EFFECT OF GLUTARALDEHYDE ON CHICKEN DRUMSTICKS INOCULATED WITH VARIOUS SALMONELLAE

by

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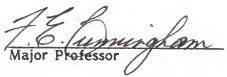

Major Professor

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INTRODUCTION

The prevalence of Salmonellosis represents a major and continuing global threat as a zoonotic disease. Salmonellae infects most species of warm and cold blooded vertebrates including food - producing animals and birds (such as cattle, swine, sheep, goats, horses, fowl and turkeys). Companion pets, such as dogs, cats and other household pets (pet birds and turtles) are likewise carriers of Salmonellae. Poultry is still considered the single most important source of Salmonellae ever since Edward reported it in 1939.

In this experiment the effect of six concentrations of glutaraldehyde (100, 300, 500, 1000, 3000 and 5000 ppm) were tested for their effectiveness in eliminating Salmonella typhimurium from chicken drumsticks infected with a known amount of the organism. Different times (15 and 30 min) and temperatures (4°C and 27°C) were used in a 6 x 2 x 2 factorial design. The three highest concentrations were also tested at unadjusted and adjusted 8.6 pH. Later, the experiments were repeated with Salmonella heidelberg and Salmonella montevideo with concentrations of 1000, 3000 and 5000 ppm at the temperatures and times used in the experiments for S. typhimurium.

An informal organoleptic evaluation was conducted for smell, appearance, color, texture, taste, and flavor of treated, fried drumsticks.

The objectives of this study were to: 1) Determine the most effective concentration of glutaraldehyde that would eliminate S. typhimurium from poultry drumsticks, 2) Study effectiveness of glutaraldehyde as a disinfectant at different temperatures for elimination of the organism, 3) Investigate the efficacy of

glutaraldehyde as a disinfectant for S. typhimurium at various time intervals, 4) Determine if glutaraldehyde activity is pH dependent, and 5) Correlate concentration of glutaraldehyde with temperature and time for effective elimination of S. typhimurium, S. heidelberg and S. montevideo.

LITERATURE REVIEW

SALMONELLAE

Salmonellosis of man is an important infectious disease in the United States. Its prevalence represents a major and continuing global threat as a zoonotic disease. The number of reported infections is estimated to be only a portion of the number of actual cases, due to the low fatality rate, mildness of the disease, and its self-limiting nature. Salmonellosis cannot, except during epidemics, be accurately diagnosed on clinical grounds alone, because bacteriological examinations are not usually undertaken. All the cases diagnosed are not reported to health authorities. Infection frequently occurs sporadically and not in association with recognized food poisoning outbreaks.

Intensified and concentrated rearing of livestock have compounded the problem in the United States and northern Europe. Environmental, as well as psychological, stresses play a definite role in the hosts' resistance to the infection. Contamination of foods during processing and preparation favors interhost transfer. Indiscriminate antimicrobial therapy may, to some degree, compound and further complicate difficulties in the control of Salmonellosis. The ubiquitousness of Salmonella, the host range of the agents, widespread reservoirs in nature, impact of environmental stresses, marked susceptibility of the young, aged, and debilitated, and the questionable efficacy of therapies indicate the eradication of Salmonellosis in food-producing animals and birds will be difficult (Morse and Duncan, 1974).

Salmonellosis may be caused by any of some 1,400 serotypes. The Animal Health Division, USDA (1974), reported the identification of 56 serotypes from chickens, 49 from turkeys, 35 from swine, 25 from cattle, 8 from sheep and goats, and 10 from horses.

Poultry is still considered to be the single most important source of Salmonellae, ever since Edwards reported it in 1939. In 1975, chickens and turkeys accounted for about 28% of the incidences of Salmonella in domestic animals and their environment, but only about 19% of the incidences of human foodborne isolations (Center of Disease Control, 1976).

A survey was conducted to determine the incidence of Salmonellae in processed, ready-to-market, whole young chickens by Green et al., (1982). Carcasses from 15 federally inspected chicken eviscerating plants were analyzed, using a carcass washing technique for determining the presence of Salmonellae. The results obtained during the 1979 incidence survey were compared to results obtained in an identical 1967 Salmonella survey. Washings from 597 eviscerated chickens analyzed in 1967 resulted in 18.6% positive Salmonella isolations. The 1979 results revealed a Salmonella incidence of 36.9%. This represented an observed increase of 8.5% between the two studies. From individual plant results, the data obtained after the 1967 survey when compared to that of 1979 showed that the lowest incidence was 7.5% in 1967 and 2.5% in 1979 and the highest incidence was 73.7% in 1967 and 87.5% in 1979.

The predominant serotypes changed from time to time in a plant survey conducted by Bryan (1968a), of turkey processing operations and turkey products. Of 23 serotypes recovered, Salmonella anatum and Salmonella sandiego were the most frequent isolates. In general, the most common isolates of Salmonella have been Salmonella typhimurium, Salmonella heidelberg, Salmonella enteriditis, Salmonella infantis and Salmonella newport. In 1965, Sadler and Corstvet also found the above serotypes as well as several others in a market survey of chickens and turkeys. S. typhimurium and S. sandiego were the most common isolates from a turkey flock. A total of more than 1000 turkeys yielded 108 positive Salmonella isolations (5.25%); for about 2800 chicken fryers, the incidence was only about 1% and Salmonellae

were recovered from 7 (0.54%) of about 1280 chicken hens.

Poultry may acquire a wide range of Salmonella serotypes from various sources including feedstuffs, breeding flocks, rodents, wild birds, and other vectors. Clinical disease is uncommon, but all infections are of importance as potential sources of food poisoning in man.

INFECTIONS

Types

In poultry there are two kinds of Salmonella infection, systemic or generalized, and enteric. Salmonella pullorum and Salmonella gallinarum are systemic infection causing organisms that are virtually host-specific and rarely infect other host species or cause food poisoning in man.

Infection with other Salmonella serotypes is usually confined to the gut but occasionally if conditions are suitable, severe systemic infection may occur. This is most likely if the infected birds are very young or if they receive a large dose of organisms. Stress-producing circumstances such as bad environment, over-crowding, or intercurrent disease may also contribute to the development of a systemic infection and young flocks may then show heavy losses. They usually show a clinical recovery by 6 to 8 weeks of age, but it is imposssible to say how many will continue to excrete Salmonellae in their faeces or for how long they will do so. Young breeding stock that suffer from systemic infection or clinical enteric infection are potentially dangerous and of considerable nuisance-value because of the possibility that they will later transmit Salmonella infection vertically by faecal contamination of the eggs (Gordon and Tucker, 1965).

Eggs

Contamination of egg contents may occur in two ways. One is by direct transmission from the ovary. For this to occur, the bird must have experienced a systemic infection. Duck eggs are classically regarded as being commonly infected by ovarian transmission with serotypes such as S. typhimurium and S. enteritidis. More common is the second method of contamination involving the faeces of a bird that has had only an enteric infection and which is a Salmonella excretor. The Salmonellae may penetrate the eggshell pores as the egg cools. Alternatively, faecal material adherent to the shell may contaminate the contents when eggs are 'broken out' in the manufacture of egg products (Borland, 1975).

Salmonella in Live Birds

The carrier rate of Salmonellae in poultry entering the processing plant varies according to the country, the method of rearing, and the degree to which the feed has been contaminated. Despite the high rate of contamination of poultry feedingstuffs in most countries, this rarely gives rise to clinical outbreaks of Salmonellosis in poultry, but in vertically integrated operations there may be a self-perpetuating cycle of infection in which waste offals that are processed for inclusion in the feed for subsequent batches of birds transmit the infection to them. This transmission of Salmonellae vertically from contaminated feed to final product has been studied by Morris et al., (1969) and Vaughn et al., (1974).

Treatment of clinical outbreaks of Salmonellosis on the farm will reduce mortality, but Knivett and Tucker (1972) showed that despite treatment with Furazolidone one half of chickens experimentally infected with S. typhimurium carried the organism in the cecum, liver, and spleen when slaughtered 2 months

after challenge.

Distribution in the Processing Plant

The control of Salmonellae is very difficult in poultry plants because of the entry of birds that are either carriers or passively contaminated. Once such birds enter the slaughter process, there is always the possibility of Salmonellae becoming widely disseminated in a plant. There is a high potential for cross contamination in the plant with high thoroughputs, repeated handling of carcasses and viscera and common scalding and chilling processes. Salmonellae spread from one consignment to another during processing and the serotypes in a plant change daily although there is no buildup in plants from day to day (Morris and Wells, 1970).

Incidence of contamination of poultry by Salmonellae in processing plants and retail markets was reported by Bryan (1968b) to vary from about 1 to 50% in various surveys made by different investigators. Processing operations, particularly defeathering by machine, are important sources for spread of Salmonellae in the plant.

The persistence of Salmonellae in flock contamination was shown by Dougherty (1976) to drop significantly during growth of broilers processed at 8 or 9 weeks. Initial contamination of chicks was 37.5%, but infection with Salmonellae had decreased to about 2.5% at processing.

From the reports reviewed, Dawson et al., (1979) and Cunningham (1982) came to the conclusion that the contamination of incoming poultry to processing plants was relatively low, but that peaks of Salmonellae occurred during processing. Market poultry becomes contaminated to varying degrees, undoubtedly due to marked differences in sanitation practices, in processing plants as well as later handling and retailing conditions.

DIFFERENT TREATMENTS USED FOR REDUCTION OR ELIMINATION OF SALMONELLA

Rapid Freeze - Thaw Treatment in Conjunction with Various Chemicals

Olson et al., (1981) performed a five cycle rapid freeze - rapid thaw process in conjunction with chemicals to reduce numbers of S. typhimurium cells on poultry meat. The second portion of chicken wings consisting of ulna and radius with attached skin and muscle was innoculated with 400-900 colony forming units of a nalidixic acid resistant strain of S. typhimurium. Chemicals used were 20 ppm chlorine, 5% potassium sorbate, 5% lactic acid, and 5% calcium propionate. The wings were either sprayed with or dipped into all chemicals before the freeze-thaw process. Wings were also chemically treated and not subjected to the freeze-thaw process. Numbers of S. typhimurium were determined by the most probable number procedure. The relative effectiveness of combinations of chemicals and the freeze-thaw process was compared to a control with the following percentage reductions of numbers of S. typhimurium cells: lactic acid, 98%; calcium propionate, 96%; and freeze-thaw process without chemicals, 95%. There were no statistically significant differences among the treatments. In a pilot plant study simulating commercial conditions, a carbon dioxide freezer was used for the rapid freeze and a microwave oven was used for the rapid thaw. Treatment of wings with 5% lactic acid plus freeze-thaw process resulted in statistically significant fewer numbers of S. typhimurium cells when compared to the freeze-thaw process without chemical treatment or to wings chemically treated without the freeze-thaw process.

Chlorine, Acid, and Heat Treatment

Thomson et al., 1976, conducted an experiment using nalidixic acid resistant strains of S. typhimurium and broiler carcasses. The carcasses were inoculated with 1/5 of a ml. of the organism suspension. The inoculated and the uninoculated (control) carcasses were placed in an experimental chiller. One hundred and fifty L of water at 18° C (64° F) was added for first stage of chilling and 150 L of 1:6 mixture of crushed ice and water for the second stage of chilling. About 50 ppm chlorine was added as sodium hypochlorite solution with concentration established initially and monitored for further addition during prechill treatment and chilling by the thiosulfate titration method. One percent succinic acid was added and the solution was agitated in the chiller for several minutes to dissolve the acid thoroughly before the carcasses were added. The carcasses were removed from the chiller after treatment and chilling, then sampled to determine whether the Salmonellae survived the treatment and chilling, and whether any of the uninoculated carcasses had become contaminated. Chlorine alone was not effective in destroying the Salmonellae inoculated on the carcasses. A high agitation prechill treatment with 50 ppm chlorine and 1% succinic acid at 55° C (131° F), plus two stage simulated commercial chilling with 50 ppm chlorine, eliminated S. typhimurium which had been inoculated on broiler carcasses before treatment and chilling, but carcass appearance was damaged.

Effect of Chlorine, Antibiotics, ß-Propiolactone and Acids

An area of the breast skin of 192 eviscerated fryer chickens was inoculated with a suspension of S. typhimurium. The area was then sprayed with a solution of 20, 100, or 200 ppm chlorine, 10 ppm Aureomycin, 0.5% ß-propiolactone, 1.0%

succinic acid, distilled water, 0.3% citric acid, or 10 ppm neomycin. The controls were left unsprayed. Half of the lot was held 5 min, then half of this 5 min group was sampled to determine surviving S. typhimurium. Culturing and counting were performed by the most-probable-numbers technique. The other half of the lot was held 30 min after spraying and treated otherwise in the same way as the 5 min group. A significant reduction in Salmonellae counts was affected by spraying all treatment solutions, including distilled water. There was no significant difference among treatment solutions, except that carcass sprayed with 100 or 200 ppm chlorine showed significantly reduced Salmonellae counts, as compared with unsprayed controls, and with carcasses sprayed with distilled water. Gentle washing after the holding periods also significantly reduced S. typhimurium counts. There was no significant difference between the group held 5 and group held 30 min (Thomson et al., 1967).

A Hot Acid Treatment

Juven et al., (1974) used hot succinic acid to test for the destruction of Salmonella on chicken broilers. Immersion in a 3% solution at 60° C resulted in some destruction of Salmonella montevideo (200 cells inoculated per leg), but at 85° C for 2-3 min was required to achieve elimination. However, when 200 cells of S. montevideo were inoculated into either a chicken meat or skin homogenate a temperature of only 60° C for 1 and 2 min, respectively, was required for elimination.

These results are in essential agreement with those reported by Bayne et al., (1965) who observed destruction of 3×10^8 cells of S. typhimurium in ground pectoral muscle after 5 min at 60° C and indicated that the test organism did not possess exceptional heat resistance.

Microbiology of Parts Dipped in Potassium Sorbate

Cunningham (1981) used fresh broiler drumsticks to study the effectiveness of a potassium sorbate dip to control bacterial growth. Some pieces were inoculated with S. typhimurium before being dipped. Other pieces, used to determine total counts, were not inoculated with Salmonella. Treated drumsticks were held at 4, 10, or 22° C. When stored at 4° C, untreated pieces had counts of 10^6 in about 6 days, but parts dipped in 10% potassium sorbate took nearly 10 days to reach the same total counts. At 22° C, potassium sorbate extended the shelf life about 1 day. Potassium sorbate solutions of 5% strength were nearly as effective as the 10% solution on drumsticks held at 4° C, but not at 22° C. The 10% dip (for 30 sec) effectively reduced numbers of Salmonella after 2 days of storage at 22° C. These data showed that sorbates can be used to control growth of spoilage organisms associated with fresh poultry parts.

Chemical Pasteurization

Teotia and Miller (1973) pasteurized turkey parts, utilizing different chemical solutions. The chemicals used were lactic acid, acetic acid, sodium carbonate, potassium hydroxide, chlorine, and sodium tetra borate (Borax). Turkey drumsticks were inoculated with Salmonella senftenberg 775W or S. typhimurium in concentrations ranging between 500,000 and 900,000 viable cells per ml. of contaminating fluid. After each treatment, samples were cultured, plated, and tested according to standard methods to determine the susceptibility of Salmonella organisms to the particular treatment. The 3% lactic acid solution eliminated Salmonella from turkey drumsticks in 90 min at 30-32° C. One percent solutions of

potassium hydroxide eliminated the test organism at 82° C in 60 sec. Various solutions of sodium carbonate under various conditions failed to reveal any consistency for eliminating Salmonella. A 3400 ppm solution of chlorine as sodium solution of Borax produced sterile drumsticks at 22° C in 9 hours. All treatments, except the sodium carbonate and Borax solutions, reduced the visual acceptability of the drumsticks.

Lysozyme, EDTA, X-Ray, Microwave and Chlorine Treatments

Lysozyme, ethylenediaminetetracetic acid (EDTA), chlorine, x-irradiation, and microwaves were used in experimental attempts to eliminate S. senftenberg 775W or S. typhimurium from turkey drumsticks and whole carcasses (Teotia and Miller, 1975). Turkey drumsticks or whole carcasses were artificially contaminated with S. senftenberg 775W or S. typhimurium in concentrations ranging between 5×10^5 to 8×10^5 viable cells per ml. of contaminating fluid. After each treatment, samples were cultured, plated, and tested according to standard methods to determine the susceptibility of Salmonella organisms to the particular treatment. A 0.1% solution of lysozyme eliminated S. senftenberg 775W at 22° C within 3 hours. A 0.5% solution of EDTA failed to destroy the test organism under the same conditions. Eighty thousand rads of X-ray eliminated the test organisms on turkey drumsticks but not from whole turkey carcasses. Microwaves eliminated S. senftenberg 775W in 150 sec from turkey drumsticks and 10 min from broiler chicken carcasses. Aqueous solutions containing 3400 and 2125 ppm chlorine failed to destroy the test organism on turkey drumsticks at 21° C in 9 and 24 hours. None of the treatments changed the appearance of the skin or meat, except that the microwaves produced partially cooked appearance and the chlorine produced off-color drumsticks.

Poly (Hexamethylenebiguanide Hydrochloride) Treatment

Broiler carcasses were each inoculated with 30 cells of marker S. heidelberg and prechilled and chilled together with uninoculated carcasses in a simulated commercial chilling system. Cross-contamination (uninoculated carcass showing contamination with marker Salmonella after chilling) was prevented and no viable Salmonellae were found on the inoculated carcasses when either 10 to 25 ppm of PHMB [(poly-hexamethylenebiguanide hydrochloride)] was added to the prechill water. Crossed-contamination was not prevented and viable Salmonellae were found on the inoculated carcasses when carcasses, each inoculated with 60,000 cells of marker Salmonella, were similarly chilled and 10 ppm of PHMB was added to the prechill water. With 60,000 cells, and 25 ppm PHMB, cross contamination was prevented, but viable Salmonella remained on the inoculated carcasses (Thomson et al., 1981).

The Efficacy of Chlorine Dioxide

A chlorine dioxide (ClO_2) treatment was performed in a commercial Ontario poultry slaughter house (15,000 birds/hr), which procured broilers from throughout southern Ontario and northeastern United States. Chicken broiler carcasses were sufficiently scalded to remove the skin cuticle (61° C for 62 sec), then automatically eviscerated, federally inspected, and chilled in one of two similar chilling systems which reduced the carcass temperature to below 4° C. The large spin type chiller was adapted so that the chill water could be treated with various levels of chlorine dioxide (ClO_2), increasing the concentration of ClO_2 from 0 to 1.39 mg/l. This reduced the bacteria count to the point where Salmonella could not be isolated from the chilled water or the chilled broiler carcasses. In addition,

coliform, psychrotroph, and aerobic plate counts were greatly reduced (< 1 log cycle) in chill water but were only slightly reduced (< .05 log cycle) in macerated chicken broiler breast skin. Shelf-life was lengthened for broiler carcasses treated with 1.33 and 1.39 mg/l ClO_2 compared to control carcasses. Sensory panelists reported no off flavor for any ClO_2 concentration but rated broiler skin as being slightly lighter in color compared to control carcasses at all concentrations of ClO_2 treatment (Thiessen, et al., 1984).

GLUTARALDEHYDE

Glutaraldehyde (Pentanedial) (a saturated dialdehyde having the formula $CHO-CH_2-CH_2-CH_2-CHO$) has been known for many years, but only recently has it been developed commercially as a chemosterilizer. It has a molecular weight of 100.12. Glutaraldehyde is supplied as stable 25 or 50% aqueous solutions. It reacts through cross-linking to impart water resistance to protein and polyhydroxy compounds. As a tanning agent it helps produce leathers with outstanding durability, uniformity, and feel. Glutaraldehyde is also a reducing agent for photochemicals and a bactericide and sterilant. Glutaraldehyde has a broad spectrum of activity, a fast rate of kill, and has been classed as a chemosterilizer. A chemosterilizer is defined as a chemical agent which, when utilized properly, can destroy all forms of microbial life, including bacterial and fungal spores, tubercle bacilli, and viruses. As bacterial spores are the most difficult to kill of all microbial forms, sporicides could be considered synonymous with chemosterilizers. Thus the ability of glutaraldehyde to kill bacterial spores is very important. It is known that glutaraldehyde is the only aldehyde to exhibit excellent sporicidial activity (Borick, 1968).

Chemistry of Glutaraldehyde

Glutaraldehyde (1, 5-pentanedial) was first synthesized by Harries and Tank (1908). The saturated 5-carbon dialdehyde is an amber colored liquid usually supplied in solutions of acidic pH. As with other aldehydes, the two aldehyde groups react readily under suitable conditions, particularly with proteins (Bowes and Cater, 1966; Hopwood et al., 1970).

Effect of Glutaraldehyde

Thomson, et al., (1977) found that S. typhimurium cells, inoculated on the skin with 200-300 cells per broiler carcass before chilling, were eliminated by a formulation containing 0.5% glutaraldehyde at pH 8.6 for 30 min in the prechill water during simulated commerical chilling. They were not eliminated when inoculum was 360,000 cells per carcass. Passage of inoculated Salmonellae from inoculated to uninoculated carcasses (cross contamination) was prevented by glutaraldehyde at 0.01% or greater in the prechill water, when inoculum level was 200-300 cells per carcass, and low, but not prevented, when inoculum was 360,000 cells per carcass. Glutaraldehyde at 0.5% adjusted to pH 8.6 for 10 min prechill during chilling extended carcass shelf-life 6 days over control carcasses at 2° C. This extension appeared to result from reduced initial total bacteria counts on glutaraldehyde - treated carcasses, and not from a reduced rate of bacterial growth after lag phase.

Laboratory tests were conducted to determine the efficacy of glutaraldehyde as a disinfectant when used in simulated commercial chilling (Mast and MacNeil, 1978). Poultry carcasses were held for 45 min in 3°C chill waters consisting of either 50 ppm glutaraldehyde, 50 ppm chlorine, or water control. Chilling reduced

the number of bacteria on all carcasses; those chilled in glutaraldehyde retained lower counts throughout 10 days of storage at 3° C. Glutaraldehyde - chilled carcasses had a shelf-life 2 days longer than chlorine - chilled carcasses and 3 days longer than controls. There were no significant differences in moisture uptake, moisture retention, or color among carcasses chilled in the three solutions.

Antibacterial Activity

One of the earliest indications of the potential antimicrobial activity of glutaraldehyde came from the results of a survey of sporicidal activity of saturated dialdehydes in a search for an efficient substitute for formaldehyde (Pepper & Lieberman, 1962). Further studies by Pepper and Chandler (1963) revealed that glutaraldehyde in alcohlic solution was superior as a sporicidal agent to both formaldehyde and glyoxal.

In their claims for glutaraldehyde as a chemical sterilizing solution, Stonehill et al., (1963) pointed out that aqueous solutions of glutaraldehyde were mildly acidic and needed to be buffered by suitable alkalinating agents to a pH of 7.5-8.5 for antimicrobial activity. Two percent (w/v) glutaraldehyde buffered to alkaline pH by addition of 0.3% (w/v) sodium bicarbonate was advocated to provide the minimum concentration and conditions necessary for rapid sporicidal activity.

The time required for sterilization by a chemical agent is based upon the killing time achieved by the agent against a reasonable challenge of spores which are considered to be the most resistant. At the use-dilution of 2% (w/v) glutaraldehyde was capable of killing spores of Bacillus and Clostridium sp. in 3 hours (Stonehill et al., 1963; Borick et al., 1964).

Vegetative bacteria are readily susceptible to the action of glutaraldehyde. A

0.02% aqueous alkaline solution is rapidly effective against Gram positive and Gram negative species. A 2% solution is capable of killing many vegetative species, including Staphylococcus aureus, Proteus vulgaris, Escherichia coli and Pseudomonas aeruginosa within 2 min (Stonehill et al., 1963). McGuchen and Woodside (1973) reported a complete kill in 10 min of E. coli (2×10^8 cells/ml) by 100 µg/ml alkaline glutaraldehyde compared with a 45% kill produced by the unactivated acid solution.

Antifungal Activity

Antifungal activity was first demonstrated by Stonehill et al., (1963), who reported the growth of Trichophyton interdigitale was inhibited by a 5 min exposure to a 2% alkaline solution and that this solution was more potent than a number of other commercially available preparations tested.

A 1% solution was also fungicidal (Dabrowa et al., 1972). Porous surfaces contaminated with Candida albicans and Microsporium gypseum were significantly more difficult to disinfect with glutaraldehyde than were smooth surfaces (Gorman et al., 1980).

Aspergillus niger is more resistant than other fungi to glutaraldehyde (Rubbo et al., 1967; Gorman and Scott, 1977a). In common with a range of other fungal species, however, both mycelial growth and sporulation are inhibited by 0.5% alkaline glutaraldehyde whole spores swelling is entirely halted by 0.5% solution (Gorman et al., 1980).

Antiviral Activity

In a review, Gorman et al., (1980) stated that the susceptibility of animal

viruses to disinfectants was thought to be intermediate between that of vegetative bacteria and bacterial spores, with the non-lipid viruses usually showing significantly more resistance than the enveloped lipophilic viruses. The problems of estimating virucidal activity and the lack of standard tests may account for the limited information available, although in recent years an increasing number of publications relate to the effects of germicides on viruses.

A number of reports showed that glutaraldehyde was effective against a range of viruses (Stonehill et al., 1963; Borick et al., 1964; Snyder and Cheatle, 1965; Blough, 1966; Graham and Jaeger, 1968; Shen et al., 1977). The enteroviruses - polio, echo, and coxsackie - showed greater resistance to disinfection with glutaraldehyde than other virus groups (Gorman et al., 1980). Subsequent studies have confirmed the virucidal activity of glutaraldehyde even in the presence of high levels of organic matter (Saitanu and Lund, 1975; Evans et al., 1977).

Factors Influencing Activity

When recommending any chemical agent as a disinfectant or chemosterilizer, it is essential to qualify the conditions under which it is effective. These conditions for use are decided as a result of extensive in vitro tests, which, as well as defining the useful biological activity, determine the influence of such factors as pH, temperature, organic matter, and in-use dilution on the activity and stability of the compound (Russell, 1974).

A complex relationship exists between the parameters of concentration, temperature, and pH for glutaraldehyde. At 20° C the order of death of a population of cells exposed to acid and alkaline glutaraldehyde is approximately exponential. The rate of kill for aqueous acid solutions is considerably lower than that for activated alkaline solution. The effect of pH on sporicidal activity is

similar (Borick et al., 1964; Thomas and Russel, 1974). As temperature is increased, however, this difference in activity between alkaline and acid solution is reduced. At 37°C, acid glutaraldehyde produces a 100% kill of Escherichia coli (2×10^8 cells/ml) within 10 min (McGuchen and Woodside, 1973).

In practice, glutaraldehyde is generally available as a 2% solution to which an "activator" is added to bring the pH to around 8 before use. This solution, used at room temperature, will disinfect within 10 min and sterlilize within 10 hours (manufacturer's literature cited by Gorman et al., 1980). It is also recommended that this solution should be discarded 14 days after activation because of the reported significant decrease in activity and free aldehyde concentration. This has led to the development of newer, more stable preparations, formulated at a lower pH, some with other potentiators included to increase the otherwise low level of activity observed at lower pH. It is claimed that these formulations retain their activity for at least 28 days (Gorman et al., 1980).

Effect of Alkalination

The enhanced biocidal activity of glutaraldehyde in alkaline solution is thought to be due to an effect on the glutaraldehyde molecule in relation to polymerization, the outer layers of the microbial cell, or a combination of both (Gorman et al., 1980). After examining the activity of various dialdehydes, Boucher et al., (1973) postulated that the two predominant factors governing activity were the distance between the aldehyde groups and their tendency to polymerize. The latter factor is the more important because it allows free aldehyde groups in glutaraldehyde to interact with the amino groups of the bacterial cell. This statement essentially agreed with the findings of Rubbo et al., (1967) that antibacterial activity was due to the two free aldehyde groups present in the molecule.

The effect of sodium bicarbonate is likely to be on the bacterial cell rather than on the aldehyde molecule (Munton and Russell, 1970). Support for this hypothesis was obtained from protein magnetic resonance (PMR) studies by King et al., (1974). Gorman and Scott, (1977b) examined the possibility of a bicarbonate effect on the glutaraldehyde molecule by estimating the degree of polymerization in acid and alkaline solutions. The degree of polymerization was extensive at alkaline pH but negligible in acid solution. This polymerization, leading to an extensive loss of aldehyde group, was however measured in weeks rather than in the short periods (min or hours) in which biocidal activity of alkaline glutaraldehyde was observed. An immediate effect was not, therefore, apparent on the glutaraldehyde molecule and consequently the primary effect of sodium must be on the bacterial cell. It appeared likely that the role of bicarbonate was to aid penetration of the glutaraldehyde molecule to the site of greatest activity.

A further interesting point was developed from a study by Munton and Russell, (1970) who found that acid glutaraldehyde did not react immediately with the outer cell layers or to the same overall extent as an alkaline solution.

Influence of Test Methods

Claims for the efficiency of a disinfectant are based on the result of laboratory tests which must fulfill a number of important criteria. They must provide accurate and reproducible results, be capable of interpretations, be relevant to in-use conditions, and be adequately controlled. These ideals are almost impossible to achieve, as so many variables are associated with test design. No single laboratory test can yield all the information required, hence the wide selection to test methods used for glutaraldehyde assessment and wide variation in results obtained (Gorman et al., 1980).

MECHANISM OF ACTION

Mode of Biocidal Actions

In mode of action studies of a disinfectant, toxic effects on quite distant and apparently unrelated cellular structures and processes can occur which may be attributed erroneously to a direct action of the chemical agent. It is therefore necessary to examine all possible effects of an agent on the microbial cell to determine the sequence in which they occur and thus decide on the actual mode of action (Hugo, 1967).

Some brief conclusions by Gorman et al., (1980), as to the mode of action of glutaraldehyde on particular groups of organisms are presented below.

(i) Gram positive bacteria

Considerable cross-linking of the abundant peptidoglycan in the cell wall has been shown to occur resulting in a strengthening and sealing effect on the wall. Intermolecular bonding of techoic acid chains and glutaraldehyde interaction with cell wall protein would also contribute to the aforementioned effects. Nutrient access to cytoplasmic enzymes would therefore be inhibited as also would passage of the products of the extracellular degradative enzymes.

(ii) Gram negative bacteria

The factors responsible for the destruction of these organisms appear to be a

combination of a partial sealing or contraction of the outer layers of the cell envelope (lipoprotein components) and an inactivation of certain cell wall - associated or peri plasmic-located enzymes which appear to be crucial to cell viability. Transport of essential nutrient is, therefore, again inhibited. Although the peptidoglycan layer, more loosely cross-linked than in the Gram positive cell, offers numerous sites for possible interaction, the glutaraldehyde molecule does not require penetration to this extent to achieve its effect.

(iii) Bacterial spores

Low concentration of glutaraldehyde prevent germination. Other evidence tends again to indicate that a sealing effect occurs leading to spore death. Penetration and reaction of the aldehyde with protein, enzymes and loosely cross-linked peptidoglycan in the context may be assisted by the action of inorganic cations. The resultant collapse of the expanded cortex could then be apparent in a contraction of the wall structures aided by glutaraldehyde interaction.

(iv) Fungi

Inhibition of spore germination and sporulation has been demonstrated. Although little research has been conducted into the mode of action of the aldehyde on these organisms, an examination of the chemical nature of the organisms indicates a possible mechanism. The principal structural wall component of many molds and yeasts is chitin, which resembles the peptidoglycan of bacteria and which is thus a potentially reactive site. Fungal enzymes are also located in the cell wall. Other active sites could include the polysaccharide - protein complexes, found in yeast cell and in which cystine residues and -s-s-bonds are abundant. The formation

of intercellular bonds in yeast causing agglutination of the cells could also be a causative factor in death.

(v) Viruses

Little work has been conducted on the mode of antiviral action of glutaraldehyde. Sangar et al., (1973), working with foot and mouth disease virus, found the glutaraldehyde - treated virus particles had a smaller sedimentation coefficient than normal particles. This, however, was in contrast to the effect observed on treated poliovirus when no change occurred (Baltimore and Huang, 1968). Sangar et al., (1973) showed that considerable alterations in the arrangement of the RNA and protein subunits occurred. The overall structural integrity of the virus particle was not maintained.

Microbiological Uses of Glutaraldehyde

Glutaraldehyde is extensively used in the leather industry as a tanning agent (Filachione et al., 1964). It has been widely used as a fixative for electron microscopy. Glutaraldehyde has been used successfully to disinfect or sterilize medical, dental, and hospital instruments. It is especially useful in areas where heat cannot be employed such as sterilization of lensed instruments, that is, cystoscopes and bronchoscopes. The main advantages claimed for its use as a chemosterilizer are, its broad spectrum of activity, especially good sporicidal properties; its activity in the presence of organic matter; its rapid antimicrobial action; its non-corrosive action towards metals, rubber, lenses and most materials (Stonehill et al., 1963; Borick et al., 1964; Borick, 1968).

Glutaraldehyde has found application for treatment of viral warts (London,

1971; Bunney et al., 1976; Bunney, 1977). Topically applied glutaraldehyde was also found to be effective in the treatment of onychomycosis, a fungal infection of the nails (Suringa, 1970).

A novel application of glutaraldehyde as the active component of oral compositions for the prevention of dental calculus formation and in reducing dental caries formation in the mouth was reported by Eigen (1970) in the patent literature. It has also been included as an ingredient of a chewing gum with anti-caries activity (Litchfield and Vely, 1972).

In the immunological field, glutaraldehyde is used in radioimmune assays for specific antigen (Kao et al., 1977).

TOXICITY

Most of the published information regarding toxicity of glutaraldeyde relates to its use as a cold sterilizing agent. A 2% solution of glutaraldehyde was considered by Stonehill et al., (1963) to be slightly irritant to the skin and severely irritant to the eye but in both cases considerably less than formaldehyde. These workers concluded that residual amounts of glutaraldehyde after a sterilizing procedure were non-irritant. Varpela et al., (1971) also concluded that sterilization of respirators by glutaraldehyde did not constitute any hazard to patients. Their test showed that approximately 10% of glutaraldehyde absorbed by rubber or plastic parts was liberated in 24 hr.

It is recommended that contact with skin should be avoided and some cases of skin sensitization have been recorded. In a report of allergic contact dermatitis due to Cidex cited by Lyon (1971), it was suggested that the patient, a dental assistant,

had failed to observe the recommended precautions and had removed small items from the sterilizing bath by hand. Sanderson and Cronin (1968) stated that two operating - room nurses, who had handled instruments disinfected in glutaraldehyde, had become sensitive to the aldehyde with developement of a rash. However 10% solution of glutaraldehyde had been employed to prevent hyperhydrosis without adverse reaction, and in some cases treatment has continued for over a year (Juhlin and Hansson 1968). Sato and Dobson (1969) found that 5% glutaraldehyde was only irritant if the epidermal barrier was not intact.

Stonehill et al., (1963) described glutaraldehyde as slightly to moderatedly toxic. They reported an LD_{50} in rats of 10 mg/kg by intravenous injection of a 2% activated aqueous glutaraldehyde solution and 250 mg/kg by oral administration. In Union Carbide Literature cited by Meltzer and Henkin (1977), an oral LD_{50} in rats for a 25% glutaraldehyde solution was approximately 60 mg/kg body weight. It was also stated in this paper that single inhalations of concentrated vapours by rats for 6-8 hrs of 25% and 50% glutaraldehyde solutions respectively did not kill any of the exposed animals.

MATERIALS AND METHODS

SOURCE OF MATERIAL

The strain of Salmonella typhimurium which was resistant to nalidixic acid was obtained as a culture on a nutrient agar slant from the United States Department of Agriculture[1].

The agar was composed as follows:

3.0 g of beef extract

10.0 g of peptone

5.0 g of sodium chloride

20.0 g of agar

1000 ml of distilled water

This was combined in a 2 L flask and brought to a boil to dissolve the agar. About 3-3.5 ml of each solution was dispensed into 13 x 100 mm screw cap tubes and autoclaved for 15 min at 121° C at 15 pounds per square inch (P.S.I.). The tubes were left upright for stabs. These were inoculated with a 18-24 hour culture and incubated for 4 hours. The tubes were sealed with paraffin wax and stored at room temperature.

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[1]Dr. N.A. Cox, Agriculture Research Service, Southern Region, Richard B. Russell, Agricultural Research Center, P.O. Box 5677, Athens, Georgia 30613.

EXPERIMENTAL PREPARATIONS

Strengthening of Culture

To strengthen the culture S. typhimurium are subjected to increasing concentrations of nalidixic acid[1], in order to make them more resistant to it. This was performed under sterile conditions using Brain Heart Infusion Broth [2] as the media throughout.

The various ppm were calculated using the following formulae.

$$\frac{\text{Weight of Substance}}{\text{Weight of Solute}} \times 10^6 = \text{ppm}$$

From the original bacterial culture, three loops were inoculated into the Brain heart infusion broth containing 10 ppm of nalidixic acid. This was incubated for 24 hr and when the broth was turbid three loops were transfered to broth containing 50 ppm of nalidixic acid. This procedure was continued with 100, 300, 500 and 1,000 ppm nalidixic acid. From each of the turbid concentrations two MacConkey agar plates with 100 ppm of nalidixic acid were streaked and after 24 hours of incubation at 37° C the typical small pink colonies were observed. This confirmed that the growth in the broth was indeed S. typhimurium and not due to some contaminant.

Preparation of MacConkey Agar[2] with 100 ppm Nalidixic Acid

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[1] SIGMA Chemical Company, P.O.Box 14508, ST.Louis MO, 63178.

[2] DIFCO Laboratories Inc., Detroit Michigan, 48201.

The MacConkey agar with 100 ppm of nalidixic acid was made as follows:

Difco MacConkey agar was rehydrated by adding 50 g to one L of distilled water and heated to boiling to dissolve completely. This was sterilized in an autoclave 15 min at P.S.I. at 121°C and then cooled in a water bath at 50°C. Ten ml of filter sterilized nalidixic acid was added to this and the media was swirled so as to make a homogeneous mixture.

The nalidixic acid solution was prepared by dissolving 0.1 gram of nalidixic acid in 6 to 7 ml distilled water and by adding 1 N NaOH (sodium hydroxide) dropwise. After the acid was dissolved the volume was brought to 10 ml with distilled water. This solution was filter sterilized using a Swinnex 25mm filter holder[1] and .22µm filter pore size. The filter paper[1] was placed into the Swinnex filter holder using tweezers and put into an autoclave bag. Three of these were prepared at a time and autoclaved together for 15 min at 121°C and 15 P.S.I.

The nalidixic acid solution was drawn into a 12cc hyperdermic syringe and using sterile procedure the syringe was fixed to the Swinnex filter holder. The solution was then carefully dispensed into the MacConkey agar by pushing the hyperdermic syringe down.

Dispensing of Agar

The sterile agar was dispensed into petri dishes using a Master flex 575 RPM fixed speed drive peristaltic pump with 4 feet of sterile tygon tubing (an internal diameter of 0.125 inches and an outer diameter of 0.251 inches). The suction end of the tubing was aseptically placed into the flask with the medium keeping the aluminum foil covering intact. The other end was hand held and used

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[1] Millipore Corporation Bedford, Massachusetts, 01730.

Figure 1. Dispensing of sterile agar into petri dishes using a Master flex 575 RPM fixed speed drive peristaltic pump with 4 feet of sterile tygon tubing.

A electronic digital timer with predetermined time to keep volumes accurate and consistent.

to dispense 10 ml of the media by hand (which has an approximate temperature of about 50-60° C) into each petri dish.

An electronic digital timer[1](Model # K-8683-10), which automatically reset itself, was used to cycle the pump for a set, predetermined time to keep volumes accurate and consistent.

Growth of Organism

S. typhimurium was grown on MacConkey agar (Difco) with 100 ppm of nalidixic acid slants for 18 hours at 37° C and then washed from the culture medium with one ml of sterile phosphate buffer (See appendix A). This suspension was further diluted with additional buffer until the optical density (O.D.) was 0.2 at 550 nm (wave length) using a Bausch and Lomb Spectronic 20 Spectrophotometer.

This method was used due to the fact that bacteria growing in a liquid culture medium exhibit two properties which make them subject to quantitation: (1) the size of bacteria is such that they tend not to settle out of suspension easily, and (2) bacteria either absorb light or diffuse light through refraction or reflection. The size and light affecting properties of bacteria make them subject (within limits) to the Beers-Lambert Law. This law in a simplistic interpretation, recognizes that the amount of light absorbed or diffused by a colored solution or bacterial suspension (O.D.) is proportional to the concentration of light-affecting material in the solution or suspension. This means, for example, that if a bacterial suspension at 2×10^7 cells/ml has an O.D. of 0.15, one division later at 4×10^7 cells/ml the O.D. would be 0.30.

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[1]Cole-Palmer Instrument Company, 7425 North Oak Park Avenue, Chicago, Illinois 60648.

Inoculation of Drumsticks with S. typhimurium Resistant to Nalidixic Acid

Seventy representative packages of six chicken drumsticks each were purchased at a local supermarket. These were brought back to the laboratory and immediately frozen. Each time a trial was performed a package of drumsticks was randomly removed and thawed for 6 hours. When the drumsticks were at room temperature, they were aseptically removed and placed on aluminum foil with the smaller surface of the drumstick facing down.

Each leg was inoculated with a suspension of S. typhimurium, resistant to 100 ppm of nalidixic acid, which had been serially diluted to give 100 organisms per ml.

This concentration was prepared fresh each day of the experiment as follows. The 18 hour stock culture of S. typhimurium was diluted as previously explained to an O.D. of 0.2 at 550 nm. This should approximate to 2.0×10^8 organisms per ml. One ml of this suspension was diluted with one ml of phosphate buffer to give 1.0×10^8 organisms per ml. One ml of this was put into a dilution bottle containing 99 ml of buffer. This was thoroughly mixed giving a dilution of 1.0×10^6 organisms per ml. This process was repeated with another dilution of 99 ml of buffer to give a concentration of 1.0×10^4 organisms per ml. From this, one ml was pipetted into a dilution tube containing 9 ml of buffer, this was vortexed to give a final dilution of 1.0×10^3 organisms per ml. It was from this suspension that 0.1 ml was used to inoculate each chicken leg. The 0.1 ml aliquot gave a final concentration of 100 organisms per ml. This was confirmed by enumerating another aliquot on three MacConkey agar pour plates with 100 ppm nalidixic acid. These were incubated at 37° C for 24 hours and were counted and averaged together, throughout the experiment all the plates were counted using the Fisher Accu-lite Bacterial Colony Counter[1]. The inoculum 0.1 ml was pipetted onto each drumstick and thoroughly rubbed into the skin using a sterile bent glass rod and left to air dry.

These were the treated drumsticks.

Six different solutions of glutaraldehyde[2] were prepared, 100 ppm, 300 ppm, 500 ppm, 1000 ppm, 3000 ppm, and 5000 ppm.

Preparation of Glutaraldehyde

To calculate ppm the equation previously defined was used (see "Strengthening of culture").

The calculations are as follows:

$$\frac{1\ \mu\ \text{litre}}{1000\ \text{ml}} = 1\ \text{ppm}$$

But since the glutaraldehyde comes 50% in water by titration:

$$\frac{2\ \mu\ \text{litre of glutaraldehyde}}{1000\ \text{ml of distilled water}} = 1\ \text{ppm}$$

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[1]Fisher Accu-lite Bacterial Colony Counter, Fisher Scientific Company, 1241 Ambassador Blvd., P.O. Box 14989, St. Louis, MO 63178.

[2]Eastman Kodak Company, Rochester, New York, 14650.

A. 0.2 ml of glutaraldehyde

$$\frac{\text{0.2 ml of glutaraldehyde}}{\text{1000 ml of distilled water}} = \text{100 ppm stock solution}$$

B. 0.6 ml of glutaraldehyde

$$\frac{\text{0.6 ml of glutaraldehyde}}{\text{1000 ml of distilled water}} = \text{300 ppm stock solution}$$

C. 1.0 ml glutaraldehyde

$$\frac{\text{1.0 ml glutaraldehyde}}{\text{1000 ml of distilled water}} = \text{500 ppm stock solution}$$

D. 2.0 ml glutaraldehyde

$$\frac{\text{2.0 ml glutaraldehyde}}{\text{1000 ml of distilled water}} = \text{1,000 ppm stock solution}$$

E. 6.0 ml glutaraldehyde

$$\frac{\text{6.0 ml glutaraldehyde}}{\text{1000 distilled water}} = \text{3,000 ppm stock solution}$$

F. 10.0 ml glutaraldehyde

$$\frac{\text{10.0 ml glutaraldehyde}}{\text{1000 ml of distilled water}} = \text{5,000 ppm stock solution}$$

The pH of each solution was checked with an Altex 71 pH meter[1].

TREATMENTS

Each treatment was replicated five times.

Treatment 1:

One treated drumstick was completely immersed into a beaker containing 100 ppm of glutaraldehyde.

Treatment 2:

One treated drumstick was completely immersed into a beaker containing 300 ppm of glutaraldehyde.

Treatment 3:

One treated drumstick was completely immersed into a beaker containing 500 ppm of glutaraldehyde.

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[1]pH Meter, Beckman Instruments, Inc., Fullerton, California 92634.

Treatment 4:

One treated drumstick was completely immersed into a beaker containing 1000 ppm of glutaraldehyde.

Treatment 5:

One treated drumstick was completely immersed into a beaker containing 3000 ppm of glutaraldehyde.

Treatment 6:

One treated drumstick was completely immersed into a beaker containing 5000 ppm of glutaraldehyde.

TIME AND TEMPERATURE

Two immersion times, 15 and 30 min, and two temperatures, 27° C (room temperature) and 4° C were used in this experiment. All the time and temperature interactions were tested with all six treatments these being:

1. 15 min at 27° C.
2. 15 min at 4° C.
3. 30 min at 27° C.
4. 30 min at 4° C.

This gave a three way factorial treatment structure in a randomized complete block design (see Appendix B).

At the end of their respective time period each drumstick was immediately taken out of the solution and put into an 18 ounce sterile Whirl Pak Bag[1] containing 50 ml of lactose broth[2]. The contents were shaken for one min and incubated at 37° C for 24 hours, after incubation each whirl pak bag was opened and using aseptic technique three loops were taken, one from each side and one from the middle of the broth. Two plates of MacConkey agar containing 100 ppm of nalidixic acid were streaked for each drumstick. After incubation for 24 hours at 37° C the presence or absence of growth of S. typhimurium was noted.

After the results were obtained from the above experiments, the experiments were further repeated with serotypes of other Salmonellae. The organisms used were Salmonella heidelberg and Salmonella montevideo which are resistant to nalidixic acid. The concentrations of glutaraldehyde used were 1000, 3000 and 5000 ppm at temperatures 4°C and 27°C and times 15 and 30 min, with adjusted and unadjusted pH.

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[1] Nasco, 901 Janeville Avenue, Fort Atkinson Wisconsin 53538. .

[2] Difco Laboratories Inc., Detroit, Michigan 48201.

RESULTS AND DISCUSSION

SALMONELLAE

The Salmonella typhimurium and Salmonella heidelberg were chosen for this experiment because they were among the most common isolates of salmonella in a turkey processing survey conducted by Bryan (1968a). Salder and Corstvet (1965) also found the above serotypes as well as several others in a survey of chickens and turkeys. Salmonella montivideo was one of the ten most common Salmonella serotypes isolated from man in the 1967 Salmonella Surveillance Report, Annual Summary NCDC Publications.

Salmonellae require pre-enrichment media for growth. Lactose broth was used for this purpose in this experiment since it was recommended by the Subcommittee on Food Microbiology (1971) of the Food Protection Committee of the National Academy of Sciences.

The times and temperatures were chosen as representing those used in commercial poultry processing plants.

Salmonellae on Salmonella-positive carcasses are extremely low, 1 to 30 per carasses (Surkiewicz et al., 1969), and less than 100/100g of skin (Mulder et al., 1977). These values were used in determining the Salmonellae levels tested in this experiment.

TREATED DRUMSTICKS

Each treated drumstick had a range of 94.6 - 100.6 S. typhimurium. After

treatment, positive growth occurred at 4 and 27°C on drumsticks treated for 15 and 30 min with the gluratarldehyde concentrations of 100, 300, 500, 1000, 3000 ppm (Table 1). Therefore, these conditions were insufficient to eliminate the Salmonellae.

At 5000 ppm gluteraldehyde, there was positive growth at both 4 and 27°C at 15 min. However, after 30 min, no viable Salmonellae were recovered at either temperature (Table 1).

The hypothesis was that the probability of a positive response would be the same for all 24 treatment combinations. This was tested by using a Chi-Square Test (Snedecor and Cochran, 1967). The observed test statistics was $X^2 = 45.82$ with 23 degrees of freedom and a probability of less than 0.01. Causing the hypothesis to be rejected. Therefore, the negative growth at 5000 ppm glutaraldehyde represented a significant difference.

When the drumsticks were treated with S. heidelberg and S. montivideo in 1000, 3000 and 5000 ppm glutaraldehyde, the results were the same as those of S. typhimurium. The treated drumsticks had a range of 97.3 - 100.3 organisms. There was positive growth in 1000 and 3000 ppm at both temperatures and times. In 5000 ppm, positive growth was observed at 15 min for 4 and 27°C and negative growth at 30 min at both temperatures (Tables 2 and 3).

All the serotypes of Salmonellae were eliminated at 5000 ppm at 30 min at both 4 and 27°C. Bailey et al., (1977) likewise found that S. typhimurium (200-300 cells per carcass) were effectively eliminated when carcasses were held for 30 min in chilled water containing 5000 ppm glutaraldehyde.

pH OF THE GLUTARALDEHYDE SOLUTIONS

The pH of the glutaraldehyde solutions at 4°C were as follows: 7.87 (100 ppm), 7.26 (300 ppm), 6.53 (500 ppm), 6.28 (1000 ppm), 4.90 (3000 ppm) and 4.69 (5000

TABLE 1. EFFECT OF GLUTARALDEHYDE CONCENTRATIONS ON THE GROWTH OF SALMONELLA TYPHIMURIUM AT 4 AND 27°C AND TREATMENT TIMES OF 15 AND 30 MIN.

Temperature (C)	Glutaraldehyde Conc (ppm)	pH	Salmonella Counts[1]	Growth[2] 15 Min	Growth[2] 30 Min
4	100	7.87	100.6	+	+
	300	7.26	97.6	+	+
	500	6.53	94.6	+	+
	1000	6.28	96.6	+	+
	3000	4.90	99.6	+	+
	5000	4.69	98.6	+	-
27	100	7.78	100.6	+	+
	300	7.66	97.6	+	+
	500	7.10	94.6	+	+
	1000	6.38	96.6	+	+
	3000	4.97	99.6	+	+
	5000	4.77	98.3	+	-

[1]Each chicken drumstick was inoculated with 0.1 ml Salmonella typhimurium.

[2] (+) = Positive Salmonella growth on drumsticks after treatment.

(-) = Negative Salmonella growth on drumsticks after treatment.

TABLE 2. EFFECT OF GLUTARALDEHYDE CONCENTRATIONS ON THE GROWTH OF SALMONELLA HEIDELBERG AT 4 AND 27°C AND TREATMENT TIMES OF 15 AND 30 MIN.

Temperature (C)	Glutaraldehyde Conc (ppm)	pH	Salmonella Counts[1]	Growth[2] 15 Min	Growth[2] 30 Min
4	1000	6.29	98.6	+	+
	3000	4.78	100.1	+	+
	5000	4.63	97.3	+	-
27	1000	6.32	98.6	+	+
	3000	4.80	100.1	+	+
	5000	4.68	97.3	+	-

[1] Each chicken drumstick was inoculated with 0.1 ml Salmonella heidelberg.

[2] (+) = Positive Salmonella growth on drumsticks after treatment.

(-) = Negative Salmonella growth on drumsticks after treatment.

TABLE 3. EFFECT OF GLUTARALDEHYDE CONCENTRATIONS ON THE GROWTH OF SALMONELLA MONTEVIDEO AT 4 AND 27°C AND TREATMENT TIMES OF 15 AND 30 MIN.

Temperature (C)	Glutaraldehyde Conc (ppm)	pH	Salmonella Counts[1]	Growth[2] 15 Min	Growth[2] 30 Min
4	1000	6.27	99.0	+	+
	3000	4.86	100.3	+	+
	5000	4.70	97.9	+	-
27	1000	6.38	99.0	+	+
	3000	4.99	100.3	+	+
	5000	4.61	97.9	+	-

[1]Each chicken drumstick was inoculated with 0.1 ml Salmonella montevideo.

[2] (+) = Positive Salmonella growth on drumsticks after treatment.

(-) = Negative Salmonella growth on drumsticks after treatment.

ppm), and 3.80 undiluted glutaraldehyde. At 27°C the pH was 7.78 (100 ppm), 7.66 (300 ppm), 7.10 (500 ppm), 6.38 (1000 ppm), 4.97 (3000 ppm) and 4.77 (5000 ppm), and 3.85 for undiluted glutaraldehyde, (Figure 2).

The largest pH difference between solutions at the two temperatures was in 500 ppm preparation. A T-Test (Snedecor and Cochran, 1967) for this resulted in a probability of 1.000. Therefore, this difference was not significant.

ADJUSTED pH

The pH of all glutaraldehyde solutions was adjusted to 8.6 in this experiment by the addition of 1N sodium hydroxide. Adjusting the pH for all three serotypes of Salmonella had no effect at 1000, 3000, nor 5000 ppm glutaraldehyde. There was positive growth at 4°C and 27°C and 15 and 30 min at 1000 and 3000 ppm. At 5000 ppm, there was positive growth at 15 min for both temperatures. But negative growth at 30 min for both temperatures (Tables 4, 5 and 6).

This experiment concluded that the effect of glutaraldehyde was the same when the pH was unadjusted and when it was adjusted to 8.6. Although Borick (1968) found the bactericidal and sporicidal activitiy of glutaraldehyde to be maximal at an alkaline pH.

In the previous research, the glutaraldehyde pH was always adjusted to 8.6 by the addition of sodium bicarbonate. Sodium bicarbonate possesses a bacteriostatic action against low numbers of vegetative bacteria.

The enhanced biocidal activity of glutaraldehyde in alkaline solutions is thought to be due to an effect on (a) glutaraldehyde molecule in relation to polymerization, (b) the outer layers of the microbial cell, or (c) a combination of both (Gorman et al., 1980).

The effect of sodium bicarbonate is likely to be on the bacterial cell rather

Figure 2. pH of diluted and undiluted glutaraldehyde at temperatures 4° and 27° C.

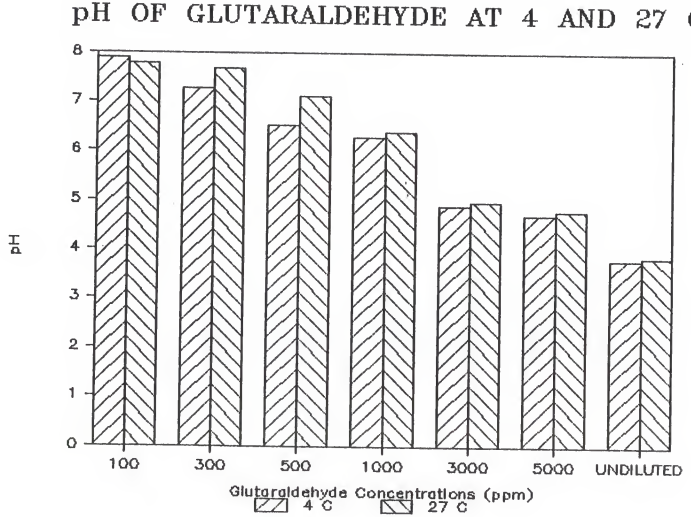

pH OF GLUTARALDEHYDE AT 4 AND 27 C
pH
8
7
6
5
4
3
2
1
0
100
300
500
1000
3000
5000
UNDILUTED
Glutaraldehyde Concentrations (ppm)
4 C
27 C

TABLE 4. EFFECT OF GLUTARALDEHYDE CONCENTRATIONS ON THE GROWTH OF SALMONELLA TYPHIMURIUM AT 4 AND 27°C AND TREATMENT TIMES OF 15 AND 30 MIN.

Temperature (C)	Glutaraldehyde Conc (ppm)	pH	Salmonella Counts[1]	Growth[2] 15 Min	30 Min
4	1000	8.6	98.0	+	+
	3000	8.6	99.6	+	+
	5000	8.6	99.6	+	-
27	1000	8.6	98.0	+	+
	3000	8.6	96.6	+	+
	5000	8.6	99.6	+	-

[1] Each chicken drumstick was inoculated with 0.1 ml Salmonella typhimurium

[2] (+) = Positive Salmonella growth on drumsticks after treatment.

(-) = Negative Salmonella growth on drumsticks after treatment.

TABLE 5. EFFECT OF GLUTARALDEHYDE CONCENTRATIONS ON THE GROWTH OF SALMONELLA HEIDELBERG AT 4 AND 27°C AND TREATMENT TIMES OF 15 AND 30 MIN.

Temperature (C)	Glutaraldehyde Conc (ppm)	pH	Salmonella Counts[1]	Growth[2] 15 Min	Growth[2] 30 Min
4	1000	8.6	96.3	+	+
	3000	8.6	98.6	+	+
	5000	8.6	97.9	+	-
27	1000	8.6	96.3	+	+
	3000	8.6	98.6	+	+
	5000	8.6	97.9	+	-

[1]Each chicken drumstick was inoculated with 0.1 ml Salmonella heidelberg.

[2] (+) = Positive Salmonella growth on drumsticks after treatment.

(-) = Negative Salmonella growth on drumsticks after treatment.

TABLE 6. EFFECT OF GLUTARALDEHYDE CONCENTRATIONS ON THE GROWTH OF SALMONELLA MONTEVIDEO AT 4 AND 27°C AND TREATMENT TIMES OF 15 AND 30 MIN.

Temperature (C)	Glutaraldehyde Conc (ppm)	pH	Salmonella Counts[1]	Growth[2] 15 Min	30 Min
4	1000	8.6	97.0	+	+
	3000	8.6	99.3	+	+
	5000	8.6	99.0	+	-
27	1000	8.6	97.0	+	+
	3000	8.6	99.3	+	+
	5000	8.6	99.0	+	-

[1]Each chicken drumstick was inoculated with 0.1 ml Salmonella montevideo.

[2] (+) = Positive Salmonella growth on drumsticks after treatment.

(-) = Negative Salmonella growth on drumsticks after treatment.

than on the aldehyde molecule (Munton and Russell, 1970). Support of this hypothesis was obtained from protein magnetic resonance studies by King et al., (1974). Gorman and Scott (1977b), also examined the possibility of bicarbonate effect on the glutaraldehyde molecule by estimating the degree of polymerization in acid and alkaline solutions. The degree of polymerization is extensive at alkaline pH but negligible in acid solution. This polymerization, leading to an extensive loss of aldehyde groups, is, however, measured in weeks rather than in the short periods (minutes or hours) in which biocidal activity of alkaline glutaraldehyde is observed. An immediate effect is not, therefore, apparent on the glutaraldehyde molecule and consequently the primary effect of sodium bicarbonate must be on the bacterial cell.

These points could explain why the results of the adjusted pH of this experiment did not vary from that of the unadjusted pH.

A further interesting point developed from a study by Munton and Russell (1973) who found that acid glutaraldehyde does not react immediately with the outer cell layers or to the same overall extent as an alkaline solution.

The concentration of glutaraldehyde when used as a chemical sterilizing agent for equipment in hospitals is typically 2% or 20,000 ppm (Spaulding, 1970). Glutaraldehyde solutions at this concentration will have acid pH. Unadjusted glutaraldehyde solutions, with a pH as low as 2.5, do exhibit microbial properties at ambient temperature; however, the sporicidal activity is greatly reduced (Freid, 1972). Such solutions exhibit a high degree of sporicidal activity in a pH range of 7.4-8.5. Since Salmonella do not form spores, this difference in sporicidal activity resulting from pH adjustment is of minor consequence.

TASTE TESTING

An informal sensory evaluation was done by untrained members of the

department on the drumsticks that were immersed in 5000 ppm glutaraldehyde for 30 min at 27°C. After frying, their odor and taste were the same as for ordinary chicken. The only problem was that the skin became dark brown, where the glutaraldehyde had penetrated and was especially noticable at the feather follicles. The texture was also tough. The dark brown color and hard texture were very superficial. This was even noticable when the drumsticks were coated with a commercial breading and fried. It had an unappetizing appearance.

Glutaraldehyde has not been approved for use on foods. Its safety is accepted for medical applications, but it will require specific pharmacological and toxicological investigations for food use.

CONCLUSIONS

The concentration of 5000 ppm glutaraldehyde eliminated Salmonella typhimurium, Salmonella heidelberg and Salmonella montivideo, when the treated drumsticks were dipped in the solution for 30 min.

The temperatures 4 and 27°C were not a significant factor in this experiment as the results were the same at both temperatures.

Salmonellae were not eliminated at glutaraldehyde concentrations lower than 5000 ppm at either temperatures or times.

Adjusting the pH to 8.6 did not have an effect in this case. The results were the same in adjusted and unadjusted pH.

REFERENCES

Bailey, J.S., J.E. Thompson, and N.A. Cox, 1977. Elimination of Salmonella and extension of shelf - life with a glutaraldehyde product on broiler carcasses. Poultry Sci. 56: 1346, S.A.A.S. Abstr.

Baltimore, D. and A.S. Huang, 1968. Isopyonic separation of subcellular components from polio virus - infected and normal hela cells. Science, New York. 162: 572-574.

Bayne, H.G., J.A. Garibaldi and H. Lineweaver, 1965. Heat resistance of Salmonella typhimurium and Salmonella seftenberg 775W in chicken meat. Poultry Sci. 44: 1281-1284.

Blough, H.A., 1966. Selective inactivation of biological activity of myxoviruses by glutaraldehyde. Journal of Bacteriology. 92: 266-268.

Borick, P.M., F.H. Dondershine and J.L. Chandler, 1964. Alkalinized glutaraldehyde a new antimicrobial agent. Journal of Pharmaceutical Science. 53: 1273-1275.

Borick, P.M., 1968. Chemical sterilizers (chemosterilizers). Advances in Applied Microbiology. 10: 291-312.

Borland, E.D., 1975. Salmonella infection in poultry. The Vet. Record. 97: 406-408.

Bryan, F.L., 1968a. What the sanitation should know about Staphylococci and Salmonellae in non-dairy products. I. Staphylococci. J. Milk Food Technol. 31: 110-116.

Bryan, F.L., 1968b. What the sanitation should know about Staphylococci and Salmonellae in non-dairy products. II. Salmonella. J. Milk Food Technol. 31: 131-140.

Boucher, R.M.G., A.J. Last and G.K. Smith, 1973. Biochemical mechanisms of saturated dialdehydes and their potentiation by ultrasound. Proceedings of the Western Pharmacological Society. 16: 282-288.

Bowes, J.H. and C.W. Cater, 1966. The reaction of glutaraldehyde with proteins and other biological materials. Journal of the Royal Microscopical Society. 85: 193-200.

Bunney, M.H., M.W. Nolan and D.A. Williams, 1976. An assessment of methods of treating viral warts by comparative treatment trials based on a standard design. British Journal of Dermatology. 94: 667-679.

Bunney, M.H., 1977. The treatment of viral warts. Drugs. 13: 445-451.

Center for Disease Control, 1976. Salmonella surveilliance. Annual summary 1975. U.S. Department of Health, Education and Welfare, Center for Disease Control, Atlanta, Georgia.

Cunningham, F.E., 1981. Microbiology of poultry parts dipped in potassium sorbate. Poultry Sci. 60: 969-971.

Cunningham, F.E., 1982. Microbiological aspects of poultry and poultry products. Journal of Food Protection. 45: 1149-1164.

Dabrowa, N., J.W. Landau and V.D. Newcomer, 1972. Antifungal activity of glutaraldehyde in vitro. Archives of Dermatology. 105: 555-557.

Dawson, L.E., J.R. Chipley, F.E. Cunningham and A.A. Kraft, 1979. Incidence and control of microorganisms on poultry products. Research Report 383, North Central Regional Research. Publication N. 260. Michigan State University, East Lansing, Michigan.

Dougherty, T.J., 1976. A study of salmonella contamination in broiler flocks. Poultry Sci. 55:1811-1815.

Edwards, P.R., 1939. Incidence of salmonella types in fowls in United States. Proc. World's Poultry Cong. 271-274.

Eigen, E., 1970. Oral compositions containing non-toxic non-volatile aliphatic aldehydes. U.S. Patent No. 3, 497, 590.

Evans, D.H., P. Stuart and D.H. Roberts, 1977. Disinfection of animal viruses. British Veterinary Journal. 133: 356-359.

Filachione, E.M., M.L. Fein and E.H. Harris, 1964. Tanning with glutaraldehyde. J. Am. Leath. Chem. Ass. 59: 281.

Freid, M.G., Jr. 1972. Activated glutaraldehyde a cold chemical sterilant. Union Carbide Tech. Bull, Union Carbide Corp., New York, N.Y.

Gordon, R.F. and J.F. Tucker, 1965. The epizootiology of Salmonella menston infection of fowls and the effect of feeding poultry food artificially infected with salmonella. Br. Poultry Sci. 6: 251-264.

Gorman, S.P. and E.M. Scott, 1977a. A quantitative evaluation of the antifungal properties of glutaraldehyde. Journal of Applied Bacteriology. 43: 83-89.

Gorman, S.P. and E.M. Scott, 1977b. Effect of alkalination on the bacterial cell and glutaraldehyde molecule. Microbios Letters 6: 39-44.

Gorman, S.P., E.M. Scott and A.D. Russell, 1980. A review of antimicrobial activity, uses and mechanism of action of glutaraldehyde. Journal of Applied Bacteriology. 48: 161-190.

Graham, J.L. and R.F. Jaeger, 1968. Inactivation of yellow fever virus by glutaraldehyde. Applied Microbiology. 16: 177.

Green, S.S., A.B. Moran, R.W. Johnston, D. Uhler and J. Chiu, 1982. The incidence of salmonella species and serotypes in young whole chicken carcasses in 1979 as compared with 1967. Poultry Sci. 61: 288-293.

Harries, C.D. and L. Tank, 1908. Conversion of cyclopentene into the mono-and di-aldehydes of glutaric acid. Berichte 41, 1701-1711.

Hopwood, D., C.R. Allen and C. McCabe, 1970. The reactions between glutaraldehyde and various proteins: an investigation of their kinetics. Histochemical Journal 2, 137-150.

Hugo, W.B., 1967. The mode of action of antibacterial agents. Journal of Applied Bacteriology. 30: 17-50.

Juhlin, H. and H. Hansson, 1968. Topical glutaraldehyde for plantar hyperhidrosis. Archives of Dermatology. 97, 327-330

Juven, B.J., N.A. Cox, A.J. Mercuri and J.E. Thompson, 1974. A hot acid treatment for eliminating salmonella from chicken meat. J. Milk Food Technol. 37: 237-239.

Kao, W.W.Y., N.A. Guzman and D.J. Prockop, 1977. Use of glutaraldehyde-fixed Escherichia coli cells as a convenient solid support for radioimmune assays. Analytical Biochemistry 81, 209-219.

King, J.A., W. Woodside and P.V. McGucken, 1974. Relationship between pH and antibacterial activity of glutaraldehyde. Journal of Pharmaceutical Sciences 63: 804-805.

Knivett, V.A. and J.F. Tucker, 1972. Comparison of oral vaccination of furazolidone prophylaxis for Salmonella typhimurium infection in chicks. Br. Vet. J. 128: 24-34.

Litchfield, J.H. and V.G. Vely, 1972. Diladehyde - containing anti-caries chewing gum compositions. United States Patent No. 3, 679, 792.

London, I.D., 1971. Glutaraldehyde solutions for warts. Archives of Dermatology. 104: 96-97.

Lyon, T.C., 1971. Allergic contact dermatitis due to Cidex. Oral Surgery. 32, 895-898.

Mast, M.G. and G.H. MacNeil, 1978. Use of glutaraldehyde as a disinfectant in immersion chilling of poultry. Poultry Sci. 57: 681-684.

McGuchen, P.V. and W. Woodside, 1973. Studies on the mode of action of glutaraldehyde on Escherichia coli. Journal of Applied Bacteriology. 36: 419-426.

Meltzer, N. and H. Henkin, 1977. Glutaraldehyde - a preservative for Cosmetics and Toiletries. 92, 95-98.

Morris, G.K., B.L. McMurray, M.M. Galton and J.G. Wells, 1969. Study of the dissemination of salmonellosis in a commercial broiler chicken operation. Am. J. Vet. Res. 30: 1413-1421.

Morris, G.K. and J.G. Wells, 1970. Salmonella contamination in a poultry - processing plant. Appl. Microbiol. 19: 795-799.

Morse, E.V. and M.A. Duncan, 1974. Salmonellosis: A current animal environmental health problem. American J. of Vet. Med. Ass. Vol. 165, No. 8., October 15.

Mulder, R.W.A.W., S. Notermans, and E.H. Kampelmacher, 1977. Inactivation of Salmonella on chilled and deep frozen broiler carcasses by irradiation. J. Appl. Bacteriology. 42: 179-185.

Munton, J.T. and A.D. Russell, 1970. Aspects of the action of glutaraldehyde on Escherichia coli. Journal of Applied Bacteriology. 33:410-419.

Munton, T.J. and Russell, A.D., 1973. Interaction of glutaraldehyde with spheroplasts of Escherichia coli. Journal of Applied Bacteriology. 36, 211-217.

Olson, V.M., B. Swaninathan, D.E. Pratt and W.J. Stadelman, 1981. Effect of five cycle rapid freeze-thaw treatment in conjunction with various chemicals for the reduction of Salmonella typhimurium. Poultry Sci. 60: 1822-1826.

Pepper, R.E. and E.R. Lieberman, 1962. Dialdehyde alcoholic sporicidal compositions. United States Patent No. 3, 016, 328.

Pepper, R.E. and V. Chandler, 1963. Sporicidal activity of alkaline alcoholic saturated dialdehyde solutions. Journal of Applied Microbiology. 11: 384-388.

Russell, A.D., 1974. Factors influencing the activity of antimicrobial agents: an appraisal. Microbios. 10: 151-174.

Rubbo, S.D., J.F. Gardner and R.L. Webb, 1967. Biocidal activities of glutaraldehyde and related compounds. Journal of Applied Bacteriology. 30: 78-87.

Sadler, W.W. and R.E. Corstvet, 1965. Second survey of market poultry for salmonella infection. Applied microbiology. 13: 348-351.

Saitanu, K., and E. Lund, 1975. Inactivation of entero virus by glutaraldehyde. Applied Microbiology. 29: 571-574.

Samonella Surveillance Report, 1967. Annual Summary NCDC Publ., Atlanta, Georgia.

Sanderson, K.V. and E. Cronin, 1968. Glutaraldehyde and contact dermatitis. British Medical Journal. 3, 802-805.

Sangar, D.V., D.J. Rowlands, C.J. Smale and F. Brown, 1973. Reaction of glutaraldehyde with foot and mouth disease virus. Journal of General Virology. 21: 399-406.

Sato, K. and R.L. Dobson, 1969. Mechanism of the antiperspirant effect of topical glutarladehyde. Archives of Dermatology. 100, 564-569.

Shen, D.T., T.B. Crawford, J.R. Gorham and T.C. McGuire, 1977. Inactivation of equine infectious anaemia virus by chemical disinfectants. American Journal of Veterinary Research. 38: 1217-1219.

Snedecor, G.W. and W.G. Cochran, 1967. Statistical Methods (6th ed.). The Iowa State University Press, Ames, Iowa.

Snyder, R.W. and E.L. Cheatle, 1965. Alkaline glutaraldehyde an effective disinfectant. American Journal of Hospital Pharmacy. 22: 321-327.

Spaulding, E.H., 1970. rode of chemical disinfection in the prevention of nosocomial infectins. Proc. Conf. on Nosocomial Infect., Center of Dis. Control, Atlanta, GA, pp. 247-254.

Stonehill, A.A., S. Krop and P.M. Borick, 1963. Buffered glutaraldehyde, a new chemical sterilizing solution. American Journal of Hospital Pharmacy. 20: 458-465.

Subcommittee on Food Microbiology, 1971. Reference methods for the microbiological examiniation of foods. Food Prot. Comm., Nat. Acad. Sci., Washington, DC.

Surkiewicz, B.F. R.W Johnston, A.B Moran and G.W Krumm, 1969. A bacteriological survey of chicken eviscerating plants. Food Technol. 23: 80-85.

Suringa, D.W.R., 1970. Treatment of superficial onychomycosis with topically applied glutaraldehyde. Archive of Dermatology. 102: 163-167.

Teotia, J.S. and B.F. Miller, 1973. Chemical pasteurization of poultry meat. Poultry Sci 52: 2094.

Teotia, J.S. and B.F. Miller, 1975. Destruction of salmonellae on poultry meat with lysozyme, EDTA, X-ray, microwave and chlorine. Poultry Sci. 54: 1388-1394.

Thiessen, G.P., W.R. Usborne and H.L. Orr, 1984. The efficacy of chlorine dioxide in controlling salmonella contamination and its effect on product quality of chicken broiler carcasses. Poultry Sci. 63: 647-653.

Thomas, S. and A.D. Russell, 1974. Studies on the mechanism of the sporicidal action of glutaraldehyde. Journal of Applied Bacteriology. 37: 83-92.

Thomson, J.E., G.J. Banwart, D.H. Sanders and A.J. Mercuri, 1967. Effect of chlorine, antibiotics, ß-propiolactone, acids and washing on Salmonella typhimurium on eviscerated fryer chickens. Poultry Sci. 46: 146-151.

Thomson, J.E., N.A. Cox and J.S. Bailey, 1976. Clorine, acid and heat treatment to eliminate salmonella on broiler carcasses. Poultry Sci. 55: 1513-1517.

Thomson, J.E., N.A. Cox and J.S. Bailey, 1977. Control of salmonella and extension of shelf-life of broiler carcasses with a glutaraldehyde product. Journal of Food Science. Vol. 42. No. 5: 1353-1355.

Thomson, J.E., N.A. Cox, J.S. Bailey and M.N. Islam, 1981. Minimizing salmonella contamination on broiler carcasses with Poly (Hexamethylenebiguanide Hydrchloride). Journal of Food Protection. 44: 440-441.

USDA, 1974. Microbiology laboratory guide book. Animal, Plant and Health Inspection Service, Washington, DC.

Varpela, E., S. Otterstrom, and R. Hackman, 1971. Liberation of alkalinized glutaraldehyde by respirators after cold sterilization. Acta Anaesthesiologica Scandinavica. 15: 291-298.

Vaughn, J.B., L.P. Williams, R. LeBlanc, H.L. Helsdon and C. Taylor, 1974. Salmonella in a modern broiler operation: A longitudinal study. Am. J. of Vet. Res. 35: 737-741.

APPENDIX

APPENDIX A

The phosphate buffer stock solution was prepared as follows:

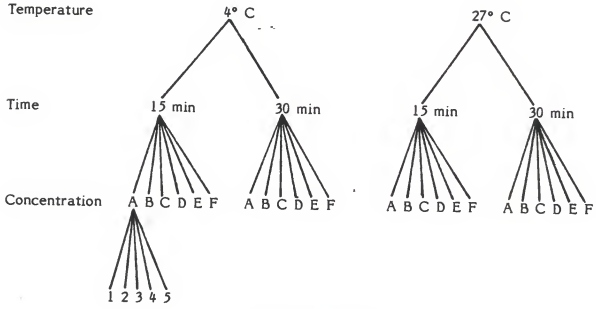
To 100 ml of deionized distilled water 8.5 gram KH_2PO_4 was added. The pH was adjusted to 7.2 using 1N NaOH and then brought to 250 ml using more water. From this stock solution 1.25 ml was added to 1 litre of water. After stirring, this was the buffer solution used throughout the experiment as the wash media and for the dilution bottles and tubes.

APPENDIX B

(A) Six different concentrations used were A = 100 ppm, B = 300 ppm, C = 500 ppm, D = 1,000 ppm, E = 3,000 ppm, and F = 5,000 ppm of glutaraldehyde.

(B) Two times 15 and 30 min.

(C) Two temperatures 4° C and 27° C



Treated drumsticks

Five replications were done on each concentration at the various temperature and time.

This led to a 3 way factorial treatment structure in a randomized complete block design.

EFFECT OF GLUTARALDEHYDE ON CHICKEN DRUMSTICKS INOCULATED WITH VARIOUS SALMONELLAE

by

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ABSTRACT

In this experiment the effect of various concentrations of glutaraldehyde, time, temperature and pH were studied on chicken drumsticks inoculated with nalidixic acid resistant Salmonella typhimurium.

Each drumstick was inoculated with a known amount of S. typhimurium and immersed in glutaraldehyde solution. Six concentrations of glutaraldehyde used were 100, 300, 500, 1000, 3000, and 5000 ppm. Two times (15 and 30 min) and two temperatures (4°C and 27°C) were also tested in a 6 x 2 x 2 factorial experimental design. After treatment, the drumsticks were removed from the beaker and incubated in an 18 ounce sterile Whirl Pak Bag containing 50 ml Lactose broth. After 24 hr at 37°C three loops were taken, one from each side and one the middle of the broth and plated on MacConkey agar containing 100 ppm nalidixic acid as a selective agent. The presence or absence of S. typhimurium was observed with positive growth indicating that the treatment conditions were insufficient.

The experiment was further repeated with an adjusted pH of 8.6 for concentrations 1000, 3000 and 5000 ppm.

After the results were obtained further experiments were conducted with Salmonella heidelberg and Salmonella montevideo with concentrations of 1000, 3000 and 5000 ppm at the temperatures and times used in the experiments for S. typhimurium.

It was shown that only the 5000 ppm solution eliminated the Salmonellae. Temperature was not a factor at any concentration. Time had no effect except for the 5000 ppm solution where only the 30 min treatment eliminated the Salmonellae. Adjusting the pH to 8.6 did not have an effect.

An informal sensory evaluation showed that it was good as any fried chicken except the fact that, where the glutaraldehyde penetrates it, it leaves a hard texture and a dark brown color which does not look appetizing.