USDA survey during the first six months of 1967. Product and environmental samples were taken from approximately 200 dry milk samples in 19 states. Results indicated that 34 (1.4%) of 3,315 product samples and 121 (8.2%) of 1,475 environmental samples were contaminated with salmonellae.

Final survey data (14, 15, 16) showed that 0.2% of all products tested contained salmonellae and these were manufactured in 13% of the plants.

According to Severs et al. (184), the Newfoundland Public Health Laboratories reported 20 isolations of *S. newport* from stool specimens examined during February 1 to March 20, 1968. The 20 patients, who came from 17 different households in the St. John's district, gave a history of diarrhea and vomiting; five preschool children and two adults required hospitalization. At least 12 other persons in the families of confirmed cases of *S. newport* infection gave a history of diarrhea and vomiting. All the families had purchased and consumed a particular brand of dried skim milk. Opened 3-lb boxes of product, all with the same batch number, were obtained from three of the homes and were found to contain *S. newport*. Dried milk from eight unopened 3-lb boxes with the same batch numbers as those taken from homes of infected persons were obtained in St. John's supermarkets and also found to contain *S. newport*. Additional details on this outbreak of salmonellosis associated with dried milk were not available to the author when this paper was prepared.

**Ice cream and related products.** Ice cream and related frozen desserts can become contaminated with salmonellae before freezing. Freezing does not destroy all of the organisms and, hence, the contaminated frozen desserts when consumed can give rise to salmonellosis. Relatively few problems apparently have been encountered with these products in the United States, but the foreign literature suggests that ice cream has been associated with a number of salmonellosis outbreaks.

During 1923 to 1941, according to Savage (171), 40 outbreaks of paratyphoid fever occurred in Great Britain. Two of the outbreaks were attributed specifically to ice cream, although a variety of dairy products was associated with 25 of the other outbreaks. Cockburn and Simpson (49) noted that salmonellae in ice cream were responsible for three disease outbreaks in England and Wales during 1931-32.

During September 1, 1939, to May 31, 1940, Santiago, Chile, experienced outbreaks of typhoid and paratyphoid fevers. Molina (132) reported that of 356 cases investigated, 107 involved ice cream as the sole source of infection and in 44 others as an auxiliary source. Fecal coliforms were also observed frequently in the ice creams from plants which had produced the product infected with salmonellae. The problem in Santiago persisted and Tellez Aguirre (205) studied outbreaks which occurred during September, 1940, to May, 1941. He noted that 140 of 472 cases were traced to ice cream. During 1940, ten of 600 fecal samples from dairy workers and ice cream vendors contained typhoid or paratyphoid bacteria. Again, many of the ice creams contained fecal coliforms.

Moore (134) described an outbreak of paratyphoid fever which involved 25 cases and was associated with ice cream from a particular delivery van. Examination of the ice cream
and vendor failed to reveal any infecting organisms, but later investigation showed that the vendor's wife was discharging S. paratyphi in her feces. It was thought that she was indirectly responsible for contaminating the ice cream. Another outbreak of salmonellosis involving 505 cases was also attributable to an ice cream vendor. According to Evans (63) all involved persons had consumed ice cream dispensed by the infected person who, upon subsequent examination, was found to be an active carrier of S. typhi. The largest number of typhoid fever cases in this outbreak occurred in the 10- to 15-year age group. Four deaths were associated with the outbreak.

Other instances of salmonellosis resulting from consumption of contaminated ice cream were reported by Leinbrock and Kirhoff (106) and Bierschenk (31). The former authors noted that ice cream contaminated with S. typhimurium during manufacture caused illness in 53 persons in a sanatorium near Bonn. The other author reported that 180 persons became ill after eating unpasteurized ice cream infected with S. infantis.

Wundt and Voss (239) studied a recent outbreak of gastroenteritis caused by commercial ice cream containing 10,000 to 100,000 cells of S. typhimurium and Salmonella bareilly per milliliter. These authors also inoculated vanilla, strawberry, and lemon ice creams with S. typhimurium and then subjected them to heat treatments of 60 to 80 C. Ten to 15 min of exposure at the lowest temperature destroyed the organisms in all types of ice cream, but from 20 to 60 min of additional exposure (depending on the type of container) was needed before the desired temperature was attained.

During 1967, 14 outbreaks of salmonellosis occurred in New York, New Jersey, Connecticut, and Maine, and were attributable to a kosher imitation ice cream (19). Although the imitation ice cream did not contain dairy ingredients, it is the type of product which could easily be manufactured in a dairy plant. Each outbreak occurred one to three days after a catered banquet at which kosher food was served. Of approximately 3,300 persons who attended the banquets, an estimated 1,800 (54%) developed diarrhea, abdominal pain or cramps, fever, chills, headache, nausea, and vomiting. The median duration of the illness was three days, and there were no deaths. Examination of stool specimens from 12 persons who represented six of the banquets revealed S. typhimurium. Product left over from the banquets also yielded S. typhimurium. Studies were then carried out at the plant which manufactured the ice cream. All environmental samples from the plant and employee stool cultures were free of salmonellae. Ingredients used in the product were also examined and all were free of salmonellae except the frozen egg yolks, which yielded S. typhimurium. The frozen egg yolks were unpasteurized and were produced at a New York City egg-breaking plant. Environmental samples taken at the egg-breaking plant revealed several serotypes, including S. typhimurium.

As a consequence of the incident just described, action was taken by the New York City Health Department which resulted in the temporary suspension of operations by the frozen dessert and egg-breaking plants. All imitation ice cream manufacturers in the city have been placed under the milk code, which will require production of a certified pathogen-free product. The incriminated egg-breaking plant has agreed to pasteurize its raw egg products. Joint action by the city health department, state officials, and U.S. Food and Drug Administration officials resulted in recovery or destruction of several thousand servings of contaminated product.

Although egg yolk was the ingredient responsible for contamination of the frozen dessert just described, other materials can also serve as vehicles for the introduction of salmonellae into these products. An example is recorded by Ahmed and Nagib (2). They suggested that guar flour be utilized as a stabilizer in ice cream, but found that the flour contained S. typhi, if not adequately processed. Other ingredients which might contaminate dairy products are discussed later in this paper.

B. Butter. Salmonellae survive for extended periods if they occur in butter and the product has been associated with some outbreaks of salmonellosis. A survey made during 1945-1950 by Iordanov et al. (93) revealed the presence of S. typhimurium in some samples of Bulgarian butter.

Outbreaks of salmonellosis. Cambessedes and Boyer (41) reported that in June, 1944, several hundred cases of typhoid fever appeared in Paris. The victims were all customers of certain stores and dairies and the only food from these sources eaten by all affected was butter obtained from one wholesale merchant. The causative agent, apparently, was not recovered from the butter.

A study made by Studney (201) on two explosive outbreaks of paratyphoid fever in Graz, Austria, during 1940 and 1945 revealed that butter was the likely source of infection. Investigations at the dairy which supplied the
area showed that each time an outbreak occurred one worker handling dairy products was suffering from paratyphoid fever. Although the causative organism was not isolated from milk, cream, or butter during the investigation, it was thought that pasteurized cream was infected before it was made into butter. Later experiments demonstrated that paratyphoid organisms could be recovered from butter made from cream contaminated artificially after pasteurization.

Survival in butter. Several investigators have demonstrated that salmonellae survive for extended periods in butter. Siegmund (185) observed that Salmonella spp. survived for an average of 52.5 days in butter held at 22 C and for 91 days when the product was stored at 4 C. He also tested Shigella spp. and found the survival of these organisms to be an average of 59.5 and 63 days at the respective temperatures cited above.

B butter was heated for 30 min at 63 C on three successive days and then inoculated with S. typhi and S. paratyphi in experiments by Guerzoni (80). Subculturing of the butter at intervals revealed that both organisms remained viable for more than 55 days. Recent tests by Zagaevskii (240, 241) revealed that S. dublin and S. typhimurium survived for up to 285 days in butter stored at 0 to 4 C. Todorov (213) made sweet- and ripened-cream butters containing S. typhimurium and S. enteritidis. When the butters were held at room temperature, salmonellae survived beyond the shelf-life of the products; whereas, at 0 C storage salmonellae persisted for 124 days in sweet-cream butter and 55 days in the ripened-cream spread. Survival of S. typhimurium in butter patties was found by Orlandella and Barresi (143) to be about 76 days.

Although free fatty acids have a destructive effect on S. typhi in ordinary media, especially at a pH value of 5 or less, tests by Brison (36) demonstrated that this was not true in rancid butter. He noted that the pH of rancid butter was near 6.0 and suggested this may have accounted for survival of the organism in short-term storage experiments.

Smirnova (186) made butter creams containing 35-41% sugar and inoculated them with S. typhimurium. The organism was able to grow in the product during conventional storage. According to this investigator, 64% sugar was needed in the aqueous phase before growth of the organism was stopped with certainty.

Cheese. Outbreaks of salmonellosis have been associated with the consumption of different cheeses. The early literature is concerned, primarily, with typhoid fever, whereas more recent investigations suggest that salmonellae other than S. typhi may sometimes be associated with cheese-induced illness.

Cheddar cheese. Gauthier and Foley (72) described an epidemic of typhoid fever in Canada during the autumn of 1941. Forty cases were involved and six deaths resulted. The only food common to all the patients was Cheddar cheese, made locally from raw milk and consumed when it was about ten days old. Although the factory where the cheese was made lacked adequate sanitation, the source of the epidemic was found to be a known typhoid carrier who, against orders from public health authorities, milked cows whose milk was used by the factory to produce Cheddar cheese.

Another outbreak of typhoid fever attributable to Cheddar cheese occurred in Quebec during February, 1944, and was described by Foley and Poisson (66). The original source of infection was never traced, although later it was found that the cheesemaker's wife had an active case. Nevertheless, the authors believe she was not responsible for infecting the cheese. Foley and Poisson (66) also recommended use of a three-month ripening period when Cheddar cheese is made from raw milk.

One hundred and eleven out of 507 cases of typhoid fever in Alberta between 1936 and 1944 were caused by Cheddar cheese, according to Menzies (123). Samples of cheese from the last three outbreaks in 1944 were recovered and tested for S. typhi. The organism was found in 30-day-old cheese, but could not be recovered from 48- and 63-day-old cheese. As a consequence of this outbreak, Alberta halted sale of cheese made from raw milk unless the cheese was ripened for at least three months.

Survival of S. typhi in Cheddar cheese was studied by Ranta and Dolman (151) and Campbell and Gibbard (42). The former authors mixed S. typhi with Cheddar cheese and found that the organism survived for one month at 20 C. Inoculation of S. typhi on to the surface of cheese was accompanied by a similar survival at room temperature, a longer survival period at refrigerator temperature, and penetration of the organism to a depth of 4 to 5 cm into the cheese after 17 days.

Campbell and Gibbard (42) inoculated milk with S. typhi and used it to make Cheddar cheese. All cheeses were ripened for two weeks at 14.4 to 15.6 C, after which one cheese from each duplicate set was transferred to storage at 4.4 to 5.6 C. At the lower temperature, seven out of ten cheeses contained viable S. typhi cells for more than ten months, whereas
at the higher temperature the organism generally disappeared after three months of ripening. Size of inoculum and acidity of the cheese did not appear to affect the longevity of *S. typhi*.

Recently Goepfert, Olson, and Marth (74, 75) investigated the behavior of *S. typhimurium* during the manufacture and ripening of Cheddar cheese. Pasteurized milk was inoculated with *S. typhimurium* when the lactic starter culture was added. A slight increase in number of salmonellae occurred during the time between inoculation and cutting of the curd, followed by a rapid increase during the interval between cutting the curd and draining the whey. After accounting for concentration of cells through coagulation, an average of three and a half generations of salmonellae developed during this period. Salting of the curd was associated with a reduction in the growth rate and ripening of the cheese was accompanied by a decline in the salmonellae population. Survival of *S. typhimurium* exceeded 12 weeks at a ripening temperature of 12.8 C and 16 weeks at 7.2 C. Limited tests demonstrated that acetate accumulating in ripening cheese may contribute to the demise of salmonellae.

**Colby cheese.** A typhoid fever epidemic started in January, 1944, in the northern part of Indiana and covered 18 to 20 counties (6, 155). Approximately 250 cases and 13 deaths were recorded in this outbreak. Thomasson (210) noted that the carrier was never traced but illness was associated with consumption of Colby cheese. The cheese, made by a single dairy in the area, was produced from raw milk preheated to 32.2 to 37.8 C and was not allowed to ripen before sale.

Wright (237) and Tucker et al. (215) record an incident in which 384 cases of illness in Kentucky were caused by consumption of 12- to 14-day-old Colby cheese infected by *S. typhimurium*. Investigation revealed that a dead mouse had been removed from a 1,000-gal vat of milk used to produce the cheese. Tests on infected cheese demonstrated that *S. typhimurium* survived for 302 days during storage at 6.1 to 8.9 C.

**Mold ripened cheeses.** Mocquot et al. (131) made blue cheese from milk inoculated with 10⁴ to 10⁵ *Salmonella* sp. cells per milliliter and observed the behavior of the organisms during manufacture and ripening. The death rate of *Salmonella* was related to the pH value of 24-hour-old cheese and increased with a decrease in the pH. Survival and death of the organism were similar in the inner and outer portions of the cheese. The percentage survival of salmonellae in six-day-old cheese was less than 0.01.

Camembert cheese was responsible for an extensive outbreak of illness in Germany, with more than 6,000 cases involved. According to Bonitz (32) the cheese was infected with *S. bareilly*, and rennet was thought to be the original source of the contaminant. After conducting additional experiments, Bonitz (33) changed his mind and postulated that infection probably entered the cheese via the glue used to fasten labels to individual cheeses.

**Other cheeses.** The occurrence of salmonellae in a variety of other ripened cheeses has been reported. Many of these cheeses are not common in the United States, but the information may be useful, in that it further demonstrates the gaps which exist in our knowledge about the behavior of salmonellae in cheese. In some instances investigators simply stated that salmonellae were recovered from cheese, without specifying the type of cheese being studied. A few examples will be given at the end of this section.

Bruhn et al. (38) studied the survival of salmonellae in Samsoe cheese. This cheese has a pH value of 5.15 to 5.20 after 24 hr and contains 44 to 46% moisture. Samsoe cheese was ripened at 16 to 20 C for five to six weeks, after which it was held at 10 to 12 C for an additional seven to ten weeks. The authors noted that a 60-day period was necessary to achieve a 10,000-fold reduction in number of viable salmonellae. The death rate was less rapid at 10 to 12 C storage than at 16 to 20 C.

In May of 1944 an unusually high number of typhoid fever cases were reported in four counties of California. Investigation, according to Halverson (84), showed that the sources of infection were Romano Dolce, Teleme, and high-moisture Jack cheeses, all made from unpasteurized milk. Over 90% of the affected persons had consumed one or several of the cheeses just mentioned (4). This outbreak provided the impetus for the state of California to pass laws controlling the manufacture and sale of cheese in that state.

Wahby and Roushdy (227) studied the survival of *S. enteritidis*, *S. typhi*, and *S. paratyphi* B in Damietta cheese, an Egyptian dairy product. When the cheese was held at 20 to 25 C, *S. enteritidis* survived for 17 days, *S. typhi* for 12 days, and *S. paratyphi* B for 27 days.

Forty samples of Kareish cheese (an Arabian product) were examined chemically and bacteriologically by Moutsy and Nasr (136). They observed that the cheeses contained from 2.33 to 11.38% salt, 0.75 to 2.7% titratable acidity,
68 million to 6.3 billion bacteria per gram, and 1,000 to 100,000,000 coliforms per gram. One sample yielded *S. typhimurium*.

The behavior of *S. typhimurium* and *S. enteritidis* in Kachkaval cheese (a hard cheese) was studied by Todorov (214). He added five million to 350 million salmonellae per milliliter of milk, which was then made into Kachkaval cheese. Heating of the curd in a water bath at 71 to 72 °C for 70 to 90 sec did not destroy the salmonellae. Their survival in the cheese ranged from four to 20 days, depending on the initial level of contamination.

Vizir (226) studied the behavior of *S. breslau*, a contaminant of Brynza cheese, in different media fortified with salt. At 12 to 18 °C the organism remained alive in milk with 5, 10, and 15% salt and in broth with 5 to 10% salt during the 45 days of the experiment. Lactic acid at a concentration of 0.9% or higher destroyed the organisms in five to ten days, regardless of temperature. Zagaevskii (241) noted that *S. typhimurium* and *S. dublin* remained viable in Brynza cheese for up to 22 months.

An outbreak of typhoid fever attributable to a cheese made in a Norwegian home was reported by Hemmes (87). A man ill with typhoid fever was a part of the household when the cheese in question was made. Studies on the aged cheese revealed that viable *S. typhi* were present after 40 but not 55 days.

Cheese produced in a factory in southern Alberta, Canada, was responsible for an outbreak of typhoid fever in March, 1944 (5). Thirty-five cases and one death were reported. The source of the infection was traced to one cheese factory and it was believed that the factory’s water supply was contaminated.

A total of 5,387 food-poisoning incidents were recorded in England and Wales during 1961. The vehicle of infection was identified in 160 of the outbreaks. Four outbreaks were caused by cheese, and salmonellae were responsible in one of these four incidents (9). Rivas et al. (160) examined 524 samples of different cheeses in Mexico between 1957 and 1962. Salmonellae were recovered from five samples and the following serotypes were noted: *S. typhimurium*, *S. enteritidis*, *Salmonella car- ran*, and *S. kentucky*.

Quarg. Quarg is a soft, unripened cheese product common in Europe and in other areas. It can be made by coagulating milk through the use of lactic acid producing microorganisms or rennet. The product is probably quite similar to dry Cottage cheese curd. D’Yasko- nova (59) noted that an outbreak of food-borne illness attributable to quarg occurred in the Saratov district of Russia in 1960. Evidence obtained suggested that the quarg was prepared from milk produced by a cow infected with *S. typhimurium*.

An earlier epidemic attributable to quarg occurred in South Baden, Germany, in 1940. Merkle (124) reported that *S. typhimurium* was recovered from the feces of 68 adults and four infants. Two of the infants died later. Quarg produced by a large dairy which supplied much of the Baden area was found to be the source of infection.

Survival of salmonellae in quarg has been studied by a number of investigators. Todorov (213) found that *S. typhimurium* and *S. enteritidis* survived for up to 20 days in rennet quarg and for more than 35 days in whey quarg. Siegmund (185) noted that salmonellae survived an average of 16.5 days in quarg at 22 °C and an average of 64.7 days when the quarg was stored at 4 °C. Rather extended survival periods were reported by Zagaevskii (240, 241). He reported that *S. typhimurium* and *S. dublin* survived up to 22 months in quarg at 0 to 4 °C and that *S. dublin* remained viable for four and three-fourths years in dried quarg stored at 0 to 4 °C.

Cream and Cottage cheeses. Reports on the occurrence of salmonellae in cream cheese are very limited. Savage (171) reported one out of 40 outbreaks of paratyphoid fever in Great Britain during 1923 to 1941 resulted from consumption of contaminated cream cheese.

Studies on the behavior of salmonellae in Cottage cheese are also quite limited. Recently, Basarab et al. (24) reported an outbreak of salmonellosis which involved 12 cases and resulted from consumption of Cottage cheese contaminated with *S. typhimurium*. The pathogen was traced to the cheesemaker, and he was responsible for infecting the product. Lyons and Mallmann (114) introduced *S. typhi* into Cottage cheese and held the resultant mixture at 37, 24, and 10 °C. Within 48 hr samples stored at 37 and 24 °C had dropped to a pH value of 3.8 and viable cells of *S. typhi* could not be recovered. Holding of the cheese at 10 °C was accompanied by a survival period of 96 hr.

Recently, McDonough et al. (120) studied the behavior of salmonellae during the manufacture of Cottage cheese by the short-set method. A mixture of salmonellae was added to the cheese milk approximately 1 hr prior to the manufacture of cheese. Although a rather high initial inoculum (200,000 to 500,000 salmonellae per milliliter) was used, there was no evidence of growth during the interval

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between inoculation and cooking of the curd. A cooking temperature of 51.7 C was needed to ensure destruction of salmonellae during the cheesemaking operation. The same investigators also added salmonellae to Cottage cheese creamed with a sweet cream dressing, a dressing containing Leuconostoc sp., and a dressing cultured with lactic acid bacteria. Salmonellae persisted for more than 12 days, regardless of the cream dressing used on the cheese when the products were held at 4.4 C. No marked decrease in numbers was noted during the storage period.

**Other Products**

*Milk shake.* A small outbreak of typhoid fever traced to a milk shake was described by Marmion and Martin (115). The product consisted of dried milk, flavoring, saccharine, and water. It was impossible to determine how the milk shake became contaminated, but the authors speculate that water or workers preparing the drink might have been sources of *S. typhi*. This outbreak consisted of 11 cases of confirmed typhoid fever and eight additional cases of diarrhea and vomiting among people who had consumed the drink.

*Eggnog.* A fatal case of *S. enteritidis* infection in an 11-year-old male resulted from a family outbreak in a rural community in Oregon in August of 1967 and was reported by Holmes et al. (89). Seven members of the family became ill with severe gastroenteritis following a luncheon. The suspected meal consisted of chocolate eggnog made with raw eggs, pancakes, and fried eggs. Normally, the family consumed eggs collected from nests on the farm; however, the suspected meal included eggs collected from the hayloft and around the farm. These eggs were highly contaminated with fecal material and dirt, and the shells were not cleaned prior to breaking the eggs during food preparation.

The deceased child became ill with abdominal cramps five hours after consuming the suspect meal. Symptoms of the central nervous system developed and the boy was brought to the doctor's office, where he was pronounced dead.

Stool cultures from all members of the family (including the deceased boy) and a blood culture from the deceased boy were found to contain *S. enteritidis*. Shells of eggs used for the eggnog, pancake batter, and the chocolate drink all yielded *S. enteritidis*.

*Cream.* Sandiford (168) reported the isolation of *S. typhi* from a can of sterilized cream imported into England. Further tests on 225 cans of the same batch revealed that 33% were not sterile. The sealing of the cans in the affected batch was imperfect, and it was suggested that they became contaminated from cooling water after sterilization. For this particular batch, the cooling water came from a shallow well and not from the regular supply.

According to Scassellati Sforzolini (172), *S. typhi* does not survive too well in frozen cream. He inoculated cream, held it at -25 C for 60 days, and found that the numbers of *S. typhi* declined by 98% during the treatment.

An outbreak of gastroenteritis associated with a cream dessert was described by Leimbrock and Ritter (107). The chocolate dessert was made from milk and cream and *S. enteritidis* was responsible for illness in at least 23 patients. The source of the contaminant was not reported by the authors.

*Whey.* Data presented by Helm and Wedemann (86) indicate that *S. paratyphi* B and *S. enteritidis* were able to survive in acid and rennet wheys for a sufficient time to constitute a possible hazard in the unpasteurized product. Recently, *S. derby* has been recovered from dried rennet whey resulting from the manufacture of Swiss cheese (21).

*Lactalbumin.* A recent report (17) has indicated that a governmental agency recovered *S. cubana* from a sample of lactalbumin.

*Salmonellae in ingredients used in some dairy products.* Salmonellae can enter dairy products from some ingredients used in their manufacture. The final section of this review will consider some of these sources.

*Coconut.* Schaffner et al. (174) have observed that raw, unprocessed coconut supports the growth of salmonellae as well as other enteric bacteria. Original contamination of the coconut does not result from carriers or polluted waters, but instead from contact with soils containing salmonellae, followed by dispersion via infected coconut milk. Since salmonellae were particularly resistant to desiccation (see earlier discussion in this paper), the authors resorted to a pasteurization treatment to destroy these bacteria in coconut. They found that heating raw coconut meat in a water bath at 80 C for 8 to 10 min effectively killed salmonellae, did not injure the product, and provided a prophylactic method now widely used by the coconut industry. Exposures of 1 min at 100 C and 5 min at 90 C destroyed salmonellae but caused product damage. It was reported that more than 75% of the *Salmonella* cultures recovered in the United States from imported coconut were *S. senftenberg*, whereas 20% were *S. cubana*. Other salmonel-
Salmonellae recovered from coconut include *Salmonella lexington*, *Salmonella stanley*, and *S. bareilly*.

*Eggs.* It is well known that salmonellae occur with some frequency in dried eggs and other egg products. In one study, Schneider (177) recovered salmonellae from 3.2% of 901 samples of high-quality dried egg. Salmonellae encountered in his work include *S. pullorum*, *S. oranienburg*, *S. tennessee*, *Salmonella oregon*, and *S. montevideo*. Other references could be cited to verify this point, but it appears unnecessary. Salmonella can grow rapidly in reconstituted dried eggs, hence such products should be used immediately after rehydration. According to Solowey and Calesnick (191), no more than 4 hr should elapse between preparation and use if the eggs are at a temperature of 25 to 45 C.

Effective July 1, 1966, egg products entering interstate commerce had to be pasteurized. It is of interest to observe that of 76 to 112 official samples of egg products tested by a governmental agency during the first and second quarters of 1966 (before pasteurization), 46 and 23%, respectively, contained salmonellae. During the third and fourth quarters (after pasteurization) this same agency tested 92 and 29 samples and recovered salmonellae from 12 and 31%, respectively (18). Although use of pasteurization has reduced the frequency with which salmonellae were observed in egg products, it apparently has not completely eliminated the problem.

*Dye.* Carmine dye is prepared from the cochineal insect, *Coccus cacti*, which lives on cactus plants in tropical areas. The insects are imported into the United States, primarily from Peru and the Canary Islands. The dye is then produced in the United States (as well as in England, France, and Germany). Group G salmonellae have been recovered from shipments of the insect and from the carmine dye manufactured in the United States and England. Lang et al. (104) reported that interest in this dye developed after an outbreak of *S. cubana* infections occurred among patients in a Massachusetts hospital who had ingested the dye for gastrointestinal studies.

Although contamination of carmine dye with salmonellae was initially discovered through use of the dye as a clinical diagnostic aid, the bulk of the dye is used to color foods, cosmetics, and drugs. Tests by federal and state laboratories in the United States (13) revealed *S. cubana* in the following items which contained the dye: carmine stock solution, pink summer coating, rainbow peach coating, rainbow yellow coating, kiddy pops, raspberry creams, other candies of various types, paprika mix, meat binder, meat preservative, and peppermint ice. Human illness was not, however, traced to any of these products.

*Fruit products.* Data by Rochaix and Jacqueson (161) indicate that fresh grape juice with pH values of 2.6 to 3.2 showed definite bactericidal properties against *S. typhi* and *S. paratyphi* A and B. These bacteria, when placed in grape juice, were destroyed in 15 min to 3 hr. Ryberg and Cathcart (166) prepared lemon, orange, pineapple, strawberry, and apricot fillings from water, sugar, fruit, salt, cornstarch, and egg yolk. They inoculated the fillings with *S. enteritidis* (approximately 50,000 to 200,000 per gram) and held them at 37 C for 24 hr. Although none of the products were free of the *Salmonella* at this point, there was a drastic reduction in numbers during the incubation period. No products contained more than 3,000 per gram (apricot and orange) and some (lemon, pineapple, and strawberry) had less than 800 salmonellae per gram. The pH values of all products ranged from 2.92 to 4.38.

*Sirup.* Lynovskii (113) inoculated the typhoid and paratyphoid bacilli into 21 samples of artificially flavored sirups containing from 30 to 53% sugar. After 24 hr, growth of these bacteria was observed in two samples.

*Products of animal origin.* During 1966 and 1967 (12, 17) governmental laboratories reported salmonellae in a variety of animal products, some used as drugs and others in the food industry. Of particular concern to the dairy industry is the recovery of salmonellae from peptic and from edible gelatin (*S. senftenberg*). During 1968 (20), *S. newington* also was recovered from unflavored, edible gelatin.

In Conclusion

This review, lengthy as it is, has not covered all aspects of the *Salmonella* problem. It has primarily addressed itself to the question of salmonellae as they affect the dairy industry. The reader interested in other aspects of this problem is referred to the books by Edwards and Ewing (60), Kaufmann (96), Van Oye (218), Seidel and Mutschler (181), Soltys (192), Weil and Saphra (231), and the chapter by Morgan (135) in Bacterial and Mycotic Infections of Man. Reviews by Bowmer (34), Buxton (40), and Prost and Riemann (150) may also prove helpful. Persons interested in methods for isolation of salmonellae will find the paper by Galton and Boring (71) to be a good starting point.

Perhaps a word should be said about control
of salmonellae. Control of these organisms is not different from that of most other bacteria. Initial contamination in raw materials should be low, the product should receive sufficient heat (or other treatment) to destroy the salmonellae, and the processed product should be protected from recontamination until it reaches the consumer. Although these general statements apply in all instances, the details of accomplishing the goal of Salmonella control will vary from plant to plant and from product to product. It is impossible to indicate all of the procedures which should be followed, but the reader can develop many of them as he considers the information given in this review.

Finally, some comments are in order about information which is missing. Obviously, much is known about salmonellae. Although we may be familiar with polar and antipolar mutants in the tryptophan operon of *S. typhimurium* (22), we do not know the frequency with which they occur nor the level of salmonellae in our raw milk supply. It is evident from this review that we really are not sure about the behavior of these organisms during the manufacture of numerous dairy products. How do various conditions associated with operating a dryer affect their survival in dried milk products? What are the relationships between starter bacteria and salmonellae? How do the salmonellae behave during the manufacture of Swiss or Brick cheese? Why are there so many viewpoints in the literature dealing with survival of salmonellae in cultured milks? How frequently do salmonellae occur in dried whey, lactalbumin, casein, and sodium caseinate? These are some of the unanswered questions. The careful reader will have many others by the time he completes this paper.

References


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