

SALMONELLA SURVEY OF PLANT  
FOODS USED IN AND AROUND  
THE HOME

by

FRANK MITTERMAYER

B.S., Wisconsin State University, LaCrosse, 1967

---

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

DIVISION OF BIOLOGY

Microbiology

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

1969

Approved by:

  
Major Professor

LD  
2668  
T4  
1969  
M57

TABLE OF CONTENTS

INTRODUCTION .....	1
REVIEW OF LITERATURE .....	2
Isolations Reported from Fertilizers and Their Ingredients .....	2
Methodology of Enrichment .....	9
Plating Media .....	16
MATERIALS AND METHODS .....	19
Procurement of Samples .....	19
Manipulation of Samples .....	20
Pre-enrichment .....	20
Selective Enrichment .....	22
Plating Media .....	23
Isolation of Suspects .....	24
Cultural Purification .....	26
Poly-H Agglutination .....	26
Biochemical Typing .....	28
Viability Tests .....	31
RESULTS .....	31
Biochemical Reactions and Poly-H Agglutination .....	32
Evaluation of Enrichment Procedures and Media .....	34
Viability Tests .....	35
DISCUSSION .....	35
SUMMARY .....	43
ACKNOWLEDGEMENTS .....	45
BIBLIOGRAPHY .....	46
APPENDIX .....	57

## INTRODUCTION

Salmonellosis has been sufficiently incriminated as a major health hazard. Presumably all of the Salmonella serotypes are potentially pathogenic.

Considerable investigative work has been done on clinical material and various food products, resulting in a better knowledge of salmonellae distribution. Despite the increased awareness of salmonellae contamination, in many outbreaks of salmonellosis the source of infection has not been established.

Some of the recent information on salmonellae contaminated materials has come from workers who have observed these organisms in substances used as fertilizers. Sickenga (1964) has pointed out that substances such as manure or organical fertilizers and dust must be considered as sources of salmonellae infection.

Plant foods or fertilizers are a possible source of infection in unexplained animal and human outbreaks.

The significance of these products as a possible mode of transmission becomes more apparent when one considers the extent of their use. Mehring (1957) found that private homes use approximately half of the plant foods purchased for non-farm purposes, most of which is obtained at hardware stores, garden supply stores, fuel dealers, supermarkets and drugstores to name a few. Millions of dollars are also spent on these materials for use by a wide variety of institutions including hospitals, public schools and various clubs and lodges. The implications of this distribution are apparent and furthermore, the trend towards non-farm use of

plant foods is increasing. In Kansas alone, Mehring (1961) estimated a total consumption of approximately ten thousand tons of fertilizers for private homes alone. This amount was less than half of that reported for certain other states. The utility of these products ranged from fertilizing lawns to potting plants in the home.

This study was conducted in order to attempt to isolate salmonellae from a variety of plant foods available at the consumer level and which are used in and around the home. As a part of this study, two enrichment media were compared and one pre-enrichment method was examined.

#### REVIEW OF LITERATURE

##### Isolations Reported From Fertilizers and Their Ingredients:

Walker (1957) in England reported that of 123 samples of organic fertilizers examined, 50 (40%) were positive for salmonellae. In that study, 40 samples of bone meal were examined and 28 (70%) were found positive and yielded a wide variety of serotypes. The evidence obtained by Walker confirmed that bone-meal and other organic fertilizers may serve as a source of salmonellae in human and animal infections. Further, organic fertilizers may account for some of the unexplained salmonellae incidents since these products had apparently not been regarded as a source of infection up to the time of Walker's study.

Salmonellae in fertilizers in Western Australia were reported by Kovacs (1959). He examined 200 mesenteric lymph glands from

cattle and pigs from a slaughterhouse and obtained 30.5% positive Salmonella cultures, indicating heavy salmonellae infection in these animals. Kovacs examined 88 specimens of fertilizer, blood and bone and demonstrated 43 positive for salmonellae (48.9%), with 24 serotypes confirmed. The results of this study suggested that using salmonellae contaminated organic fertilizers is as great a potential risk as foodstuffs themselves, and Kovacs suggested bacteriological control of these products. The path of infection postulated by Kovacs suggests that animal fodder and organic fertilizer are a source of salmonellae for animals and man becomes infected via animals. The cycle is renewed through sewage to birds, rodents, fodder, etc. Incidentally, somewhat similar modes of transmission were proposed by Bowmer (1965) and Hanks (1967). Nancy Atkinson (1964) states that in Australia organic fertilizers, meat meal, etc., pet foods and coconut, because of their salmonellae loads, must be considered as dangerous sources of human and animal salmonellosis.

Galbraith et al. (1962) in England investigated garden fertilizer samples and found 13% of these contaminated with salmonellae. The possible importance of these materials as sources of human salmonellosis was discussed. It is significant that in many cases fertilizers are sold in stores which also sell a wide variety of food products and possible contamination could result from handlers carrying the salmonellae from one product to another.

Workers in some countries have reported isolating new serotypes of Salmonella which may have been introduced into their

country by imported materials, including fertilizers. (Lachowicz, 1964).

In England, Timbury, Forsyth, and Stevenson (1966) examined garden fertilizers as a source of salmonellae. In particular, they examined bone-meal, dried blood and hoof-and-horn meal. Ninety-four percent of bone-meal samples purchased over the counter yielded salmonellae, while 4% of dried blood and 20% of hoof-and-horn meal samples were positive for salmonellae. An average of 4 serotypes were isolated from the bone-meal samples, with S. senftenberg being the most common of the species isolated. All samples of steamed bone-meal examined were free from contamination, therefore it was suggested that bone-meal can be made safe by heating.

Worthy of consideration when dealing with fertilizers is the fact that soils which are used in fertilizers may possibly be implicated as a source of pathogenic organisms, including salmonellae. The possible connection between soil and disease has been discussed by Armstrong (1964). A wide variety of organisms in different soils which might give an association between soil and human disease is presented, with the understanding that such a connection could become very complex. Bergner-Rabinowitz (1956) described a reduction of numbers of salmonellae in Israeli soil which had been irrigated with salmonellae contaminated raw sewage.

Dust particles often result from the presence of plant foods and fertilizers and the mechanics of their use. Dust may contaminate human food and if salmonellae are present they may multiply under certain conditions (Sickenga, 1964). Dust has been

incriminated in Salmonella infection. Rubbo (1948) and Mushin (1948) confirmed the presence of S. derby from dust samples during an infection outbreak in a children's ward of a hospital. They discussed the epidemiological aspects of this incident. Bate and James (1958) produced evidence strongly suggesting Salmonella infection via contaminated dust from patient to patient in a hospital. Datta, Pridie, and Anderson (1960) traced the spread of infection of an S. typhimurium hospital outbreak to infected dust. Szmuness (1966) has also reported the possibility of dust-born salmonellosis. At present, however, the importance of fertilizers as a source of human salmonellosis mediated by dust has not been clearly defined.

Fertilizers may be of human, animal, plant, or purely chemical origin. Plant foods of human origin are derived from such places as sewage-works and cesspits while those of animal origin are derived from manure, urea and remains such as bone, horn, blood, hoof and "meat". Fish remains are also used.

Many of the animals whose by-products may ultimately be used in fertilizers are well established as sources of salmonellae (Harvey and Phillips, 1961; Wilson et al., 1961). Thus any non-sterile animal products may be contaminated with salmonellae at various times (Harvey et al., 1961). In fact, present evidence indicates animals are the main salmonellae reservoir and that transmission, either direct or indirect through contaminated products of animal origin, is secondary to the animal cycle (Steele, 1966). Apparently, even though animal feeds are treated at temperatures high enough to destroy salmonellae, they may become recontaminated

by dust and containers. (Report, 1959).

Kovacs (1959) has pointed out that a likely source of salmonellae in meat meal, organic fertilizers etc. was the animal supplying the raw material.

Various fowl are often infected with salmonellae and their droppings are sometimes used in fertilizers. Edwards, Bruner, and Moran (1948) have described salmonellae infections in fowls, including the turkey. Indeed, observations suggest that fowl may be the most common carriers of salmonellae (Goddard, 1967).

An unfortunate result of animal infection is the contamination of abattoirs with its possible significance in subsequent human infection (Galton et al., 1954; Harvey et al., 1961, Harvey, Mahabir, and Price, 1966). McCoy (1963) stated that salmonellae originating from animals and present in towns are derived from trades and industries processing animals and animal products for animal or human use. Those sources include: abattoirs, butcher shops, poultry processing plants, meat meal and bone meal plants, and fertilizer plants among others.

Various surveys of rendered animal by-products have shown from 12 to 50 percent of finished products contaminated with salmonellae (Shotts, Martin, and Galton, 1961; Morehouse and Wedman, 1961; Moyle, 1966). Burr and Helmboldt (1962) have demonstrated salmonellae in three specific animal by-products, fish meal, meat scraps, and poultry by-products.

Fertilizer products are often derived from raw products which are also used in making supplements for animal feeds. The 1963 Annual Salmonella Surveillance Report numbers 24 isolations



from products labeled as fertilizers. Such a small number indicated at that time that fertilizers were not often checked for salmonellae. Since that time there have been only infrequent reports of isolations from fertilizers or compost products as such (CDC Report No. 25, June 8, 1964 and CDC Report No. 63, June, 1967).

A Council Report of the J.A.V.M.A. (1966) states that since salmonellae are found in the intestinal tracts of live animals and fowl, transmission may occur when conditions permit fecal contamination of foods, feeds, and environment. Among waste materials being treated and disposed of as fertilizers and compost are animal manure and poultry manure (Hart and Turner, 1965) and municipal garbage, rubbish, and sewage treatment residue (Committee on Refuse Disposal, APWA, 1961). Golueke, Card, and McGahey (1954) pointed out the increased interest in compost materials in this country. Substances often indicated as inocula essential to composting include animal manure, garden soil, decomposing material, and a variety of bacterial cultures. The safety of composting of night soil is unclear and although the process is not important in the United States at present, it is of considerable interest to some countries (Hanks, 1967).

Animal manures have certainly been known to contain salmonellae and if used to inoculate for composting, use of compost for enhancing soil fertility could possibly be a mode of dissemination. Other inocula might also be incriminated as sources of salmonellae.

McCoy (1963) has stated that salmonellae can survive on vegetation and in the soil from 3 to 280 days. He suggested that disappearance of salmonellae from polluted vegetables is slow and irregular, an important factor being the number of bacteria contaminating the vegetation or soil. McCoy (1963) also reported that of pathogenic organisms found in crude sewage, salmonellae are the most common.

Scheffer, Schutz, and Brune (1955) studied the viability of pathogenic intestinal bacteria in sludge and liquid manure and found that although some Salmonella species died off, others persisted. Despite modern biological treatment of sewage, removal of pathogenic bacteria is far from complete (Van der Schaaf and Atteveld, 1965).

The United States also imports some products which are used as plant foods, such as bone meal and fish meal. Present evidence indicates a high percentage of both domestic and imported bone meal, meat meal, and fish meal are salmonellae contaminated. (Galton, Boring, and Martin, 1964). Newell et al. (1959) found that fish meal and bone meal used to feed pigs contained salmonellae. Hobbs (1964) in England has shown that meat and bone meals used as fertilizers and incriminated as sources of salmonellae infection. Gray, Lewis, and Gorrie (1958) have indicated that salmonellae will survive in bone meal for long periods (13 months in one study) and suggested that contamination in bone meal is probably not a recent development. Bone meal is often distributed as a fertilizer which is used on a wide variety of gardens. Thus bone

meal is an example of a product often proven to be contaminated, which is used in apparently unrelated industries, such as in gelatin for human consumption, in plant foods, and in animal feeding stuffs (McCoy, 1962).

Other materials used as plant foods, including fish meal, coconut meal and blood meals have been shown to contain salmonellae (Gray, Harley, and Noble, 1960). Often fish meal is contaminated with many Salmonella serotypes, usually obtained from polluted waters and processing plants (CDC Report No. 7, 1962). Boring (1958) also reported salmonellae obtained from domestic fish meal.

Methodology of Enrichment: The use of enrichment media for isolating salmonellae was originally contrived for fecal specimens. However, this method has since been generally found to be better than direct plating for other types of samples. Watt and Carlton (1945) and Galton and Hardy (1948) demonstrated the significance of utilizing enrichment techniques, as did McCullough and Byrne (1952).

Most of the enrichments used are highly selective and enhance the growth of salmonellae while retarding growth of common contaminating organisms such as Proteus, Pseudomonas, and the coliforms. It has been shown (Thomson, 1955) that salmonellae recovery without selective enrichment is difficult even if the coliform to salmonellae ratio is of the low order of 10 to 1. However, some of the enrichments used are not selective.

The most popular selective media for recovering salmonellae are tetrathionate broth (Mueller, 1923), tetrathionate brilliant

green broth (Kauffmann, 1935), and selenite F broth (Leifson, 1936). In many investigations, the use of both selenite and tetrathionate broths has been reported to give more sensitive results, especially when salmonellae are present in low numbers (Jameson, 1963). Smith (1952) has reported the extreme sensitivity of both selenite and tetrathionate media for isolating salmonellae from animal feces. He also noted the superiority of these media over others. Increased yields of salmonellae from samples heavily contaminated have been reported (McCoy, 1962; James, 1962, 1963; Galton et al., 1964) upon secondary enrichment. The secondary enrichment involves subculture of the initially incubated enrichment medium into another tube of the same medium.

Various modifications of the enrichment media have resulted from finding that certain concentrations of organic material affected their selectivity (Hurley and Ayres, 1953; Dack, 1955; Silliker and Taylor, 1958; Galton, 1961). Also growth of contaminating organisms was not always properly retarded. Miller and Banwart (1965) have further pointed out the inhibitory effects of different combinations of brilliant green and bile salts contained in these media. Changes in selenite broth have involved the addition of cystine (North and Bartram, 1953), brilliant green (Stokes and Osborne, 1955), sulfa pyridine (Osborne and Stokes, 1955), and sterilized feces filtrate by Silliker, Deibel, and Fagan (1964a, 1964b). Mueller's (1923) original tetrathionate broth was modified by Kauffmann (1930, 1935, 1954) by addition of bile salts and brilliant green. Galton et al. (1950) added 0.125 mg. of sodium sulfathiazole per 100 ml. of tetrathionate broth and noted retarded

growth of Proteus while examining canine fecal swabs. After quantitative studies on tetrathionate, Knox, Gell, and Pollock (1943) suggested using balanced tetrathionate mediums containing little excess sodium thiosulfate. Rolfe (1946) reported success with this modification. Galton, Lowery, and Hardy (1954) found greater dispersion and emulsification of fat in fatty materials by addition of a wetting agent (Tergitol No. 7 or Tween 80) to tetrathionate broth. Dack (1955) also reported that d-tergitol (sodium heptadecyl sulfate) was more effective than other substances used to emulsify fat. It was found that 6 ml. of a 10 percent solution of d-tergitol added to 100 ml. of enrichment containing 30 grams of pork sausage was better than previous techniques. Silliker and Taylor (1958) described a centrifugation method of separating bacteria from soluble food matter, permitting restoration of the function of enrichment media. Forty micrograms of novobiocin per ml. of medium has been reported (Jeffries, 1959) to be of greater selectivity when isolating salmonellae from feces with large Proteus populations.

Knox et al. (1943) observed that S. schottmuelleri, most of the common salmonellae, and Proteus spp. were strong tetrathionate reducing organisms. S. typhosa was less effective while S. paratyphi, Escherichia coli, Aerobacter Aerogenes, dysentery bacilli and most fecal organisms were unable to reduce tetrathionate. Edwards et al. (1948) advocated use of Kauffmann's (1930) modified medium (1-100,000 brilliant green in tetrathionate broth) for examining fowl fecal material. McCullough and Byrne (1952) reported the efficiency of this medium for isolating certain strains of

Salmonella. North and Bartram (1953) compared the effectiveness of selenite broths of varying compositions and warned of possible false negative results.

Thomson (1955) claimed that nutrient broth was equal, or superior to selenite broth in isolating salmonellae from feces. Media for the detection and enumeration of salmonellae in dried egg albumen containing both salmonellae and coliforms have been evaluated by Taylor, Silliker, and Andrews (1958). They found that mannitol or dulcitol instead of lactose in conventional cystine selenite 'F' broth gave no improved results. Selenite brilliant green sulfa pyridine medium, used simultaneously, was also not very productive.

Taylor, Hobbs, and Smith (1964) reported that nutrient broth enrichment cultures were satisfactory for plating onto some agars. However, tetrathionate enrichment was necessary for plating onto brilliant green agar because of nonpathogens which grow abundantly on that medium.

Incubation times for enrichment broths may vary. More samples yield salmonellae however, if subculturing is performed following a minimum of two different periods of incubation. It is common practice to subculture after 24 and 48 hours or 24 and 72 hours. Guinee, Kampelmacher, and Hoejenbos (1965) reported more positive isolations upon subculturing to plates after 24 and 72 hours incubation. The same results were obtained by inoculating separate plates from duplicate enrichment cultures after 24 hours incubation but this method did not improve results.

Different temperatures of incubation have been advocated for enrichment broths. Most often, 35-37°C is used, however a selective temperature of 43°C for incubating selenite broth was suggested by Harvey and Thomson (1953) and Georgala and Boothroyd (1965). Their reports state that 43°C inhibits coliform growth but salmonellae growth continues. Elliot and Heiniger (1965) have reported maximum growth temperatures for 34 strains of Salmonella between 43.2 and 46.2°C. In contrast, McCoy (1962) observed that tetrathionate was toxic for salmonellae if incubated at 43°C. Spino (1966) isolated salmonellae from surface water by incubating selenite brilliant green sulfa and tetrathionate enrichment broths and brilliant green agar plating media at 41.5°C. He did not detect salmonellae by incubating similar samples at 37°C. The explanation for different recovery efficiencies with various samples at different temperatures is still unclear.

Toxic affects of tetrathionate and selenite broths have been reported for some Salmonella serotypes (Smith, 1962; Leifson, 1936; Banwart and Ayres, 1953). Banwart and Ayres (1953) also compared the growth of several Salmonella species in various enrichment media and found nutrient broth was best. Tetrathionate, although inhibitory to S. paratyphi and destructive to S. anatis, was reportedly not as inhibitory as selenite 'F'.

Rappaport, Konforti, and Navon (1956) reported a new enrichment medium containing malachite green as an inhibitory agent and comparatively high concentrations of magnesium chloride, which neutralizes the dye's toxic effect. Collard and Unwin (1958)



reported superior recovery results with the new medium. However, Jacobs et al. (1963) compared salmonellae recovery using Rappaport medium and tetrathionate broth, with and without brilliant green and found no significant differences. Selenite brilliant green broth gave results similar to the other two. In isolations made from dessicated coconut, Iveson, Kovacs, and Laurie (1964) obtained more salmonellae recoveries with Rappaport's medium than with either selenite or tetrathionate. Rappaport and Konforti (1959) have found that the Rappaport medium supports growth of S. paratyphi.

Raj (1966) described an enrichment medium for isolating salmonellae from fish homogenate. The medium contained sodium selenite and dulcitol and was reported to be highly sensitive for recovery of salmonellae present in small numbers. Two to seven Salmonella were detected in the presence of  $10^4$  to  $10^6$  competitive cells. Introduction of food to this "dulcitol selenite enrichment" (Raj and Liston, 1965) did not seem to affect the sensitivity, selectivity, or productivity of the medium. Abrahamsson, Patterson, and Riemann (1968) reported using dulcitol selenite enrichment in a rapid single-culture technique for detecting salmonellae in food. The new procedure gave good sensitivity and complete correlation between fluorescent-antibody staining and recovery on brilliant green agar.

Schneider (1946) suspended egg powder in distilled water and then added a sample of this to double strength selenite 'F' medium before plating. Byrne, Rayman, and Schneider (1955) used a similar technique but transferred from the distilled water suspension to replicate tubes of cystine selenite 'F' broth. Slocum (1955)



emphasized the value of pre-enrichment for increasing the numbers of salmonellae before transfer to selective media. Tricarboxylic acid intermediates have been used (Taylor et al., 1958) for pre-enrichment of positive food samples prior to cystine selenite 'F' enrichment. No improvement in isolation rate was reported. North (1961) reported the enhancing effect of lactose as a pre-enrichment prior to tetrathionate or selenite-cystine enrichment in detecting low numbers of salmonellae in dried egg products. He suggested the lactose pre-enrichment restored debilitated cells to an actively growing state. When a mixed flora is present, lactose fermentation lowers the pH enough to inhibit other microorganisms with no obvious effect on salmonellae. Montford and Thatcher (1961) changed the technique for dried egg white by suspending the sample in phosphate buffer before mixing with lactose broth. Dulcitol, mannitol or lactose pre-enrichment were found to give superior results to distilled water reconstitution of albumen (Taylor and Silliker, 1961). The type of carbohydrate was not significant. Brilliant green tetrathionate enrichment broth was shown to be superior to cystine-selenite in this and later experiments (Taylor, 1961).

Galton (1961) recommended brilliant green tetrathionate enrichment for isolating salmonellae from packed foods. Adinarayanan, Foltz, and McKinley (1965) reported mannitol purple broth pre-enrichment followed by selective enrichment in cystine selenite 'F' sulfa pyridine was effective in recovering salmonellae from marketed foods. In comparing direct enrichment in selenite broth (Hobbs, 1963) or tetrathionate (Galton et al., 1964) with pre-

enrichment in non-selective nutrient broth, the latter method was found superior. Sugiyama, Dack, and Lippitz (1960) observed efficient recovery of salmonellae from egg albumen by using non-selective broth plus polyvalent Salmonella H antiserum. The broth was incubated and centrifuged, the sediment being reincubated after addition of selenite-cystine broth.

Mossel, Visser, and Cornelissen (1963) have reported the use of a buffered brilliant green-bile-glucose broth which can be used as a non-selective pre-enrichment for food samples containing salmonellae. Gorichter and Sechter (1966) compared selenite-cystine broth and Kauffmann-Muller medium as selective liquid media for isolating salmonellae from bone meal. They found that selenite cystine medium gave better results when small inocula were used but when small numbers of salmonellae and large numbers of coliforms were present, Kauffmann-Muller medium was best. Grinding the bone meal increased the number of serotypes isolated.

Banwart and Ayres (1953) explained the difficulty of suggesting that one type of broth is better for all types of samples or for all species of Salmonella because of the variance in samples used to evaluate these media. Jameson (1962) has discussed why many isolation methods used together should yield more salmonellae recoveries than separate procedures used alone. In fact, most researchers in the field have suggested the use of more than one enrichment medium for obtaining the highest recovery rates.

Plating Media: Many differential and selective plating media have been utilized for isolating salmonellae from food products and clinical samples. These media, which are available in the

dehydrated state, are economical and time saving.

Of the differential media, MacConkey's agar (Mac) (1908), as well as Leifson's desoxycholate agar (1935), utilize the selective action of bile salts for gram negative bacteria. Eosin-methylene blue agar is available for distinguishing lactose and non-lactose fermenters, while inhibiting gram-positive bacteria. Since differential media often do not give sufficient inhibition of other gram negative bacteria, more selective media are frequently streaked from enrichment broth. Of the selective media available, the more commonly used are bismuth sulfite agar (BS) (Wilson and Blair, 1927, 1931), brilliant green agar (BG) (Kristensen, Lester, and Jurgens, 1925), Salmonella-Shigella agar (SS) and desoxycholate citrate agar (DC). DC agar was initially reported by Leifson (1935) and later modified by Hynes (1942). The preparation and composition of these media are outlined in the Difco Manual (1953). Even these media, however, were not always found adequate for isolating salmonellae. As a result, many modifications have been made to facilitate salmonellae recovery.

Banwart and Ayres (1953) observed abundant growth of S. paratyphi, S. typhi-murium, S. oranienberg, S. pullorum, S. anatis and S. worthington on BGA. These organisms were inhibited on BSA, DCA, and SS agar. Pseudomonads, which present a problem when examining certain samples, have been found to be greatly inhibited by addition of 8-16 mg. of sodium sulfadiazine per 100 ml. of BG agar (Galton et al., 1954). This BGS agar also markedly inhibits coliform growth. While recommending this medium when food samples are examined, Galton (1961) noted a possible changed appearance

of Salmonella colonies with heavy coliform contamination. Osborne and Stokes (1955) added 0.1 percent sulfapyridine to BGA for examining egg products and reported greater selectivity for salmonellae and marked inhibition of coliforms. Byrne et al. (1955) found BGA inhibitory to S. pullorum while BSA was not. They advised using many plating media for more salmonellae recoveries. BGA was found superior to SS and BSA (Taylor et al., 1958) for differentiating between coliforms and non-coliforms. On comparing selective media, Wells, and Forsythe (1958) found BGA, BGS and BSA gave satisfactory results for salmonellae from egg white solids. BGA reportedly gave results slightly superior to the other two. For isolating salmonellae from foods, Georgala and Boothroyd (1965) obtained consistently good recoveries on BGA inoculated from selenite broth. In isolating salmonellae from prepared foods, Adinarayanan et al. (1965) found BGA a better plating media than Mac, EMB, BSA, DCA, or SS agars. Edwards and Ewing (1962) suggested the use of BGA for salmonellae other than S. typhi, but warned of atypical colonial forms which could be difficult to distinguish. They also recommended the use of tetrathionate enrichment containing 1 to 100,000 brilliant green prior to plating on BGA. Earlier reports of the superiority of BGA for plating clinical or food specimens were stated. (Galton, Scatterday, and Hardy, 1952; Galton et al., 1954; Galton, Harless, and Hardy, 1955; Osborn and Stokes, 1955).

Taylor et al. (1964) mentioned overgrowth and spreading of Proteus on BGA. This situation might be due to incorrect sterilization of the medium, which should be exactly 15 minutes at 15

pounds pressure in the autoclave (Galton, Morris, and Martin, 1968). Jameson and Emberley (1956) suggested adding anionic detergent, Teepol (secondary sodium alkyl sulfate), instead of bile salts in Mac agar for retarding the swarming of Proteus. Guinee and Kampelmacher (1962) obtained good salmonellae growth and inhibition of Proteus and coliforms. Their brilliant green was not sterilized, although brilliant green is commonly sterilized as part of a complete rehydrated medium. Gerichter and Sechter (1966) reported that, in their laboratory, BGA gave better results than SS agar for isolations from bone meal. BGA has been recommended for isolating salmonellae from animal feeds and meat byproducts (Grumbles et al., 1967).

Variability in plating efficiency of salmonellae on different lots of commercial BGA has been examined (Read and Royes, 1968; Morris, 1968) and inadequacies noted.

#### MATERIALS AND METHODS

Procurement of Samples: Bags, boxes, and packages of unopened, undamaged plant foods were purchased from grocery stores, seed dealers, nurseries, and department stores in Manhattan, Kansas. In those instances where the same type of plant food was available from different companies, samples from each source were purchased. Packages of appropriate size were purchased in order that triplicate 100 gm. samples could be run on each type of plant food. Ten samples at a time were examined until a total of 100 had been surveyed. The examination of samples was begun within a few days of the time they were purchased. In most cases, the contents of

the packages were outlined on printed wrappers. All packages were stored at room temperature before and after use. The samples obtained were quite evenly divided between those containing animal, plant, chemical, or soil substances, often with a mixture of two or more of those types of materials. Only a few plant foods of bacterial composition and fish products were available for sale. Of the plant food products of animal origin, 15 contained bone-meal and/or blood-meal as a constituent. Some of the bone-meal samples were advertised as steamed products.

Manipulation of Samples: The plant food containers were handled with aseptic precautions while materials were being removed for examination. The containers were opened with a flamed knife by cutting along the tops of the packages. The metal tops found on certain containers were flamed before and after opening. Specimens were transferred to enrichment media by use of wooden tongue depressors, which had been wrapped in Kraft paper and sterilized in the hot air oven. All specimens were extracted from the center of the samples tested. After the specimens were obtained, the packages were resealed with adhesive tape and kept in a room separate from the laboratory.

Pre-enrichment: Lactose broth (Difco) was used as a pre-enrichment medium for the first 30 samples examined. The medium was prepared one day prior to use by dissolving 13 grams of dehydrated Bacto-Lactose Broth in 1,000 ml. of distilled water, distributing 330 ml. portions into quart jars with rubber sealed covers, and sterilizing in the autoclave for 15 minutes at 15 pounds pressure (121°C). The final pH of the medium was 6.7. After

cooling, the jars were incubated overnight and then only that media which was visibly uncontaminated was used. In a few instances when 330 ml. of broth did not give a good suspension of the sample, more sterile lactose broth was added aseptically until a good dispersion was obtained.

The quart jars were weighed on a balance and then approximately 100 grams of each specimen were added using aseptic technique. To those samples containing a high content of fat, 6 ml. of a 10% solution of Tergitol No. 7 were added for every 100 ml. of lactose broth, the jar lids were tightened and the mixture shaken vigorously until a homogenous suspension was attained. The jar lids were loosened and the suspended sample was incubated at 37°C for 24 hours with occasional mild agitation to resuspend any sediment. Following incubation, a 6 mm. loopful of the lactose broth culture was streaked to brilliant green agar plates containing 8 mg. of sodium sulfadiazine per 100 ml. of agar. The lactose jars were tilted while obtaining a loopful of broth to avoid any froth on the liquid surface. The samples were then reincubated for 24 hours at 37°C.

After 48 hours incubation, a 6 mm. loopful of lactose broth was streaked on BGS agar and also 1 ml. of broth was subcultured in 10 ml. of brilliant green tetrathionate broth (BGTET). After 24 and 48 hours incubation at 37°C, a loopful of the BGTET subculture was streaked onto brilliant green agar (BGA) plates. These plates were also incubated 24 and 48 hours at 37°C before examination and then held one more day at room temperature before discarding. In those cases when BGS agar was streaked, these plates



were incubated in the same manner as the BGA plates. The lactose pre-enrichment method gave unsatisfactory results and was discontinued after the first 30 samples.

Selective Enrichment: Tetrathionate broth ("Difco") was prepared according to the specifications of the manufacturer and used for selective enrichment of all samples examined. Brilliant green was added to the tetrathionate broth to give a final concentration of 1:100,000 (BGTET). This medium was used both for subculturing from lactose pre-enrichment broth and for direct enrichment of samples. BGTET was prepared the day it was to be used, except in cases when subculturing from lactose pre-enrichment or secondary enrichment were employed. In these instances, the tetrathionate base, without the iodine solution added, was stored in the refrigerator. The BGTET selective enrichment, for the first 30 samples tested, was incubated at 37°C for 24, 48, and 72 hours prior to plating. The 72 hour plating was discontinued, however, for the remaining 70 samples since no substantial change in results was observed.

Secondary enrichment was employed on all samples enriched in BGTET. This was accomplished by subculturing 1 ml. of the 24 hour BGTET broth to another tube containing 10 ml. of BGTET and incubating at 37°C for 24 and 48 hours before plating. Sediment which tended to accumulate in the bottom of these tubes was resuspended at convenient intervals over the incubation period.



The first 30 samples were also examined using Dulcitol Selenite Enrichment broth, which had the following composition:

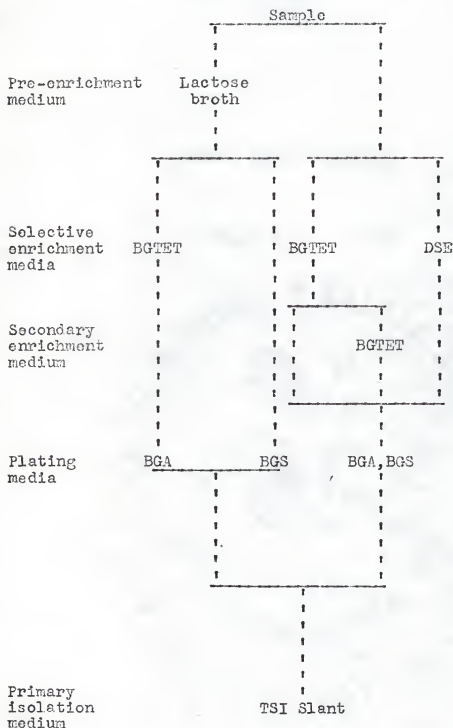
Proteose peptone	4 grams
Yeast extract	1.5 grams
Dulcitol	4 grams
$\text{Na}_2\text{HPO}_4$	1.25 grams
$\text{KH}_2\text{PO}_4$	1.25 grams
Sodium selenite	5 grams
Distilled water	1 liter

The constituents were heated to facilitate dissolution and the prepared medium was adjusted to pH 6.9 with Normal sodium hydroxide solution. The medium was sterilized by steaming without pressure for 30 minutes. Samples examined by Dulcitol Selenite Enrichment (DSE) were run ten at a time, by first investigating 30 gram samples and later 100 gram samples. Thirty gram samples were enriched in 100 ml. of medium and 100 gram samples were suspended in 330 ml. of enrichment broth. Six ml. of 10% solution of Tergitol No. 7 were added to samples with high fat content and each suspension was shaken before incubation. Incubation was at 37°C for 24, 48, and 72 hours before plating. This enrichment was not continued for the last 70 samples because of fewer isolations with this medium than with BGTEF.

Plating Media: Two plating media were utilized for examining the first 30 samples. The differential brilliant green agar (BGA) was prepared and used according to the directions of the manufacturer. BGA was sterilized in 100 ml. amounts in screwcapped bottles. A sterilization time of exactly 15 minutes at 15 pounds pressure

in the autoclave was used, following which the agar was allowed to harden and the bottles were then stored in the dark. Before use, the agar was remelted in the Arnold steamer, poured into sterile petri-dishes, and upon solidification were stored in a metal box in the refrigerator. For a more selective plating medium, brilliant green sulfadiazine agar (BGS) was used. BGS was prepared by melting brilliant green agar and then cooling it to 45°C in the water bath, followed by addition of 0.5 ml. sulfadiazine per 100 ml. of BGA. The sulfadiazine was placed in the 45°C water bath for temperature equilibration before addition to BGA. If not used immediately, these plates were also stored in the refrigerator. Before use, plates of both BGA and BGS were incubated at 37°C overnight and let stand at room temperature until used. This procedure provided both a sterility check and a dry surface for streaking. All plates were used within 2 weeks after their preparation. For isolating directly from lactose pre-enrichment broth, BGS was used, while both BGA and BGS were streaked from the selective enrichment media. BGS was not used for examining the last 70 samples because the BGTET-BGA combination provided adequate selectivity and differentiation of the organisms. The scheme of media utilized in isolating salmonellae is shown in Fig. 1.

Isolation of Suspects: The inoculated plates of differential media were incubated at 37°C for 24 hours and then examined for suspect Salmonella colonies. The plates were reincubated for 24 hours, then re-examined and held at room temperature for one more day and additional isolations made if indicated. On plates showing characteristic growth, a minimum of at least 4 colonies were



Key: BGTET= Brilliant Green Tetrathionate  
 DSE = Dulcitol Selenite Enrichment  
 BGA = Brilliant Green Agar  
 BGS = Brilliant Green Sulfadiazine Agar  
 TSI = Triple Sugar Iron Agar

Fig. 1. Flow diagram showing media utilized for pre-enrichment, selective enrichment, secondary enrichment, and isolation of salmonellae from plant food samples.

picked for further examination. The center of suspect colonies was gently touched with the tip of a sterile inoculating needle, which was then first streaked on the slant and then stabbed into the butt of a Triple Sugar Iron agar (TSI) slant. The TSI slants were incubated at 37°C for 24 hours and then separated according to reaction and held at room temperature until identification was completed. All isolated cultures were catalogued with a serial number, the enrichment and isolation media used, the sample number, the isolation date, and the reaction on TSI. Only those slants which were H<sub>2</sub>S positive with an acid butt and alkaline slant or H<sub>2</sub>S negative with an acid butt and alkaline slant were held for further identification.

Cultural Purification: TSI cultures were streaked onto MacConkey's agar (Difco) plates and incubated 24 hours at 37°C. Isolated colonies were picked and reinoculated on TSI agar slants. After incubation, the slants were checked for reactions identical to those on the original TSI slants. The plates were then discarded.

Those cultures which were suggestive of salmonellae were subjected to poly-H agglutination and a representative number of isolates from each sample were typed biochemically. The scheme presented in Fig. 2 was used for identifying cultures.

Poly-H Agglutination: Difco commercial Salmonella 'H' Antiserum Poly (2406) was used for a slide agglutination test. Clean glass slides were divided into sections with a glass marking pencil. A small drop of antisera was placed on a section of the slide and a suitable amount of culture from a 24 hour TSI slant

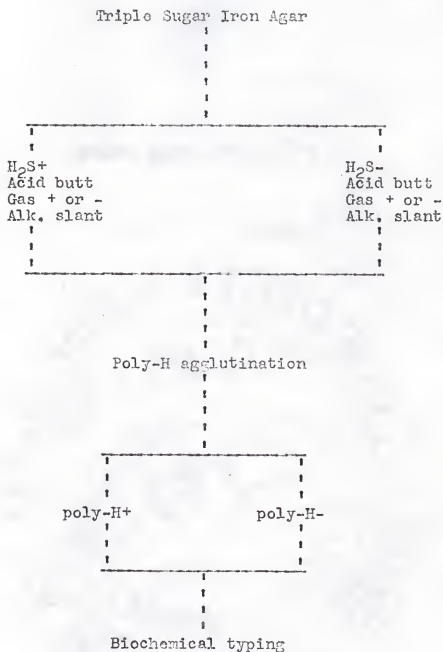


Fig. 2. Diagram of preliminary identification scheme for isolated cultures.

was added with an inoculating needle and mixed. The slide was warmed gently over a flame and rocked in a circular manner to facilitate the reaction. Results of the test were read by observing the slide with light transmitted from a table lamp. Positive agglutination was clearly seen and without further reaction after 15-20 seconds. Occasionally very slow, weak cross reactions were observed and discounted. The poly-H test was not regarded as unconditional, however, because of the innate incompleteness of an antigen test of this type. The Difco H. antiserum (2406) contained antibodies for antigens a, b, c, d, eh, en, enx, fg, fgt, gm, gmq, gms, gp, gpr, gq, gst, gt, i, k, lv, lw, lz<sub>13</sub>, lz<sub>28</sub>, mt, r, y, z, z<sub>4</sub><sup>z</sup><sub>23</sub>, z<sub>4</sub><sup>z</sup><sub>24</sub>, z<sub>4</sub><sup>z</sup><sub>32</sub>, z<sub>6</sub>, z<sub>10</sub>, z<sub>29</sub>, 1, 2, 1,5, 1,6, and 1,7 (Difco, May 1964).

Biochemical Typing: At least 5 isolated cultures from each presumable positive sample were subjected to biochemical testing. The appropriate media were inoculated from 24-hour-old TSI agar slant cultures. The fermentation, indole, motility, and urea media were prepared, processed, and interpreted as stipulated by Edwards and Ewing (1962). Malonate medium, Simmon's citrate agar, and the decarboxylase differential media were obtained from Difco. Preparation and interpretation of these media were as indicated in Difco literature (Difco, 1953; Difco, June 1964). The results of the biochemical reactions were recorded on a chart similar to that shown in Fig. 3.

Those isolates which could still be considered as possible salmonellae after biochemical tests were forwarded to the Kansas State Department of Health, Division of Public Health Laboratories,

SourceSample No.TSI No.TSI Reaction:      Slant \_\_\_\_\_ Butt \_\_\_\_\_ H<sub>2</sub>S \_\_\_\_\_Gram StainPoly-H AgglutinationFermentationsDays

	1	2	3	4	5	21
Sucrose						
Lactose						
Xylose						
Dulcitol						
Dextrose						
Salicin						

Fig. 3. Table implemented to record characteristics of isolated organisms.

Fig. 3. (concl.)

Malonate utilization

Indole

Motility

Ornithine decarboxylase

Arginine decarboxylase

Lysine decarboxylase

Simmon's citrate

Urea

Final Identification:



Topeka, Kansas for final identification.

Viability Tests: The first 7 positive samples were held at room temperature in sealed containers and re-examined culturally at intervals of 4 and 8 months from the date of initial isolation.

## RESULTS

One hundred samples of a wide variety of plant foods of various origins were examined bacteriologically. Nine of these were positive for salmonellae. The source of the major ingredient of these samples, the number of samples examined, and the number of salmonellae containing samples of each type is shown in Table 1.

Table 1. The source and number of samples examined and the number which contained salmonellae.

Source of major ingredient	Number examined	Number yielding salmonellae
Animal	28	7
Soil	28	2
Chemicals	21	0
Plant	17	0
Bacteria	4	0
Fish	2	0
Total	100	9

It should be noted that in many cases, the plant foods contained a mixture of ingredients from various sources. Twenty-eight plant foods of mainly animal origin were examined. Of these, 10 were bone meal samples, 4 (40%) of which yielded salmonellae. The 4 salmonellae containing samples were advertised as "steamed" bone meal. The serotypes isolated were S. meleagridis, S.

worthington, S. fresno, S. cerro, S. cubana, and S. anatum. Of the remaining 18 samples of animal origin, 5 were advertised as Rose Food and contained bone meal and blood meal as part of their ingredients. Three of these samples yielded salmonellae. The serotypes isolated were S. cerro, S. melasgridis, and S. anatum. Twelve samples of manure products, originating from cattle, sheep, and turkeys were negative. The 28 soil-type of samples investigated contained a wide variety of minor ingredients. Two of these samples were positive. One sample of African Violet Potting Soil, which contained composted manure as a constituent, yielded S. cerro. The printed wrapper on this package noted that the product had been sterilized. The other Salmonella positive soil sample, which sold as a Planter and Potting Soil, contained organic compost as an ingredient. The serotype isolated was S. anatum. None of the 21 samples whose principal constituents were chemicals yielded salmonellae. The remaining 23 samples, which were derived from plant, bacterial, and fish sources did not yield salmonellae. All of the various types of samples which contained salmonellae were products of different companies. Details of these results are summarized in Table 2.

Biochemical Reactions and Poly-H Agglutination: The biochemical reactions and antigenic structures of the various Salmonella serotypes are outlined in the Appendix. Poly-H agglutination was carried out as part of the isolation routine. This study, however, was not concerned with the number of different serotypes isolated, but rather with the number of salmonellae containing plant foods. Therefore, only a few, random, isolated cultures from each sample

Table 2. Type and number of samples examined, the number yielding salmonellae, and Salmonella serotypes isolated from the various samples.

Type of sample	Number of samples examined	Number containing salmonellae	Serotypes isolated
Bone meal	10	4	<u>S. meleagridis</u> <u>S. worthington</u> <u>S. fresno</u> <u>S. cerro</u> <u>S. cubana</u> <u>S. anatum</u>
Rose food	5	3	<u>S. cerro</u> <u>S. meleagridis</u> <u>S. anatum</u>
Manure	12	0	
Blood meal	1	0	
Potting soils	28	2	<u>S. cerro</u> <u>S. anatum</u>
Solid chemicals	17	0	
Liquid chemicals	4	0	
Mixed plant products	11	0	
Humus	3	0	
Moss	2	0	
Peat	1	0	
Bacterial inoculants	4	0	
Fish emulsion	2	0	
Total	100	9	

were sent to the Kansas State Board of Health Laboratory for final identification by serotyping.

Evaluation of Enrichment Procedures and Media: The first 30 samples, including 7 positive samples, which were examined by the lactose pre-enrichment method gave no positive results. Neither direct plating onto BGS nor plating on BGA after subculture in tetrathionate yielded salmonellae isolations. The number of coliforms and other contaminants present after lactose pre-enrichment of these samples greatly reduced the number of isolated, suspect colonies appearing on both plating media. Thus, the lactose pre-enrichment method, as used in this study, did not provide an effective means of achieving Salmonella isolations from a contaminated environment.

On the other hand, direct inoculation of both brilliant green tetrathionate and dulcitol selenite selective enrichments gave sufficient inhibition of contaminants to allow fishing out of salmonellae. Dulcitol selenite enrichment gave greater suppression of contaminants. However, it was felt that possibly some salmonellae were also being inhibited. All DSE isolates were forwarded for serotyping and these yielded only 2 serotypes, S. macleagris and S. worthington. Both serotypes were obtained from bone meal and no salmonellae were isolated from positive Rose Food samples. BGA was more effective than BGS for seeding DSE cultures. Brilliant green tetrathionate in combination with BGA gave the best results of the enrichment methods utilized. A random sampling of isolates obtained by this method yielded 6 serotypes from bone meal and 3 from Rose Food, which were not detected by DSE. It was

found also that BGA worked just as well as BGS in conjunction with BGTET. The secondary enrichment of BGTET into BGTET provided excellent results during this study and when followed by plating to BGA, Salmonella colonies were easily differentiated from other organisms. Of the contaminants, Proteus, Pseudomonas, and the coliforms were found to be most common. When found in the presence of excess coliforms, salmonellae colonies on BGA were whitish and surrounded by the yellow zone of coliform lactose fermentation.

Since BGTET selective enrichment followed by seeding BGA plating media appeared to give the best means of salmonellae isolation with the first 30 plant food samples, this method alone was retained for examining the other 70 samples.

Viability Tests: All positive bone meal and Rose Food samples continued to yield viable salmonellae after 8 months. Since the salmonellae positive soil samples were obtained late in the study, viability studies over a significant duration were not carried out. However, the samples which were tested were dry products and retained salmonellae in a viable state for a relatively long time at room temperature.

#### DISCUSSION

In the isolation of salmonellae, or any other organisms, one of the most important criteria is the use of appropriate techniques. Essential to this criterion is the employment of suitable media and the recognition of their capabilities by the researcher. When salmonellae are the organisms under survey, it is imperative that the worker understands the variation and effectiveness which can

be obtained by the media employed. Since most of the original procedures for isolating salmonellae were designed for use with fecal specimens, many modifications of both procedure and media have resulted for examining other samples. Pre-enrichment has been found necessary for many samples containing small numbers of salmonellae, often in a debilitated state, in order that the numbers be increased. As a means of obtaining better yields of salmonellae, wetting agents which gave dispersion of materials in enrichment media were employed. Adjustments had to be made to compensate for the untoward effects of large concentrations of organic material. Certain of the enrichment media inhibited some of the salmonellae as well as the contaminants and others did not provide adequate retardation of contaminant growth. The same problem was encountered with plating media and often differentiating salmonellae colonies was extremely difficult. Thus, despite the many improvements in procedure and media, there is still ample room for modification and evaluation of techniques. The main object of this survey was to isolate salmonellae from plant foods which are available at the consumer level. The particular procedures used were aimed at accomplishing this, with an awareness of the inherent difficulties involved.

The pre-enrichment method used as part of this study was decided upon after contemplating the reports of various researchers in the field. The deleterious effect of large amounts of organic matter on enrichment media was shown by Hurley and Ayres (1953), Galton, Lowery and Hardy (1954), Dack (1955), Stokes and Osborne (1955), Silliker and Taylor (1958), and Galton (1961). Various

workers suggested pre-enrichment of samples before selective enrichment (Dack, 1955; Slocum, 1955; Edwards and Ewing, 1962; Galton et al., 1968). North (1961), in isolating salmonellae from dried egg albumen, found increased salmonellae recovery by the lactose pre-enrichment method. He noted that although tetrathionate gives just as good recovery following lactose pre-enrichment, it involves the disadvantage of a longer incubation time. Taylor and Sillicker (1961) reported that lactose, dulcitol and mannitol used in pre-enrichment media gave similar results. After considering these reports, a decision was made to use the lactose pre-enrichment method in this study in an attempt to facilitate better recovery of salmonellae. The lactose broth was prepared by rehydrating the "Difco" lactose broth. A 100 gram sample of the plant foods suspended in 330 ml. of lactose broth gave satisfactory distribution of material. All of these samples were directly plated to BGS and subcultured to selective enrichment following incubation and any excess of organic matter was adequately compensated for. However, this method did not provide satisfactory results for the samples examined. In spite of the fact that subculturing from lactose to BGTEF reduced the number of contaminants, it was difficult to achieve isolated, characteristic colonies on the plating media. Apparently the numbers of contaminating organisms in the plant foods were high enough so that they retained a numerical advantage even after lactose incubation.

A direct selective enrichment method using both brilliant green tetrathionate and dulcitol selenite enrichment broths was selected in order to detect organisms which might be isolated by



one medium and not the other. The effectiveness of these two media in examining the plant foods could thus be evaluated. For both direct selective enrichment and selective enrichment from lactose pre-enrichment cultures, brilliant green was incorporated into the tetrathionate medium. It was reported that tetrathionate was inhibitory to certain salmonellae (Banwart and Ayres, 1953; Smith, 1952). McCullough and Byrne (1952) reported good salmonellae recovery with BGTET. Stokes and Osborne (1955) and Osborne and Stokes (1955) had used brilliant green in tetrathionate and reported its efficiency in inhibiting contaminants. Edwards and Ewing (1962) and Galton (1961) have obtained excellent results with brilliant green tetrathionate in salmonellae isolations from different sources. Secondary enrichment was used in this study, as suggested by Jamieson (1961). Dulcitol selenite enrichment was employed because of its reported sensitivity (Raj and Liston 1965; Abrahamsson et al., 1968) in detecting very low numbers of salmonellae in the presence of large numbers of mixed flora common to foods. In this study, however, DSE seemed to be inhibitory to some salmonellae in view of the fact that BGTET facilitated more isolations from the same samples. However, those salmonellae colonies which were obtained from DSE were often the only type of growth appearing on the plating. BGTET provided adequate selective action so that the number of contaminants was small enough to enable the plating media to differentiate Salmonella. Thus, despite the fact that BGTET was less selective than DSE, in combination with plating media BGTET yielded more isolations. Particularly good results were obtained following secondary enrichment of BGTET into another tube of BGTET.



Jameson (1961, 1962) discussed the affects of secondary enrichment and McCoy (1962) suggested that secondary enrichment may need to be prolonged for 4 days for best results. Secondary enrichment was used in this study because it offers a further dilution of organic matter and may favorably affect the growth of the desired organism by some, as yet unclear, favorable enrichment dynamics.

The choice of plating media was made on the basis of results obtained by previous workers. Banwart and Ayres (1953) obtained better results with BGA than with deoxycholatecitrate agar (DCA), bismuth-sulfite agar (BSA), or Salmonella-Shigella agar (SS). Although Byrne et al. (1953) found BGA an unsuitable medium for S. pullorum, other workers (Taylor et al., 1958; Wells et al., 1958) have obtained good results with BGA. Edwards and Ewing (1962) suggested BGA for general use along with BSA. Adinarayanan et al. (1965) found BGA to be the plating medium of choice in isolations made from foods. Gerichter and Sechter (1966) obtained best isolation frequencies from bone meal with BGA. Galton et al. (1968) suggested BGA as a plating medium for examining food and feed samples. Galton, Lowery and Hardy (1954) reported improved inhibition of contaminants upon addition of 8-16 mg. of sodium sulfadiazine per 100 ml. of BG agar and recommended this medium for examining food samples. Grumbles et al. (1967) recommended BGS for examining animal feed and meat by products. In this study it was found that both BGA and BGS gave similar results as far as obtaining salmonellae from positive samples. However, the increased selectivity of BGS was found not to be required following selective enrichment in BGTTT, since well isolated colonies could

be achieved on BGA. Cultures from the plating media were transferred to Triple Sugar Iron Agar as originally suggested by Hajna (1945).

Most of the contaminated plant foods examined contained a majority of ingredients of animal origin as shown in Table 1. Bone meal and Rose Food which contained bone meal and blood meal provided all but two of the salmonellae positive samples. These results confirm the contamination of bone meal as reported by workers from other countries (Walkor, 1957; Galbraith et al., 1962; Report, 1959; Timbury et al., 1966). As shown in Table 2, two potting soils yielded salmonellae. Each of these soils contained animal manure as a minor ingredient. It is interesting to note that all of the bone meal samples yielding salmonellae were steamed products. This is in contradiction to results obtained by Timbury et al. (1966) who found that all samples of steamed bone flour examined were free of salmonellae. The African Violet Potting Soil which yielded Salmonella was advertised as a sterilized plant food. In fact most of the heat-treated plant foods examined provided abundant growth of bacteria even after selective enrichment. Although none of the plant foods of mostly chemical, plant, bacterial, or fish origin yielded salmonellae, the number of samples tested which contained bacterial compositions and fish waste was small.

Although only a few, random isolates were subjected to final serotyping, of these, the order of frequency of serotypes in bone meal was S. worthington (5), S. cubana (4), S. melsburgidis (3), S. anatum (3), S. fresno (1). The frequency in Rose Food was S.

meleagridis (5), S. cerro (2), and S. anatum (1), while S. cerro (1) and S. anatum (1) were obtained from the soil samples. The significance of similar serotypes from different sources is not readily apparent. The number of serotypes obtained is most likely an underestimate of the extent of contamination of bone meal since few isolates were serotyped. In England, Timbury et al. (1966) while examining bone meal, blood meal and hoof-and-horn meal samples isolated 66 Salmonella serotypes. Four of the more common types they obtained were S. anatum, S. meleagridis, S. cubana, and S. worthington. The results of this study are similar, except that they did not isolate S. fresno or S. cerro. These 2 serotypes have been reported in bone meal, however, by Walker (1957) and Galbraith et al. (1962). Thus, some of the bone meal sold in the United States is contaminated with salmonellae, and perhaps imported bone products are obtained from sources also furnishing other countries.

The results of this study provide evidence that plant foods purchased at the consumer level and containing at least some material of animal origin could contain a potentially pathogenic Salmonella.

The viability trials performed during this study indicate that salmonellae can survive in dry plant food products for a relatively long time at room temperature. As used in this study, the slide poly-H agglutination test on suspect isolates gave a useful means of preliminary verification of salmonellae. All positive poly-H cultures were ultimately shown to be salmonellae and the reactions with "H" antiserum were clear cut and rapid so there

was little difficulty in reading the test. Of course, the limitations to the range of "H" antigens covered by the antiserum must be borne in mind when evaluating these results.

Evaluating the hazard of plant foods as a source of human salmonellosis is difficult. However, packages of plant foods may be kept in kitchens or around the home, where dust from them could contaminate food. Every package of bone meal examined in this study leaked dust and all salmonellae containing samples liberated a cloud of dust upon opening the containers. Distributing plant foods and potting plants are often done by hand and could easily lead to contamination of food. It was observed that in most stores which sold plant foods, human food products were sold close by. In fact some of the plant foods purchased for this survey were handled by store employees who also handled human food products. Perhaps this possible mode of transmission is not fully appreciated. Most of the serotypes isolated as part of this study are often involved in food-poisoning incidents, and yet many outbreaks of salmonellosis have gone unexplained. Possibly plant foods or fertilizers are a source of infection for certain of these incidents. Since many of the samples investigated are sold by chain stores, they are widely distributed throughout the United States. It appears from this survey that a majority of plant foods are probably uncontaminated, with those containing animal products and particularly the popular bone meal as being the most likely to contain salmonellae.

## SUMMARY

One hundred different samples of plant foods obtained at various stores in Manhattan, Kansas were examined bacteriologically to determine the incidence of salmonellae contamination. Nine of the investigated samples yielded salmonellae, including 4 of steamed bone meal, 3 of Rose Food, and 2 of Potting Soils. The serotypes isolated were: S. meleagridis, S. worthington, S. fresno, S. cerro, S. cubana, and S. anatum.

A lactose broth pre-enrichment method was tested for isolating salmonellae. Both direct plating of lactose pre-enrichment and subculture in selective enrichment prior to plating were examined, and both gave unsatisfactory results in this study. Two selective enrichment media were compared for efficiency of salmonellae recovery. Both brilliant green tetrathionate and dulcitol selenite enrichment gave adequate inhibition of contaminating organisms, however DSE also reduced the number of salmonellae isolations. Brilliant green tetrathionate was found to give more satisfactory results. The BGTET-BGA combination used gave the most salmonellae recoveries and was found to adequately limit the numbers of contaminants. The more selective brilliant green sulfadiazine agar was not necessary for facilitating isolation. Especially advantageous throughout this work was the secondary enrichment method of subculturing BGTET into another tube of BGTET for recovery of salmonellae.

Salmonellae in plant foods remained viable 8 months after the initial isolation date. The possible hazard of plant foods,

especially bone meal or products containing other animal waste,  
as a source of salmonellae is cited.

## ACKNOWLEDGEMENTS

The author would like to express his deep and sincere appreciation to his major advisor, Professor V. D. Foltz, for the highly regarded counsel and concern offered by him throughout my graduate work and especially during this study. Grateful acknowledgement is extended to Dr. J. J. Iandolo for his advice both professionally and otherwise. The author is also sincerely appreciative of the guidance of Dr. J. O. Harris during the course of this graduate study.

Thanks are extended to Nicholas D. Duffet, Director, Kansas State Department of Health Laboratories, Topeka, Kansas and his staff for the final identification of cultures forwarded to them. Thanks are also due Mrs. Celia Stankorb for typing the manuscript.

This writer is particularly indebted to his wife, Sandy, for her understanding and patience.



## BIBLIOGRAPHY

- Abrahamsson, K., G. Patterson, and H. Riemann. 1968.  
Detection of Salmonella by a single-culture technique.  
Appl. Microbiol. 16: 1695-1698.
- Adinarayanan, N., V. D. Foltz, and Flora McKinley. 1965.  
Incidence of salmonellae in prepared and packaged foods.  
J. Infect. Dis. 115: 19-26.
- Armstrong, R. W. 1964.  
Environmental factors involved in studying the relationship between soil elements and disease. Am J. Public Health. 54: 1536.
- Atkinson, N. 1964.  
Salmonellosis in Australia, In The World Problem of Salmonellosis. p. 581. Ed. E. Van Oye. The Hague: Dr. W. Junk.
- Banwart, G. J., and J. C. Ayres. 1953.  
Effect of various enrichment broths and selective agars upon the growth of several species of Salmonella. Appl. Microbiol. 1: 296-301.
- Bate, J. G., and U. James. 1958.  
Salmonella typhimurium infection dust-borne in a children's ward. Lancet 2: 713-715.
- Bergner-Rabinowitz, S. 1956.  
The survival of coliforms, Streptococcus faecalis, and Salmonella tennessee in the soil and climate of Israel. Appl. Microbiol. 4: 101-106.
- Boring, J. R. 1958.  
Domestic fish meal as a source of various Salmonella types. Vet. Med. 53: 311.
- Bowmer, E. J. 1965.  
Salmonellae in food - a review. J. Milk and Food Tech. 28: 74-86.
- Burr, W. E., and C. F. Helmboldt. 1962.  
Salmonellae species contaminants in three animal by-products. 6: 441-443.
- Byrne, A. F., M. M. Rayman, and M. D. Schneider. 1955.  
Methods for the detection and estimation of numbers of Salmonella in dried eggs and other food products. Appl. Microbiol. 3: 368-371.

- Collard, P., and M. Unwin. 1958.  
A trial of Rappaport's medium. J. Clin. Path.  
11: 426-427.
- Committee on Refuse Disposal, APWA. 1961.  
Municipal Refuse disposal, APWA. Research Foundation  
Project 104. Pub. Admin. Service, Chicago, Illinois.
- Council Report. 1966.  
Salmonellosis. J.A.V.M.A. 149: 1079-1080.
- Dack, G. M. 1955.  
Symposium on methodology for Salmonella in food. Bact.  
Rev. 19: 275-276.
- Datta, N., R. B. Pridie, and E. S. Anderson. 1960.  
An outbreak of infection with Salmonella typhimurium in  
a general hospital. J. Hyg., Camb. 58: 229-237.
- Difco Laboratories. 1953.  
Difco Manual, 9th ed. Difco Laboratories Inc.,  
Detroit 1, Michigan.
- Difco Laboratories. 1964.  
Decarboxylase differential media for the Enterobacteria-  
ceae. Difco Laboratories Inc., Detroit 1, Michigan.
- Difco Laboratories. 1964.  
Identification of the Salmonella serotypes. Difco Labor-  
atories Inc., Detroit 1, Michigan.
- Edwards, P. R., D. W. Bruner, and A. B. Moran. 1948.  
Salmonella infections of fowls. Cornell Vet. 38:  
247-256.
- Edwards, P. R., and W. H. Ewing. 1962.  
Identification of Enterobacteriaceae. Burgess Publishing  
Co., Minneapolis 15, Minnesota.
- Elliot, R. P., and P. K. Heiniger. 1965.  
Improved temperature-gradient incubator and the maximal  
growth temperature and heat resistance of Salmonella.  
Appl. Microbiol. 13: 73-76.
- Galbraith, N. S., C. E. D. Taylor, P. Cavanagh, J. G. Hagan,  
and J. L. Patton. 1962.  
Pet foods and garden fertilizers as sources of human  
salmonellosis. Lancet i: 372-375.
- Galton, M. M., and A. V. Hardy. 1948.  
Studies of acute diarrheal diseases. XXI. Salmonellosis  
in Florida. Public Health Reports 63: 847-851.

- Galton, M. M., C. L. Stucker, H. B. McElrath, and A. V. Hardy. 1950.  
A preliminary study of tetrathionate brilliant green bile broth with added sulfathiazole for the isolation of Salmonella from dogs. Bact. Proc. p. 115.
- Galton, M. M., J. E. Scatterday, and A. V. Hardy. 1952.  
Salmonellosis in dogs. J. Infect. Dis. 91: 1-18.
- Galton, M. M., W. D. Lowery, and A. V. Hardy. 1954.  
Salmonella in fresh and smoked pork sausage. J. Infect. Dis. 96: 232-235.
- Galton, M. M., W. V. Smith, H. B. McElrath, and A. B. Hardy. 1954.  
Salmonella in swine, cattle and the environment of abattoirs. J. Infect. Dis. 95: 236-245.
- Galton, M. M., M. Harless, and A. V. Hardy. 1955.  
Salmonella isolations from dehydrated dog meals. J.A.V.M.A. 126: 57-58.
- Galton, M. M. 1961.  
Laboratory procedures for the isolation of Salmonella from human and animal food products. Proceedings 65th Annual Meeting of the United States Livestock Sanitary Association, Minneapolis, Minnesota, p. 434-440.
- Galton, M. M., J. R. Boring, and W. T. Martin. 1964.  
Salmonellae in Foods - A review of methods for isolation and a suggested procedure. National Communicable Disease Center, Atlanta, Georgia.
- Galton, M. M., G. K. Morris, and W. T. Martin. 1968.  
Salmonellae in Foods and Feeds - Review of isolation methods and recommended procedures. National Communicable Disease Center, Atlanta, Georgia.
- Georgala, D. L., and M. Boothroyd. 1965.  
A system for detecting salmonellae in meat and meat products. J. Appl. Bact. 28: 206-212.
- Gerichter, C. B., and I. Sechter, 1966.  
Comparison of methods for the isolation of Salmonella from bone meal. Appl. Microbiol. 14: 711-715.
- Goddard, J. L. 1967.  
Incident at Selby Junior High. Nutrition Today. 2: 2-9.
- Golueke, C. G., B. J. Card, and P. H. McGauhey. 1954.  
A critical evaluation of inoculums in composting. Appl. Microbiol. 2: 45-53.

- Gray, D. F., P. F. Lewis, and C. J. R. Gorrie. 1958.  
Bonemeal as a source of bovine salmonellosis. Austral. Vet. J. 34: 345-351.
- Gray, D. R., O. C. Harley, and J. L. Noble. 1960.  
The ecology and control of Salmonella contamination in bone meal. Austral. Vet. J. 36: 246-252.
- Grumbles, L. C., T. Bartram, E. M. Ellis, M. M. Galton, W. Henderson, B. S. Pomeroy, and J. E. Williams. 1967.  
Recommended procedure for the isolation of Salmonella organisms from animal feeds and meat by products for use in the cooperative state/federal Salmonella control program. 71st Annual USLSA Meeting, Phoenix, Arizona, October 16, 1967.
- Guinee, P. A. M., and E. H. Kampelmacher. 1962.  
Influence of variations of the enrichment method for detection of salmonella. Antonie Leeuwenhoek 28: 417-427.
- Guinee, P. A. M., E. H. Kampelmacher, and H. H. Hoejenbos (1965).  
Further studies on the influence of variations in the enrichment method for the detection of Salmonellae. J. Microbiol. and Serology 31: 1.
- Hajna, A. A. 1945.  
Triple sugar iron agar medium for the identification of the intestinal group of bacteria. J. Bact. 49: 516-517.
- Hanks, T. G. 1967.  
Solid waste/disease relationships. U. S. Dept. H. E. W. Bureau of disease prevention and environmental control national center for urban and industrial health, Cincinnati, Ohio.
- Hart, S. A., and M. E. Turner. 1965.  
Lagoons for livestock manure. J. Water Poll. Con. Fed. 37: 1578.
- Harvey, R. W. S., and S. Thomson. 1953.  
Optimum temperature of incubation for isolation of salmonellae. Monthly Bull. Minist. Health (Lond.) 12: 149-150.
- Harvey, R. W. S., and W. P. Phillips. 1961.  
An environmental survey of bakehouses and abattoirs for salmonellae. J. Hyg., Camb. 59: 93-103.
- Harvey, R. W. S., D. E. Mahabir, and T. H. Price. 1966.  
A method of secondary enrichment for salmonellae independent of selectively toxic chemicals. J. Hyg., Camb. 64: 361.

- Hobbs, B. C. 1963.  
Techniques for the isolation of Salmonella from eggs and egg products. Ann. Inst. Pasteur 104: 621-637.
- Hobbs, B. C. 1964.  
Contamination of meat supplies. Royal Society of Health Publications. 34/64.
- Hurley, N. A., and J. C. Ayres. 1953.  
A comparison of six enrichment media for the isolation of Salmonella pullorum from egg products. Appl. Microbiol. 1: 302-306.
- Hynes, M. 1942.  
The isolation of intestinal pathogens by selective media. J. Clin. Path. 54: 193-207.
- Iveson, J. B., N. Kovacs, and W. Laurie. 1964.  
An improved method of isolating salmonellae from contaminated coconut. J. Clin. Path. 17: 75-78.
- Jacobs, J., P. A. M. Guinee, E. H. Kampelmacher, and A. van Keulen. 1963.  
Studies on the incidence of Salmonella in imported fish meal. Zentralblatt für Veterinärmedizin 10: 542-550.
- Jameson, J. E., and N. W. Emberley. 1956.  
A substitute for bile salts in culture media. J. Gen. Microbiol. 15: 198-204.
- Jameson, J. E. 1961.  
A study of tetrathionate enrichment techniques with particular reference to two new tetrathionate modifications used in isolating salmonellae from sewer swabs. J. Hyg. 59: 1-13.
- Jameson, J. E. 1962.  
A discussion of the dynamics of Salmonella enrichment. J. Hyg., Camb. 60: 193-205.
- Jameson, J. E. 1963.  
A note on the isolation of salmonellae. J. Appl. Bact. 26: 112-114.
- Jeffries, L. 1959.  
Novobiocin-tetrathionate broth: A medium of improved selectivity for the isolation of salmonellae from faeces. J. Clin. Path. 12: 568-571.
- Kauffmann, F. 1930.  
Ein kombiniertes Anreicherungsverfahren für Typhus und Paratyphusbazillen. Zbt. f. Bakt. I. 119: 148-153.

- Kauffman, F. 1935.  
J. Hyg. Infektionskr. 117: 26-32. Cited by M. M. Galton,  
G. K. Morris, and W. T. Martin. 1968.
- Kauffmann, F. 1954.  
Enterobacteriaceae, 2nd edition. E. Munksgaard,  
Copenhagen.
- Knox, R., P. G. H. Gell, and M. R. Pollock. 1943.  
The selective action of tetrathionate in bacteriological  
media. J. Hyg. 43: 147-158.
- Kovacs, N. 1959.  
Salmonellae in dessicated coconut, egg pulp, fertilizer,  
meat-meal, and mesenteric glands: preliminary report.  
Mod. J. Austral. 1: 557.
- Kristensen, M., V. Lester, and A. Jurgens. 1925.  
Use of trypsinized casein, brom-thymol-blue, brom-  
cresol-purple, phenol-red and brilliant green for  
bacteriological nutrient media. Brit. J. Exp. Path.  
6: 291-299.
- Lachowicz, K. 1964.  
Salmonella Infections in Eastern Europe, in The World  
Problem of Salmonellosis. p. 309,313. Ed. E. van Oye.  
The Hague: Dr. W. Junk.
- Leifson, E. 1935.  
New culture media based on sodium desoxycholate for the  
isolation of intestinal pathogens and for the enumeration  
of colon bacilli in milk and water. J. Clin. Path.  
40: 581-599.
- Leifson, E. 1936.  
New selenite enrichment media for the isolation of typhoid  
and paratyphoid (Salmonella) bacilli. Amer. J. Hyg.  
24: 423-432.
- MacConkey, A. T. 1908.  
Bile salt media and their advantages in some bacteriolo-  
gical examination. J. Hyg. 8: 322-334.
- McCoy, J. H. 1962.  
The isolation of salmonellae. J. Appl. Bact.  
25: 213-224.
- McCoy, J. H. 1963.  
River Pollution: Bacteriological aspects and their sig-  
nificance. Royal Soc. of Health J. 83: 154-162.

- McCullough, M. B., and A. F. Byrne. 1952.  
Relative efficiency of different culture mediums in  
isolation of certain numbers of the Salmonella group.  
J. Infect. Dis. 90: 71-75.
- Mehring, A. L. 1957.  
The characteristics of the non-farm fertilizer market.  
Plant Food Review 3: 15.
- Mehring, A. L. 1961.  
Fertilizer use by non-farm homes. Plant Food  
Review 7: 20.
- Miller, V. R., and G. J. Banwart. 1965.  
Effect of various concentrations of brilliant green and  
bile salts on salmonellae and other microorganisms.  
Appl. Microbiol. 13: 77.
- Montford, J. and F. S. Thatcher. 1961.  
Comparison of four methods of isolating salmonellae from  
foods, and elaboration of a preferred procedure. J. of  
Food Science 26: 510-517.
- Morehouse, G. L. and E. E. Wedman. 1961.  
Salmonellae and other disease producing organisms in  
animal by-products. J.A.V.M.A. 139: 989-995.
- Morris, G. K. 1968.  
Symposium on laboratory problems in detecting salmonellae.  
Ninth Biennial Veterinary Conference, National Communica-  
ble Disease Center, Atlanta, Georgia, May 8, 1968.
- Mossel, D. A. A., M. Visser, and A. M. R. Cornelissen. 1963.  
The examination of foods for Enterobacteriaceae using a  
test of the type generally adopted for the detection  
of salmonellae. J. Appl. Bact. 26: 444-452.
- Moyle, A. I. 1966.  
Salmonellae in rendering plant by-products. J.A.V.M.A.  
149: 1172-1176.
- Mueller, L. 1923.  
Un nouveau milieu d'enrichissement pour la recherche du  
Bacille typhique et des paratyphiques. Compt. rend. Soc.  
de biol. 89: 434-437.
- Mushin, R. 1948.  
An outbreak of gastro-enteritis due to Salmonella derby.  
J. Hyg., Camb. 46: 151-157.



- Newell, K. W., R. McClarin, C. R. Murdock, W. N. MacDonald, and H. L. Hutchinson. 1959.  
Salmonellosis in Northern Ireland, with special reference to pigs and salmonella-contaminated pig meal. *J. Hyg., Camb.* 57: 92-105.
- North, W. R., and M. T. Bartram. 1953.  
The efficiency of selenite broth of different compositions in the isolation of Salmonella. *Appl. Microbiol.* 1: 130-134.
- North, W. R. 1961.  
Lactose pre-enrichment method for isolation of Salmonella from dried egg albumen. *Appl. Microbiol.* 9: 188-194.
- Osborne, W. W. and J. L. Stokes. 1955.  
A modified brilliant green medium for the isolation of Salmonella from egg products. *Appl. Microbiol.* 3: 295-299.
- Raj, H. and J. Liston. 1965.  
A highly sensitive enrichment medium for Salmonella from foods. *Bacteriological Proceedings, 65th Annual Meeting*, p. 3.
- Raj, J. 1966.  
Enrichment medium for selection of Salmonella from fish homogenate. *Appl. Microbiol.* 14: 12-20.
- Rappaport, F., N. Konforti, and B. Nevon. 1956.  
A new enrichment medium for certain salmonellae. *J. Clin. Path.* 9: 261.
- Rappaport, F., and N. Konforti, 1959.  
Selective enrichment medium for paratyphoid bacteria. *Appl. Microbiol.* 7: 63-66.
- Read, R. B., and A. L. Reyes. 1968.  
Variation in plating efficiency of salmonellae on eight lots of brilliant green agar. *Appl. Microbiol.* 16: 746-748.
- Report. 1959.  
Salmonella organisms in animal feeding stuffs and fertilizers. *Mon. Bull. Minist. Health Lab. Serv.*, 18: 26-35.
- Rolfe, V. 1946.  
A note on the preparation of tetrathionate broth. *Mon. Bull. Minist. Hlth. Lab. Serv.* 5: 158-159.
- Rubbo, S. D. 1948.  
Cross-infection in hospital due to Salmonella derby. *J. Hyg., Camb.* 46: 158-165.

- Salmonella Surveillance Report No. 7, 1962.  
Communicable Disease Center, Atlanta, Georgia.
- Salmonella Surveillance Annual Report, 1963.  
Communicable Disease Center, Atlanta, Georgia.
- Salmonella Surveillance Report No. 25, June 8, 1964.  
Communicable Disease Center, Atlanta, Georgia.
- Salmonella Surveillance Report No. 63, June 1967.  
Communicable Disease Center, Atlanta, Georgia.
- Scheffer, F., F. Schutz, and H. Brune. 1955.  
The viability of pathogenic intestinal bacteria in  
sludge and liquid manure. J. Soils and Fertilizers  
19: 91.
- Schneider, M. D. 1946.  
Investigation of Salmonella content of powdered whole  
egg with not more than two percent moisture content.  
II. General survey on occurrence of species of Salmonella  
in high quality egg powder. Food Res. 11: 313-318.
- Shotts, E. B., W. T. Martin, and M. M. Galton. 1961.  
Further studies on salmonellae in human and animal foods  
and in the environment of processing plants. Proceedings  
65th Annual Meeting of the United States Livestock Sanitary  
Association, Minneapolis, Minnesota, p. 309-318.
- Sickenga, F. N. 1964.  
Transmission of salmonellae and pathogenesis of salmonellosis in man, in The World Problem of Salmonellosis.  
p. 205. Ed. E. van Oye. The Hague: Dr. W. Junk.
- Silliker, J. H., and W. I. Taylor. 1958.  
Isolation of salmonellae from food samples. II. The  
effect of added food samples upon the performance of  
enrichment broths. Appl. Microbiol. 6: 228-232.
- Silliker, J. H., R. H. Deibel, and P. T. Fagan. 1964a.  
Enhancing effect of feces on isolation of salmonellae from  
selenite broth. Appl. Microbiol. 12: 100-105.
- Silliker, J. H., R. H. Deibel, and P. T. Fagan. 1964b.  
Isolation of salmonellae from food samples. VI. Comparison of methods for the isolation of salmonellae from  
egg products. Appl. Microbiol. 12: 224-228.
- Slocum, G. G., 1955.  
Symposium on methodology of Salmonella in food.  
Bact. Rev. 19: 275-276.

- Smith, H. W. 1952.  
The evaluation of culture media for the isolation of salmonellae from faeces. J. Hyg. 50: 21-36.
- Spino, D. F. 1966.  
Elevated temperature technique for the isolation of Salmonella from streams. Appl. Microbiol. 14: 591-596.
- Steele, J. H. 1966.  
Salmonellosis Proceedings 70th Annual Meeting of the United States Livestock Sanitary Association Minneapolis, Minnesota, p. 457-464.
- Stokes, J. L., and W. W. Osborne, 1955.  
A selenite brilliant green medium for the isolation of Salmonella. Appl. Microbiol. 3: 217-218.
- Sugiyama, H., G. M. Dack, and G. Lippitz. 1960.  
Agglutinating antiserum for the isolation of Salmonella with special reference to isolation from egg albumen. Appl. Microbiol. 8: 205-209.
- Szmunes, W. 1966.  
The microbiological and epidemiological properties of infections caused by Salmonella enteritidis. J. Hyg., Camb. 64: 9.
- Taylor, W. I., J. H. Silliker, and H. P. Andrews, 1958.  
Isolation of salmonellae from food samples. I. Factors affecting the choice of media for the detection and enumeration of Salmonella. Appl. Microbiol. 6: 189-192.
- Taylor, W. I. 1961.  
Isolation of salmonellae from food samples. V. Determination of the method of choice for enumeration of Salmonella. Appl. Microbiol. 9: 487-490.
- Taylor, W. I., and J. H. Silliker. 1961  
Isolation of salmonellae from food samples. IV. Comparison of methods of enrichment. Appl. Microbiol. 9: 484-486.
- Taylor, W. I., B. C. Hobbs, and M. E. Smith. 1964.  
Comparison of two methods for the isolation of salmonellae from imported foods. Appl. Microbiol. 12: 53-56.
- Thomson, S. 1955.  
The numbers of pathogenic bacilli in faeces in intestinal diseases. J. Hyg. 53: 217-224.
- Timbury, M. C., W. C. Forsyth, and J. S. Stevenson. 1966.  
Garden fertilizers as a source of salmonellae. Lancet 2: 955-956.

- van der Schaaf, A., and J. C. Atteveld. 1965.  
Frequency of salmonellae in effluents of modern plants for sewage treatment and possibilities for improvement. Antonie van Leeuwenhoek 31: 221.
- Walker, J. H. C. 1957.  
Organic fertilizers as a source of Salmonella infection. Lancet 273: 283-284.
- Watt, J., and E. Carlton. 1945.  
Studies of the acute diarrheal diseases. XVI. An outbreak of Salmonella typhimurium infection among newborn premature infants. Public Health Repts. 60: 734-740.
- Wells, F. D. Berquist, and R. H. Forsythe. 1958.  
A comparison of selective media for the isolation of Salmonella from commercial egg white solids. Appl. Microbiol. 6: 198-201.
- Wilson, E., R. S. Paffenbarger, M. J. Foter, and K. H. Lewis. 1961.  
Prevalence of salmonellae in meat and poultry products. J. Infect. Dis. 109: 166-171.
- Wilson, W. J., and E. M. Blair. 1927.  
Use of a glucose bismuth and sulphite iron medium for the isolation of Bacillus typhosus and Bacillus proteus. J. Hyg. 26: 374-391.
- Wilson, W. J., and E. M. Blair. 1931.  
Further experience of the bismuth sulphite media in the isolation of Bacillus typhosus and Bacillus paratyphosus from faeces, sewage, and water. J. Hyg. 31: 138-161.

## APPENDIX

Table 3. Biochemical reactions of the various serotypes of Salmonella isolated from plant foods.

Name of organism	TSI Agar Slant:Butt:H <sub>2</sub> S			Gram's Stain	Fermentation Reactions					
					Sucrose	Lactose	Xylose	Dulcitol	Dextrose	Salicin
<u>Salmonella</u> <u>meleagridis</u>	Alk.	A	+	Gram Negative Rods	-	-	Ag	Ag	Ag	-
<u>Salmonella</u> <u>worthington</u>	Alk.	A	+		-	-	Ag	Ag	Ag	-
<u>Salmonella</u> <u>fresno</u>	Alk.	A	+		-	-	Ag	Ag	Ag	-
<u>Salmonella</u> <u>cerro</u>	Alk.	A	+		-	-	Ag	Ag	Ag	-
<u>Salmonella</u> <u>cubana</u>	Alk.	A	+		-	-	Ag	Ag	Ag	-
<u>Salmonella</u> <u>anatum</u>	Alk.	A	+		-	-	-	Ag	Ag	-

Alk. = alkaline  
+ = positive

A = acid  
- = negative

Ag = acid and gas

Table 3. (continued)

	Malonate utilization	Indole	Motility	Ornithine decarboxylase	Arginine decarboxylase	Lysine decarboxylase	Simon's citrate	Urease	Serology		
									Somatic	Flagellar Phase I	Flagellar Phase II
<i>Salmonella</i> <i>moleagris</i>	-	-	+	+	+	+	+	-	E <sub>1</sub>	e,h	1,w
<i>Salmonella</i> <i>worthington</i>	-	-	+	+	+	+	+	-	G	z	1,w
<i>Salmonella</i> <i>fresno</i>	-	-	+	+	+	+	+	-	D	z <sub>38</sub>	-
<i>Salmonella</i> <i>cerro</i>	-	-	+	+	+	+	+	-	K	z <sub>4</sub> , z <sub>23</sub>	-
<i>Salmonella</i> <i>cubana</i>	-	-	+	+	+	+	+	-	G	z <sub>29</sub>	-
<i>Salmonella</i> <i>anatum</i>	-	-	+	+	+	+	+	-	E <sub>1</sub>	e,h	1,6



SALMONELLA SURVEY OF PLANT  
FOODS USED IN AND  
AROUND THE HOME

by

FRANK MITTERMEYER

B.S., Wisconsin State University, LaCrosse, 1967

---

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

DIVISION OF BIOLOGY

Microbiology

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

1969

Salmonellosis is considered to be a major international health hazard. Plant foods and fertilizers may be implicated as a source of infection in certain of the unexplained outbreaks of salmonellosis. As a means of determining the incidence of Salmonella contamination in plant foods used in and around the home, a bacteriological survey was carried out on various samples available to the consumer.

As a part of this study, a lactose pre-enrichment method was tested for its efficacy in isolating salmonellae from plant foods. Lactose broth cultures were both directly inoculated onto plating media and subcultured in selective enrichment media prior to plating. This pre-enrichment method, as used in this study, was found to be unsatisfactory for recovery of salmonellae. Two selective enrichment media were also compared for their efficiency in recovering salmonellae. Both brilliant green tetrathionate and dulcitol selenite enrichment gave sufficient inhibition of contaminating bacteria, however DSE also was found to reduce the number of salmonellae isolations. BGTET yielded more salmonellae recoveries than DSE. The BGTET-BGA combination used yielded the highest number of salmonellae recoveries and yet was adequately selective to limit the number of contaminants. The more selective plating medium, BGS, was unnecessary for streaking from BGTET enrichment. Particularly useful in this study was the secondary enrichment of BGTET cultures into another tube of the same medium, which gave many isolations.

One hundred plant food samples were examined and 9 were found to contain salmonellae. Of 10 bone meal samples processed, 4 (40%)

were steamed products and yielded 6 serotypes: S. meleagridis, S. worthington, S. fresno, S. cerro, S. cubana, and S. anatum. Therefore, the labeling on these packages is erroneous. Of 5 Rose Food samples investigated 3 were found to harbor the serotypes S. cerro, S. meleagridis, and S. anatum. Two soil samples, which contained manure as an ingredient, were positive. One sample of African Violet Potting Soil yielded S. cerro and one Planter and Potting Soil yielded S. anatum. None of the plant foods of plant, chemical, bacterial, or fish origin were found to contain salmonellae.

Viability trials showed salmonellae in plant foods to be viable 8 months after the date of initial isolation. The possible hazard of plant foods, especially bone meal or products containing animal waste, as a source of salmonellae in the United States should be carefully considered.