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A Scanning Electron Microscopic, Chemical and Microbiological Study of Two Types of Chicken Skin

by

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ABSTRACT

Evaluation of several methods of fixing chicken skin for scanning electron microscopy (SEM) indicated standard chemical fixation using glutaraldehyde and osmium tetroxide followed by chemical dehydration with 2,2-dimethoxypropane to be the method of choice. SEM revealed that chicken skin has a convoluted surface. Two types of chicken skin, distinguishable on the basis of chemical composition and appearance were observed. Type I has a filamentous surface with 55% moisture and 25% fat, whereas Type II skin has a globular appearance, 52% fat and 33% moisture. The fatty acid profiles of Types I and II skin are the same. Bacteria have greater affinity for Type II than Type I skin. Attachment studies indicated that *Salmonella typhimurium* quickly attach to the skin surface and cannot be removed easily by washing with water or with water containing a surfactant.

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INTRODUCTION

Poultry meat forms an important part of the diets of many of the world's population, and production is increasing to satisfy demand. Poultry is frequently associated with food-borne disease, with *Salmonella* sp., *Staphyloccocus aureus* and *Clostridium perfrigens* being the main etiological agents.

An increase in poultry-associated food-borne disease, in particular salmonellosis, can be related to the increased consumption of poultry meat. This disease is caused by ingestion of foods contaminated with bacteria of the genus *Salmonella*. In Canada, 164 of the 1440 cases of food-borne outbreaks reported between 1973 and 1975, were associated with cooked poultry (Todd, 1980).

The incidence of *Salmonella* contamination of poultry caracasses is of major concern. Various studies (Duitschaever, 1977; Wilder and MacCready, 1966) have reported levels as high as 50% for market broilers. Processing of broilers has been reported (Surkiewicz *et al.*, 1969; Dougherty, 1974; Campbell, 1979; McBride *et al.*, 1980) to be a major factor in the contamination of poultry meat.

The most probable sites of contamination are thought to be the scalding-defeathering, eviscerating and chilling operations (Mulder *et al.*, 1978; McBride *et al.*, 1980). In order to decrease *Salmonella* contamination of poultry carcasses, the mechanisms of invasion and attachment of bacteria to poultry carcasses must be elucidated.

The object of the present study was to develop the methodology for the examination of chicken skin by scanning electron microscopy, since the skin microtopography may yield information on the modes of attachment of *Salmonella* to poultry carcasses. In addition, the attachment of *Salmonella typhimurium* was studied in model systems in an attempt to provide information that can be applied toward the development of methods for decreasing *Salmonella*-contamination on poultry carcasses at the processing level.

LITERATURE REVIEW

A. *Scanning Electron Microscopy*

Scanning electron microscopy has been commercially available since 1965, but has found limited application in food microbiology. However, it could well provide information about the ecology of food borne microorganisms and the spatial relationship between the bacteria and its substrate (McMeekin *et al.*, 1979).

When this research was commenced, no work had been published on the microstructure of chicken skin. But, recently several authors have reported studies on the topography of chicken skin. McMeekin *et al.* (1979) examined breast skin from poultry carcasses. Two methods of fixation were employed: (i) fixation by immersion in glutaraldehyde (4% w/v in 0.2M phosphate buffer, pH 7.0) for 12 h and (ii) fixation in osmium tetroxide vapors (1% w/v). Skin samples were then washed in distilled water, and post-fixed in glutaraldehyde prior to washing in distilled water. Dehydration with a graded series of ethanol followed. The SEM results indicated that chicken skin has many *"crevices"* and *"channels"* of capillary size, in which bacteria may become trapped. Microorganisms were clearly visible on the skin after storage of the carcasses for 10 days at $5^{o}C$. At high magnifications, fibrils connecting individual bacterial cells could be seen. The authors also pointed out the importance of using macerated samples, rather than swabs or rinses for accurately determining viable bacterial counts.

Suderman and Cunningham (1980) studied the effects of age, method of chilling, and scald temperatures on the adhesion of coatings to poultry skin. Samples for scanning electron microscopy were prepared by three methods: (i) glutaraldehyde fixation, with ethanol dehydration and critical point drying; (ii) fixation with glutaraldehyde, post-fixation with osmium tetroxide, dehydration with ethanol followed by critical point drying. In both these methods the samples were coated with carbon and then with gold-platinum. The third method involved freeze-drying. The samples were fixed in glutaraldehyde, and frozen in isopentane prior to immersion in liquid nitrogen and freeze-drying.

Their results show that after the cuticle was removed, *"numerous protrusions of the epidermis are evident along with microholes, elevations and recessions"*. Comparisons of the three techniques indicated that freeze-drying after a glutaraldehyde fixation yielded micrographs with good clarity and resolution of detail. Both of the other previously mentioned methods resulted in electron charging of the sample, a problem frequently encountered with tissues with high fat content.

Thomas and McMeekin (1980) used scanning (SEM) and transmission electron microscopy (TEM) to examine aspects of contamination of broiler skin by bacteria during processing. Breast and leg skin samples were excised and fixed overnight at $4^{o}C$ in either osmium tetroxide vapour or 5% glutaraldehyde and then

dehydrated through a graded series of ethanol, infiltrated with amyl acetate and critical point dried. The skin samples were found to be rough and folded as shown by McMeekin *et al.* (1979).

B. *Microbiological Sampling of Chicken Carcasses*

Numerous studies have been published dealing with the spread of contamination on eviscerated and non-eviscerated carcasses during processing.

It has been shown (Surkiewicz *et al.*, 1969; Dougherty, 1974; Campbell, 1979; McBride *et al.*, 1980) that poultry carcasses may become contaminated with *Salmonella* during processing operations whereupon non-infected birds acquire *Salmonella* organisms from a contaminated environment (Wilder and MacCready, 1966).

Dissemination of *Salmonella* starts on the farm. Bryan *et al.* (1967) examined the sources of *Salmonella* contamination of turkey products. Turkeys and farm environments were evaluated, as well as plant surveys on processing equipment and turkey carcasses. They found that feed, feed ingredients, fecal droppings and trough water were sources of *Salmonella*. The predominant serotype of *Salmonella* isolated from the plant environment changed as a new flock was introduced. Defeathering machines were found to be a source of initial *Salmonella* transfer from carcass to carcass. Subsequent spray washing did not remove all *Salmonella*, and therefore processing equipment became contaminated. Carcass contact with contaminated equipment caused cross contamination as the next birds were processed.

A study by Zottola *et al.* (1970) indicated that *Salmonella* is present in the *"live bird"* area of processing plants. Ziegler *et al.* (1954) found that the area under the wing and that around the vent to be the most heavily contaminated parts of the skin and the visceral cavity, respectively.

Different areas of the processing plant have been implicated in the dissemination of *Salmonella* within the poultry plant. Van Schothorst *et al.* (1972), using *E. coli K12* as an indicator organism, investigated the problem of contamination of chickens during defeathering and subsequent cleaning and chilling. They found a definite spread of contamination during feather removal, which could be decreased somewhat in later stages of processing.

Patrick *et al.* (1973) compared the effectiveness of water - cooling and steam scalding in decreasing *Salmonella* contamination. The number of *Salmonella* contaminated chickens approximately doubled between scalding and defeathering and between defeathering and chilling, indicating that scalding and defeathering are important vectors for the spread of *Salmonella*. They also found that water scalded carcasses had a much higher level of contamination than did steam scalded carcasses.

Mulder *et al.* (1978) also studied cross-contamination of birds during the scalding and plucking operations. They contaminated poultry carcasses, both internally and externally with *E. coli K12*, resistant to nalidixic acid concentrations up to 200 mg/L. Their

results indicated that the number of *E. coli K12* on externally contaminated broilers decreased 1000-fold during scalding. This decrease was not only due to the high temperatures used in scalding, but also to the washing effect which occurs during scalding. More cross-contamination during scalding at temperatures between 52-54^{o}C was noted than when a higher scald temperature (60^{o}C) was used. In contrast, cross-contamination during plucking was slight after internal contamination (Mulder *et al.*, 1978).

Wilder and MacCready (1966) found that *Salmonella* was distributed throughout the poultry plant environment and reached their highest levels in areas maintained at low sanitary conditions, and in those areas where the poultry undergoes extensive human handling.

Surkiewicz *et al.* (1969) found that eviscerated chickens had aerobic plate counts of $1.5 \times 10^{4}/cm^{2}$, six *E. coli*/cm^{2}, and three *S. aureus*/cm^{2}. *Salmonella* were found to be present on 20.5% of the birds sampled. Passage through the continuous counterflow chiller diminished the total bacterial load but did not significantly reduce the incidence of *Salmonella* contamination.

Finlayson (1977), in a survey of Alberta poultry processing plants, found that salmonellae were common in breeding flocks and in processing plants. The most frequent sources of *Salmonella* in the plant were the defeathering area and the drains.

The chilling operation is also very important in the cross-contamination of poultry. Numerous studies have focused on this stage of poultry processing. Peric *et al.* (1971) showed that spin

chilling initially caused a decline in the surface bacterial counts of broilers early in the day, but as the day progressed, an increase in bacterial counts was noticed. The time that the increase occurred was found to be dependent upon the bacterial load of the carcasses before spin chilling, the number of carcasses processed and the amount of water used in the chiller. They also found that spray cooling was more effective than spin chilling.

Mead and Thomas (1973a) found that chlorine in the chill water destroyed virtually all the bacteria present in the chill water and therefore aided in the prevention of cross-contamination during the cooling operation. The majority of all bacteria were destroyed by the use of 45 to 50 ppm total chlorine in the chill water at a volume rate of 5 L of water per carcass. If the water rate was increased to 8 L of water per carcass, 25 to 30 ppm residual chlorine was sufficient.

In a companion paper, Mead and Thomas (1973b) studied the effects of a three-stage chiller. Total viable counts at 20 and 37^{o}C, as well as the levels of Coli-aerogenes bacteria were decreased by one log cycle. They concluded that the main effect of the chlorine in the water was to inactivate the organisms that were washed from the carcasses, thereby avoiding recontamination.

Notermans *et al.* (1973), using a nalidixic resistant strain of *E. coli K12*, found that cross-contamination can occur either in the spin chiller or in spray cooling. However, the possibility of

cross-contamination was higher in the spin chiller, than in the spray cooler.

McBride *et al.* (1980) studied the incidence of *Salmonella* at three stages of processing: (i) before scalding, (ii) after evisceration and (iii) after chilling. The average incidence of *Salmonella* was found to be between 1.2 and 74.4%, and flocks with a high incidence of *Salmonella* before scalding were still contaminated after chilling. They concluded that it is possible to predict the incidence of *Salmonella* at one site from the incidence at another site in the processing operation.

C. *Enumerating Bacteria on Poultry Skin*

There are several methods for determining the numbers of bacteria on poultry meat and carcasses. Walker and Ayres (1956) used cotton swabs to sample 2 cm^2 of both internal and external surfaces. The area was delineated by sterile metal guides. Fromm (1959) quantitatively compared four methods: direct contact plating, swab sampling, rinse sampling and skin tissue removal. His results indicated that skin tissue removal was the most accurate. The major disadvantage of this technique is that it lowers the carcass grade and therefore may not be suitable for routine quality control analysis. It may be better in such cases to employ swab techniques.

Clark (1965) used a non-destructive method, in which a known area of skin was sprayed with a fluid under constant pressure. The bacteria were recovered from a measured volume of the fluid.

Avens and Miller (1970) compared a skin *"blending"* technique to the cotton swab sampling method on turkey carcass skin. The blending technique involved the use of a laboratory blender to release the bacteria from skin samples suspended in 0.1% peptone broth or physiological saline as a diluent. The skin blending method permitted enumeration of significantly more bacteria than did the swab method. The swab method was found to yield incomplete and inconsistent results.

Thomson *et al.* (1976) compared the use of a lucite template anchored to the poultry skin by means of stainless steel pins, to a fiberboard template. The lucite template prevented skin slippage but the bacterial counts did not differ significantly from those obtained using the fiberboard template.

D. Bacterial Adhesion

Notermans and Kampelmacher (1974), concluded that attachment of bacteria to poultry skin was largely dependent on the presence of flagella on the bacteria. Studies using a non-flagellated *E. coli K12 97+* mutant indicated minimal attachment compared to that of the flagellated *E. coli K12*. They also noted that pH and temperature were important parameters. By lowering the pH of the attachment medium, the attachment rate was decreased due to the decreased motility of the organisms. The optimal temperature for the attachment of the various bacterial strains studied was 21^{o}C. Increasing

the temperature from 0^{o} to $21^{o}C$ was found to increase the attachment rate. This seemed to be due to the increased metabolic activity of the organism and the flagella (Notermans and Kampelmacher, 1974).

Notermans and Kampelmacher (1975a) also found that a proportion of the bacterial flora of skin was present in the surrounding water film and could be removed by adequate rinsing. The remainder of the flora are very difficult to remove, even with mechanical cleaning, such as spin chilling. The bacteria present in the water film played a key role in the attachment of bacteria to the skin. The attachment was time dependent and proportional to the number of bacteria present (Notermans and Kampelmacher, 1975a).

In contrast, McMeekin *et al.* (1978) found that motility had a negligible effect on the number of organisms retained on the skin. They observed that there was no preferential accumulation of motile bacteria on the skin surfaces. The time of immersion was also of minor importance when compared to the effect of population densities on retention.

Bacterial attachment seems to take place in two separate stages. Marshall *et al.* (1971), in their work with marine bacteria, separated the attachment process into an instantaneous phase and a time-dependent irreversible phase. In the first phase, the bacteria were attracted to a surface and then weakly held by a balancing of London - van der Waals attractive forces and the electrical repulsive charges of two surfaces, or a gain in entropy. In the second phase,

the bacteria became irreversibly attached by the formation of polymeric bridges between the bacteria and the surface.

This *"polymeric bridging"* was visualized by Fletcher and Floodgate (1973). Transmission electron microscopy, after the samples were stained with ruthenium red and alcian blue, demonstrated the presence of an extracellular polysaccharide layer which was involved in the adhesion of marine bacteria to surfaces. This adhesive substance was found to be present before attachment, but a secondary fibrous acidic polysaccharide was produced once natural attachment had occurred.

McCowan *et al.* (1978) used both SEM and TEM to investigate the mechanisms of attachment of bacteria to the reticulo-rumen of cattle. Bacteria could avoid being washed out of the rumen by adhering to the mucosa. TEM of the ruthenium red stained samples showed that the attachment was mediated by glycocalyx and carbohydrate coat of the bacteria. The glycocalyx aided in the attachment of bacteria to epithelium, food particles and to other bacteria. Intermittent colonization of the epithelia was observed, as well as microcolonies formed by groups of bacteria.

Costerton *et al.* (1978) attested that bacterial adhesion was mediated by the formation of glycocalyx. It is this *"mass of tangled fibers of polysaccharide"* which help the bacteria survive in a competitive environment. The glycocalyx seems to serve many purposes. It not only positions the bacteria, but may conserve and

concentrate the digestive enzymes released by the bacteria against the host cell. It may function as a food reservoir and protect the organism against predatory bacteria and bacterial viruses.

This leads to the concept that if adhesion of bacteria can be prevented, perhaps bacterial invasion of poultry skin could also be prevented. Costerton *et al.* (1978) suggested three methods of achieving this: firstly, by disrupting glycocalyx synthesis; secondly, by preventing attachment of glycocalyx to the bacteria or thirdly, by preventing attachment of glycocalyx to the host.

Notermans *et al.* (1979) examined the attachment of bacteria to cows' teats. They studied the attachment at different storage temperatures and found results similar to their earlier work on poultry skin (Notermans and Kampelmacher, 1974). After the initial attachment, the strength of adhesion increased. This increase was faster at higher storage temperatures, perhaps due to an increase in bacterial metabolism and a subsequent increase in the formation of glycocalyx. After longer periods, the strength of adhesion decreased, due to the formation of colonies. As the bacterial numbers increase, more are attached to each other and not to the surface, and therefore can be easily removed.

This was further demonstrated by Firstenberg-Eden *et al.* (1979). Scanning electron miscroscopy showed that during storage, polymers in the form of thin fibers, were produced. These fibers thickened to form slime. This work supported the observations of Notermans *et al.* (1979) and showed the existence of microcolonies of bacteria.

Butler *et al.* (1979) examined the attachment of micro-organisms to pork skin and to the surfaces of beef and lamb carcasses. The authors developed a model system in which the samples were embedded in solidified wax with the skin surfaces exposed. The wax cubes were then dipped into attachment media. They reported that different organisms have different attachment rates. Motile gram-negative organisms such as *E. coli*, *P. putrefaciens*, *E. herbicola* exhibited greater attachment than did non-motile gram-positive organisms such as *Lactobacillus* and *S. aureus*. There is also a direct relationship between the bacterial counts on the skin and the concentration of the bacteria in the attachment medium which is in agreement with the work of Notermans and Kampelmacher (1974) and Notermans *et al.* (1975b). The effects of temperature and pH were not significant. These discrepancies could be due to the fact that Butler *et al.* (1979) used pork skin whereas Notermans and Kampelmacher (1974) and Notermans *et al.* (1975) used chicken skin.

E. Inoculation of Chicken Skin

Several authors have studied model systems for the introduction of bacteria to food myosystems.

Clark (1965) developed a method of using a spray gun to uniformly inoculate nutrient surfaces with a bacterial suspension. The uniformity of inoculation was shown to be affected by the ionic strength of the bacterial suspension, the distance between the spray nozzle and the sample surface, the rate of air flow, and the exposure

time. It was reported that the use of ionic strengths less than 0.25 resulted in uneven inoculation and a 25-50% decrease of bacterial numbers when compared to ionic strengths between 0.25 and 1.5. A minimum exposure time of 30 sec was reported to be required for obtaining a consistent, uniform inoculation on all six plates. The density of the inoculum could be satisfactorily controlled by the exposure time and the concentration of the inoculating suspension.

Notermans and Kampelmacher (1974) employed the use of a physiological solution (NaCl, 8.7 g/L) containing phosphate buffer (pH 7.2) and EDTA. They immersed whole chicken carcasses into 25 L baths of attachment medium inoculated with a known number of bacteria.

Butler *et al.* (1979) embedded their samples in liquid wax with a piece of sterile string. This sample was then immersed in 180 mL of sterile attachment medium similar to that used by Notermans and Kampelmacher (1974).

Barrow *et al.* (1980) used a quite different approach. Epithelial cells were obtained from the gut of a 4 week old pig and suspended in phosphate buffered saline. Equal volumes of bacterial and epithelial cell suspensions were mixed and incubated on a rotating platform at 37°C for 30 min. The number of attached microorganisms per 20 epithelial cells was counted by phase contrast microscopy.

MATERIALS AND METHODS

Chicken carcasses (42 to 45 day old broilers) were obtained from a local poultry processing plant, placed in sterile polyethylene bags (1 Mrad γ irradiation, Gammacell 200 (Atomic Energy of Canada Ltd.)) and transported on ice to the laboratory.

A. Scanning Electron Microscopy

Three methods of preparation of chicken skin for scanning electron microscopy (SEM) were employed. They were (i) standard chemical fixation; (ii) thiocarbohydrazide fixation (TCH) and (iii) freeze-drying. In addition, two methods of dehydration were compared: standard ethanol dehydration with amyl acetate infiltration and chemical dehydration using 2,2-dimethoxypropane.

In all the fixation procedures, the chicken skin was first excised into 6 cm x 6 cm pieces. Large pieces were cut since curling occurred during fixation. The skin samples contracted in all directions and had to be kept flat with forceps until immersed in fixative.

1. Standard chemical fixation

The samples were fixed in 6.3% (v/v) electron microscopic grade glutaraldehyde (CAN-EM Chemicals, Guelph, Ont; Marivac, Halifax, N.S.) in Millonig's phosphate buffer at $4^{o}C$ overnight (Dawes, 1971).

After fixation in glutaraldehyde the tissue was washed three times in Millonig's phosphate buffer prior to post-fixation in 1% (w/v) osmium tetroxide (CAN-EM Chemicals, Guelph, Ont; Marivac, Halifax, N.X.) in Millonig's buffer for 1 h. Samples were then rinsed three times in Millonig's buffer prior to dehydration and critical point drying (Figure 1).

2. *Standard dehydration*

The samples were dehydrated through an ascending series of ethanol: 50%, 70%, and 80% (v/v) ethanol for 5 min each, two changes of 90% ethanol for 10 min each and finally three changes of 100% ethanol for 20 min each. All ethanol dilutions were made with distilled deionized water. This was followed by amyl acetate (Fisher Scientific Co., Fairlawn, NJ) infiltration. A graded series of amyl acetate solutions in 100% ethanol was used: one change of 10 min duration each in 25%, 50% and 75% amyl acetate and finally 100% amyl acetate for 1 h (Figure 2).

3. *Chemical Dehydration*

Samples were dehydrated using acidified 2,2-dimethoxypropane (2,2 DMP) following the method of Maser and Trimble (1976). 2,2 DMP reacts with water to form methanol and acetone. Two changes of 15 min duration of 2,2 DMP were used (Figure 2).

Samples were critical point dried in a Parr bomb (Parr Instruments Co., Moline, IL) and mounted on aluminum stubs with

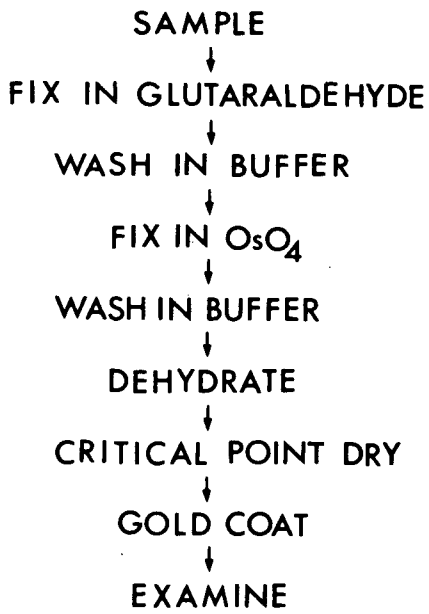


FIGURE 1: Flow sheet of the standard chemical fixation procedure.

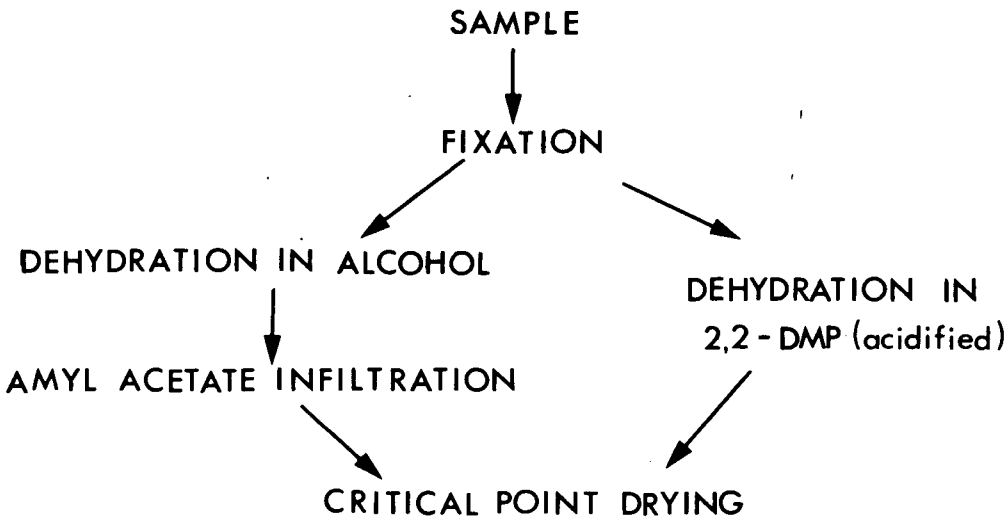


FIGURE 2: Flow sheet of the dehydration procedures.

epoxy cement and silver paste (Structure Probe Inc., West Chester, PA). Samples were then coated with gold in a sputter coater (Technics Inc., Alexandria, VA) and examined with a Hitachi S-500 Scanning Electron Microscope (Hitachi, Tokyo, Japan) at an accelerating voltage of 20 kV. Images were recorded on Ilford Pan F 135 fine grain black and white film (Ilford Ltd., Essex, England).

4. *Thiocharbohydrazide fixation*

Sample preparation was performed according to the method of Malik and Wilson (1975). The chicken skin was fixed in 6.3% (v/v) glutaraldehyde in Millonig's phosphate buffer (as described previously) and washed three times in the phosphate buffer. Following post-fixation in 1% oxmium tetroxide for 3 h, samples were rinsed six times with distilled deionized water. The samples were then placed in 1% aqueous solution of thiocarbohydrazide (TCH, Eastman Chemicals, Rochester, NY) for 30 min, after which they were again rinsed six times with distilled deionized water. Another fixation with 1% osmium tetroxide for 2 h followed. This step was then repeated, giving a total of three fixations in osmium tetroxide and two treatments with TCH (Figure 3).

5. *Freeze-drying*

Initially, samples of chicken skin were dipped into liquid N_2 and then freeze-dried. This method was then modified such that the samples were dipped in isopentane (Fisher Scientific,

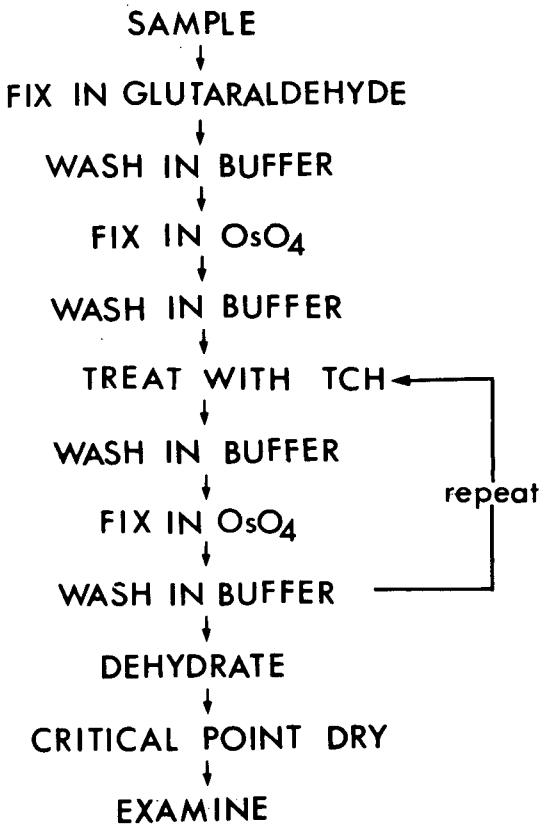


FIGURE 3: Flow sheet of the thiocarbohydrazide fixation procedure.

Co., Fairlawn, NJ) which was cooled in a liquid N_2 bath. This method did not yield satisfactory results either, so another modification was necessary. The samples were placed in wells of an aluminum block (Figure 4). The block was cooled in liquid N_2, and the samples were allowed to freeze by contact with the cold block (Figure 5).

B. *Proximate Analysis*

Chicken skin samples were obtained from Site A (before scalding) and Site C (chill tank) (see Figure 6). Five samples were taken from each site.

1. *Moisture determination*

Moisture content was determined by lyophilization of finely chopped pieces of chicken skin. Analyses were carried out in duplicate on five samples of each skin type from carcasses taken at Site A and C.

2. *Crude fat determination*

Crude fat was determined, after lyophilization of the skin samples, by the use of a Goldfish extractor (AOCS, 1975). Petroleum ether was used as the extracting solvent and the extraction was continued for 6 h. Following extraction, the petroleum ether was allowed to evaporate from the extraction flasks at room temperature overnight.

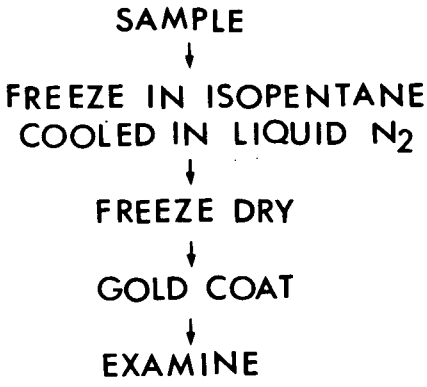


FIGURE 4: Flow sheet of the freeze-drying procedure.

FIGURE 5: Aluminum block used in the freezing of chicken skin samples.

3. *Protein determination*

Protein content of the skin samples was determined by the rapid micro-Kjeldahl method of Concon and Soltess (1973), using the Technicon Auto Analyzer (Technicon Instruments Corp., Tarrytown, NY). Analyses were carried out in duplicate on triplicate 10 mg lyophilized samples. Results were expressed as percent protein wet weight, using a nitrogen to protein conversion factor of 6.25 (Coleman, 1968).

4. *Total carbohydrate*

Total carbohydrate content of the skin samples was determined by the modified phenol-sulfuric acid method (Dubois *et al.*, 1956). Analyses were performed on 10 mg of lyophilized skin. The skin samples were mixed with 2 mL of distilled deionized water in a test tube. The tubes were stoppered and placed in a 100°C water bath for 1 min. Upon cooling to room temperature, 0.05 mL of 80% (w/v) phenol was added. Five mL of concentrated sulfuric acid was then added rapidly. In order to complete hydrolysis of the skin tissue, vortexing of the mixture was required immediately after the addition of sulfuric acid. After standing at room temperature for 10 min, the tubes were again vortexed, and subsequently incubated at 25°C for 15 min. The absorbance at 485 nm was then measured with a Unicam SP 800B spectrophotometer. Quadruplicate analyses were carried out on triplicate skin samples. Total carbohydrate

was estimated from a standard curve for glucose. Total carbohydrate was expressed as percent wet weight.

C. *Fatty Acid Composition*

1. *Preparation of methyl esters*

i) Purification of crude lipid

The crude lipid was purified by the method of Sahasrabudhe *et al.* (1979) using a biphasic separation of chloroform, methanol and water (2:1:0.8). The chloroform layer was then filtered through phase separating filter paper (Whatman, Ltd., England) to remove any traces of water and then concentrated with a rotary evaporator at room temperature. This extract was stored under N_2, at $4^{o}C$, overnight.

ii) Transesterification

Purified lipid (50 mg) was refluxed with 40 mL of H_2SO_4-methanol solution (2.0 mL concentrated H_2SO_4 in 230 mL methanol:benzene 3:1) in a flask placed in a sand bath at $140^{o}C$ for 4 h. The solution was stored under N_2, at $4^{o}C$, overnight. Then 50 mL of water was added and the mixture extracted twice with 50 mL portions of petroleum ether. The combined extracts were washed with distilled deionized water until free from acid as evidenced by an external methyl red indicator. The extract was then filtered through anhydrous sodium sulfate to remove any remaining moisture. The petroleum

ether was then removed under N_2 with a rotary evaporator at room temperature (Dr. M. R. Sahasrabudhe, Food Research Institute, personal communication).

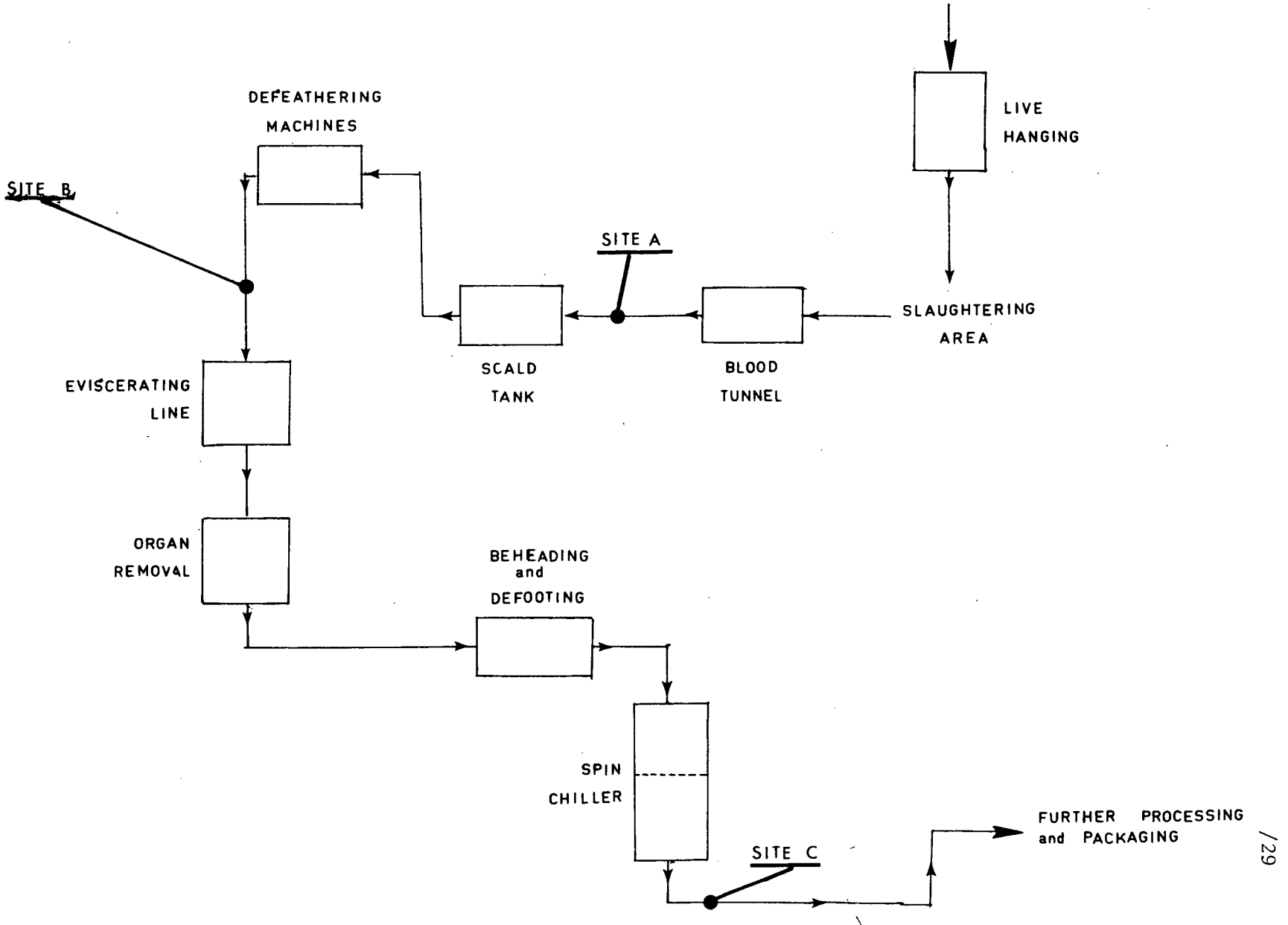
2. *Gas chromatography*

Fatty acid methyl esters were analysed with a Tracor model MT 200 gas chromatograph (Tracor, Austin, TX) equipped with dual flame ionization detectors. The column was 6 ft x 1/8 in stainless steel packed with GP 5% DEGS-PS on a 110/120 mesh support (Supelco Inc., Bellefonte, PA) and operated on a temperature program starting at 150°C and ending at 200°C, increasing by 4°/min. Fatty acids were identified by retention times and percentages of each fatty acid were calculated using response factors determined on known standards (Supelco Inc., Bellefonte, PA). Peaks were recorded and analysed using a Hewlett Packard Model 3390A Reporting Integrator (Hewlett Packard, Avondale, PA).

D. *Bacterial Load of Chicken Carcasses*

Chickens were obtained from Site B (after defeathering) and Site C (chill tank, see Figure 6). The chickens were placed in sterile polyethylene bags (1 Mrad γ radiation) and transported on ice to the laboratory. Samples of chill water were taken by dipping a sterile beaker into the chill tank. The chill water was then transferred to a sterile Whirl-pak bag (Arnold Nasco Ltd., Guelph, Ont.) and transported on ice to the laboratory.

FIGURE 6: Sampling sites in the poultry processing plant.


LIVE
HANGING
DEFEATHERING
MACHINES
SITE B
SITE A
SLAUGHTERING
AREA
SCALD
TANK
BLOOD
TUNNEL
EVISCERATING
LINE
ORGAN
REMOVAL
BEHEADING
and
DEFOOTING
SPIN
CHILLER
SITE C
FURTHER PROCESSING
and PACKAGING

Bacterial enumeration was performed on a per cm^2 basis. This was done by cutting a 12.95 cm^2 area from locations 1 and 2 (see Figure 7). The area was inscribed by the use of a round metal cookie cutter (radius ≃ 2 cm). This area was measured by the use of a electronic planimeter Model EDGC (Nemonics Corp, USA).

Each skin sample was placed in a sterile Stomacher bag to which 50 mL of sterile 0.1% peptone broth (Difco, Detroit, MI) containing 1% Tween 80 (Difco, Detroit, MI) was added. Tween 80 was added as an aid in homogenizing fat in the chicken skin (Emswiler *et al.*, 1977). The sample was then stomached for 2 min in a Colworth stomacher Lab blender 400 (A.J. Seward, London, England). Appropriate serial decimal dilutions of the macerated sample were prepared with 0.1% peptone broth and 0.02 ml aliquots, in duplicate were plated on separate sectors of Brilliant green agar (BGA, Difco. Detroit, MI) and nutrient agar (NA, Difco, Detroit, MI) using the modified drop plate technique (ICMSF, 1978) (Figure 8).

E. Attachment Studies

1. Preparation of inoculum

A freeze dried culture of *Salmonella typhimurium* (ATCC 14028) was prepared as described by American Type Culture Collection (1976). The culture was maintained on NA slants at 4°C. Prior to inoculation of the chicken samples, a loopful of *S. typhimurium* was transferred from the NA slant to several tubes containing 10 mL nutrient broth (NB) (Difco, Detroit, MI) and grown for 18-24 h at 35°C. Subseqently, 2 mL of this culture was

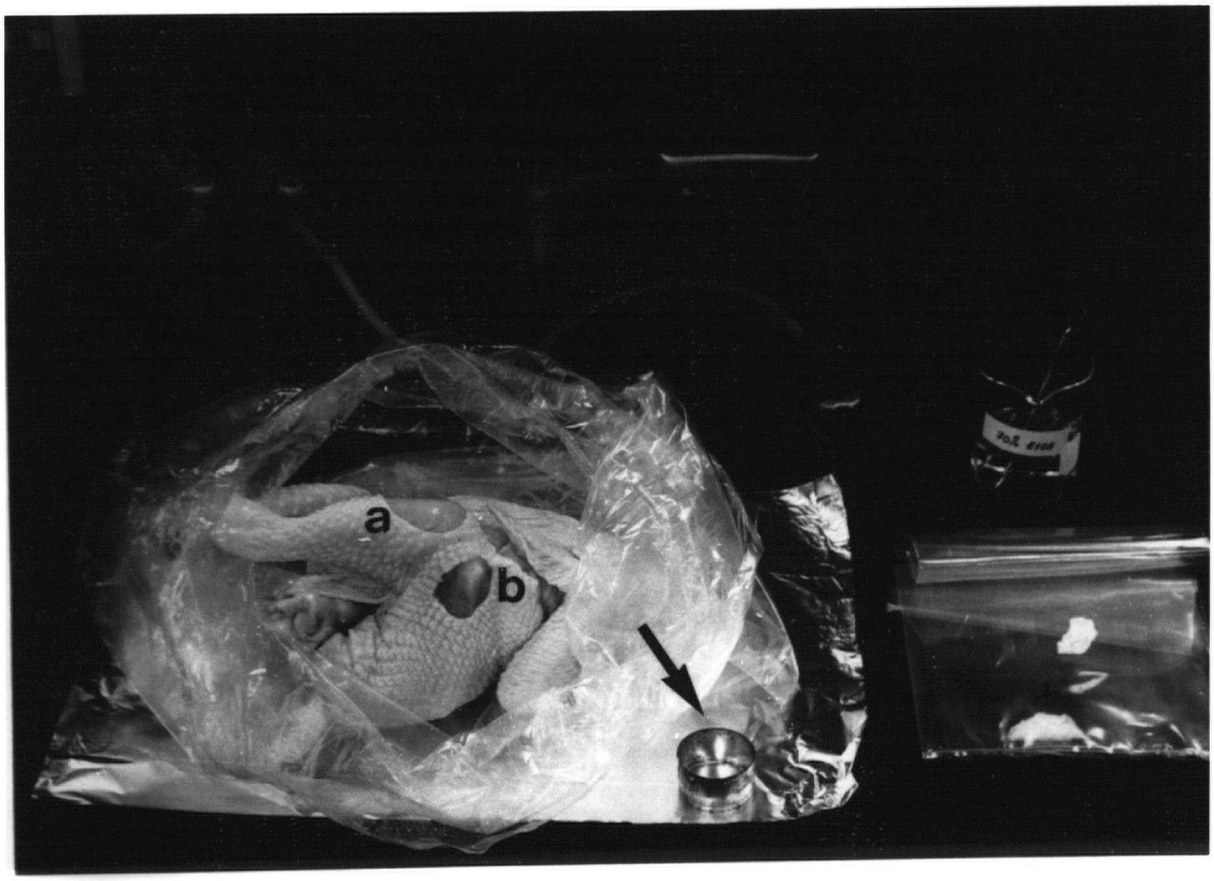


FIGURE 7: Metal template used for inscribing circles on chicken skin (arrow). Skin is cut from (a) location 1 and (b) location 2.

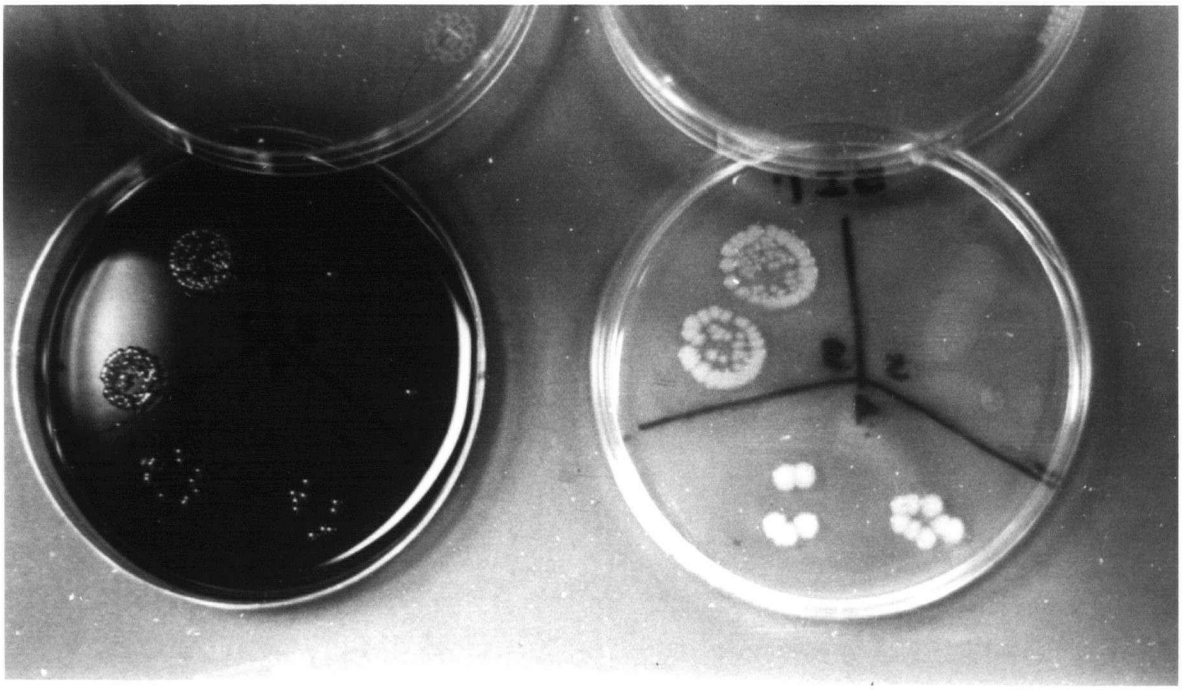


FIGURE 8: Brilliant green agar (left) and nutrient agar (right) plates. Each plate shows three dilutions, each done in duplicate, using the drop plate technique.

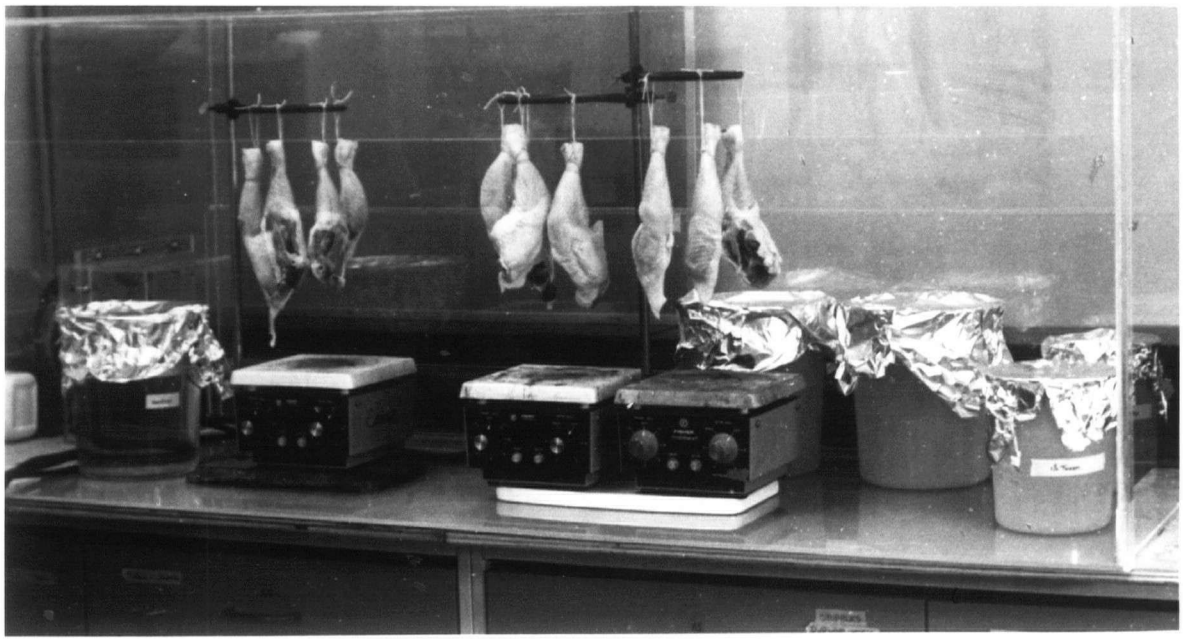
transferred to each of several bottles containing 200 mL NB. The cultures were grown at 35°C in a shaking water bath for 18-24 h. This was done in order to obtain maximum cell densities.

Approximate concentrations of the bacterial suspension were determined using an HF turbidimeter (Model DRT-1000, H.F. Instruments, Bolton, Ont.). Appropriate aliquots were then taken and centrifuged for 10 min at 5000 g, in order to remove the nutrient broth. The pellet was resuspended in 50 ml of sterile attachment medium (pH 7.2) consisting of 0.150 M NaCl, 0.0062 M Na_2HPO_4, 0.0021 M NaH_2PO_4 and 0.001 M EDTA (Notermans and Kampelmacher, 1974).

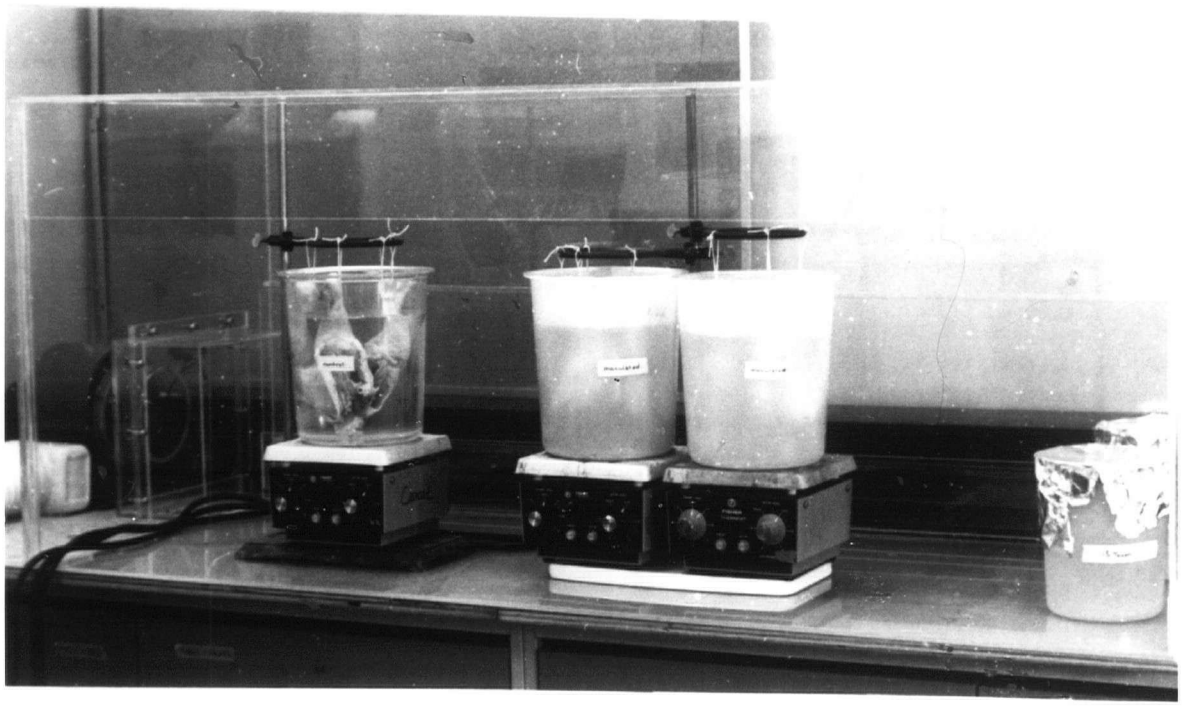
This was shaken vigorously in order to obtain a uniform suspension. The solution was centrifuged for 7 min at 5000 g. The centrifugation and resuspension in sterile attachment medium was repeated twice to ensure complete removal of the nutrient broth. After completion of the washing, the pellet was resuspended in 10 mL of sterile attachment medium and then added to the inoculating bath.

2. *Preparation of skin samples*

Chicken legs were aseptically removed from chicken carcasses obtained from Site C of a local poultry processing plant and transported on ice to the laboratory. These legs were tied with sterile string, then hung from a ring stand (Figure 9a).



(a)



(b)

FIGURE 9. Apparatus for the attachment studies. Chicken legs hanging from ring stands in a sterile hood (a). Chicken legs immersed in attachment medium (b).

They were then immersed for 15 min in 3 L of attachment medium, containing approximately 10^8 *S. typhimurium* cells/mL. Control samples were also prepared by dipping the chicken legs into 3 L of sterile attachment medium (Figure 9b).

After 15 min immersion in the attachment medium, the chicken legs were removed from the media and allowed to hang. After each of the desired time intervals (0, 5, 10 and 15 min), two legs were removed for each of the following procedures (Figure 10).

i) Inoculated samples

Skin samples were cut from locations 1 and 2 (see Figure 11) directly after hanging for the desired length of time.

ii) Washed inoculated samples

The inoculated legs were dipped quickly 15 times into 1.5 L of sterile attachment medium before skin samples were taken.

iii) Surfactant-washed inoculated samples

The inoculated legs were dipped quickly 15 times into 1.5 L sterile attachment medium containing 1% Tween 80.

3. *Bacteriological analysis*

Skin samples were placed in 100 mL of sterile 0.1% (w/v) peptone broth containing 1% Tween 80 in a sterile stomacher bag. It was then stomached for 2 min in a Colworth Lab blender 400.

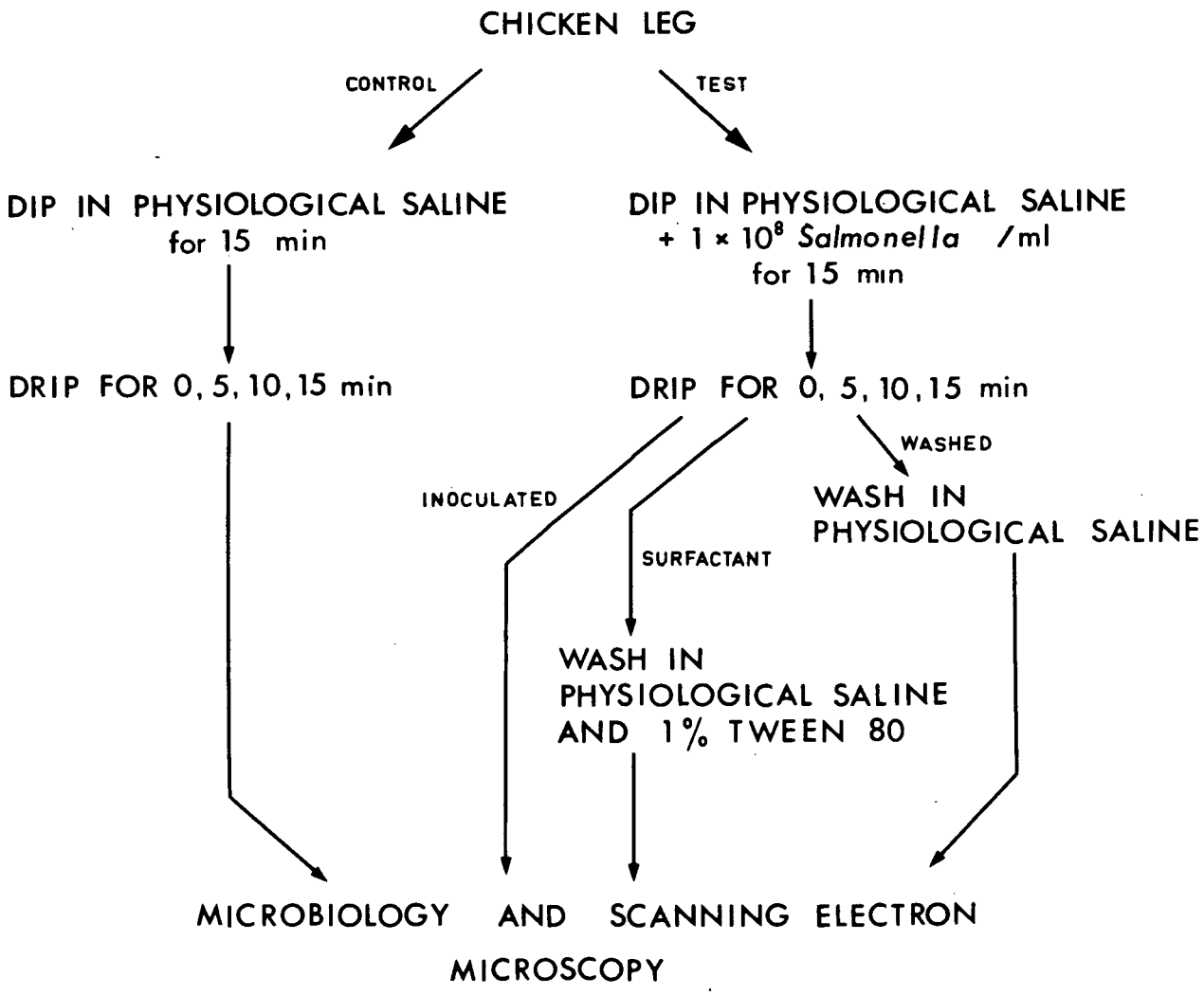


FIGURE 10: Flow sheet of the attachment procedure.

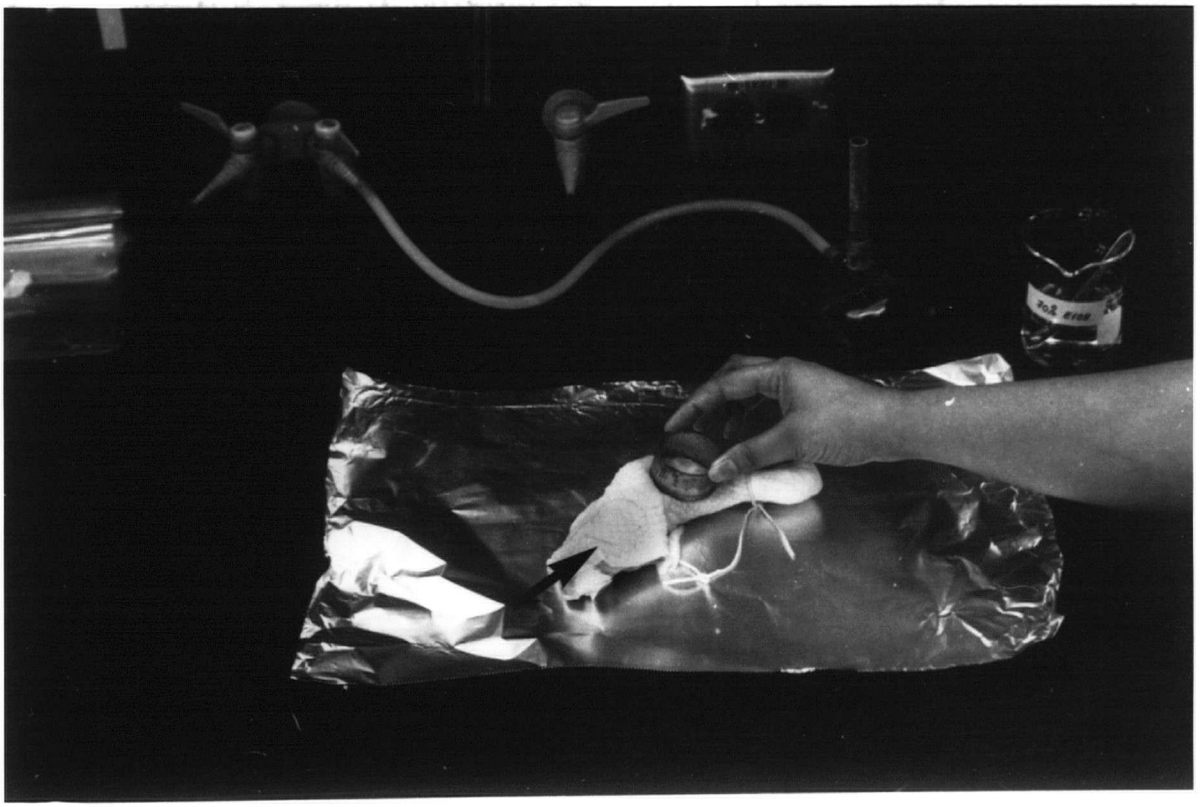


FIGURE 11: Metal template used to inscribe chicken skin shown at location 1. Location 2 is indicated by by arrow.

Appropriate serial decimal dilutions of the macerated sample were prepared with sterile 0.1% peptone broth and duplicate 0.02 ml aliquots were plated on separate sectors of BGA and NA using the modified drop plate technique (ICMSF, 1978).

Bacterial enumeration was also performed on the attachment medium by preparation of serial decimal dilutions in 0.1% peptone broth and drop-plated on BGA and NA.

All BGA plates were incubated for 24 h at 35^{o}C and the nutrient agar plates were incubated for 48 h at 35^{o}C.

4. *Statistical analysis*

The data was first analyzed by analysis of variance for a balanced 4 x 4 x 2 factorial design (Mendenhall, 1968; Londgren and McElrath, 1969) for each trial. Each of the variables was first expressed as a logarithm to the base ten. There were four treatment levels: control, inoculated, washed and surfactant and four time levels: 0, 5, 10 and 15 min after removal from attachment media. There were two skin types: Type I and Type II. Each cell had four observations giving a total of 128 observations for each trial.

The data from the two trials was then combined and analyzed as a 4 x 4 x 2 x 2 balanced factorial design. Replication was the fourth factor, with 2 levels: Trial 1 and Trial 2. This resulted in a total of 256 observations. The data was analyzed on Amdahl V8 computer (Amdahl, Sunnyvale, CA) using UBC MFAV

(Le, 1980a) which calculates analysis of variance, Neuman-Keuls's multiple range test and UBC BMD 02V (Le, 1980b) which also calculates marginal means and interaction effects.

RESULTS AND DISCUSSION

A. *Scanning Electron Microscopy*

Scanning electron microscopy can provide valuable information on the micro-topography of chicken skin, a substrate for food-borne bacteria. Poultry skin structure may play a role in the attachment of bacteria to poultry carcasses (McMeekin *et al.*, 1979).

In order to examine the involvement of the surface microstructure in the adhesion of bacteria to poultry skin, several methods of preparation of chicken skin were evaluated to determine the best method to visualise the microstructure of poultry skin.

The micrograph of chicken skin prepared by standard chemical fixation and dehydration (Figure 12) reveals that chicken skin does not have a smooth surface, but rather it is convoluted with many crevices which may serve to entrap bacteria. The structures shown are comparable to those reported by McMeekin *et al.* (1979), Thomas and McMeekin (1980), and Suderman and Cunningham (1980). McMeekin *et al.* (1979) and Thomas and McMeekin (1980) used osmium tetroxide vapors for fixation, whereas Suderman and Cunningham (1980) used phosphate buffered glutaraldehyde. Similar structures (Figure 13) can also be observed using light microscopy.

The second method of fixation to be investigated was the thiocarbohydrazide method. Most biological specimens cannot conduct electrons and therefore must be treated specifically for SEM. In the standard method, a coating of gold-palladium is evaporated or

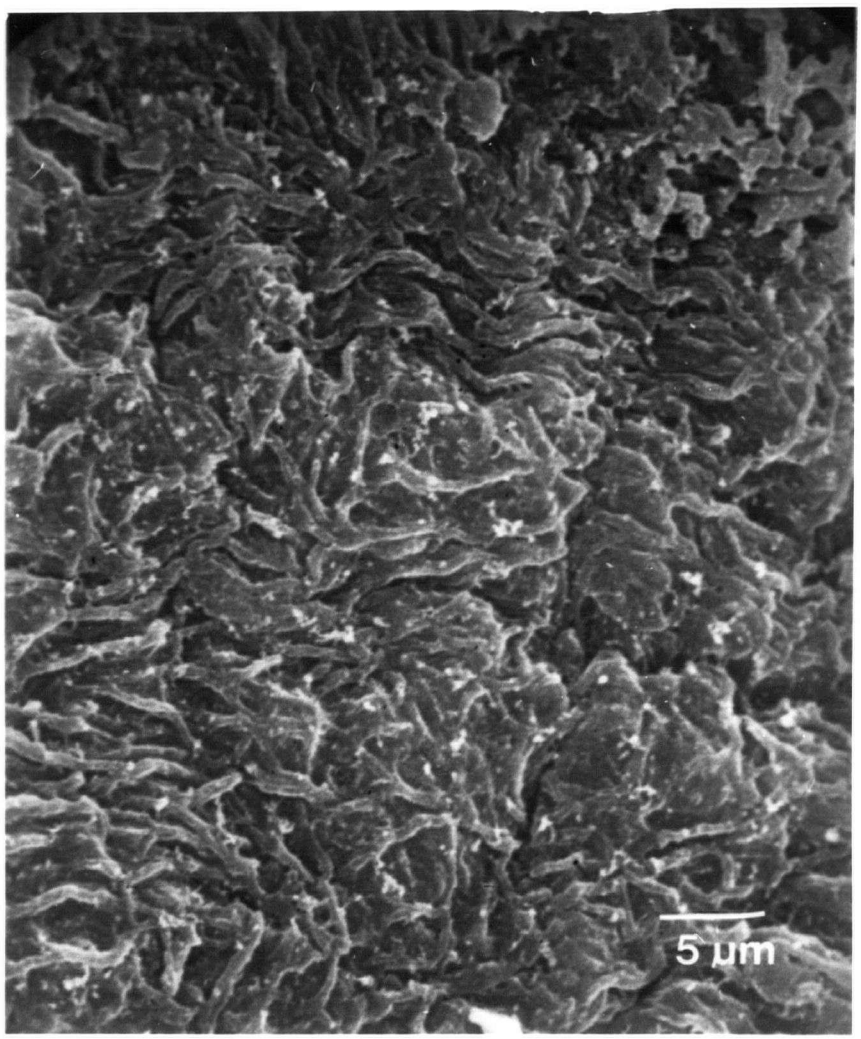


FIGURE 12: SEM micrograph of chicken breast skin prepared by standard chemical fixation and conventional ethanol dehydration and amyl acetate infiltration.

FIGURE 13: Chicken breast skin viewed under the light microscope.

sputtered onto the surface to be examined. Since biological specimens have complex topographies, uneven coating frequently results. If the coating is inadequate, charging effects will occur. These are evidenced by either abnormally bright areas or as discharges which produce dark areas lacking in detail. In addition, the use of a coating adds 100 - 200 Å to the surface being examined (Kelley *et al.*, 1973; Sweeny and Shapiro, 1977). This additional layer could mask the finer detail of the surface structure (Hayat, 1978).

The thiocarbohydrazide method employs the use of thiocarbohydrazide and osmium tetroxide in addition to the routine glutaraldehyde fixation and osmium tetroxide post-fixation. TCH is used to render the tissue conductive and acts by bridging one molecule of OsO_4 to another molecule of OsO_4 (Figure 14). This enhances contrast (Hayat, 1978). McCowan *et al.* (1978) have used the thiocarbohydrazide procedure in the fixation of rumenal epithelial cells and found that the thiocarbohydrazide treatment reduced charging while the absence of a gold layer gave clearer micrographs as compared to the standard method.

In the fixation of chicken skin, however, the TCH method did not yield superior results. This procedure is very tedious and time-consuming. A comparison of specimens prepared by the standard method and the thiocarbohydrazide method (Figure 15) reveals that the standard method is better. The same stringy structures are again evident but the resolution and definition are not as clear in the TCH fixed samples. The cracks and crevices are again present on the surface of the skin samples.

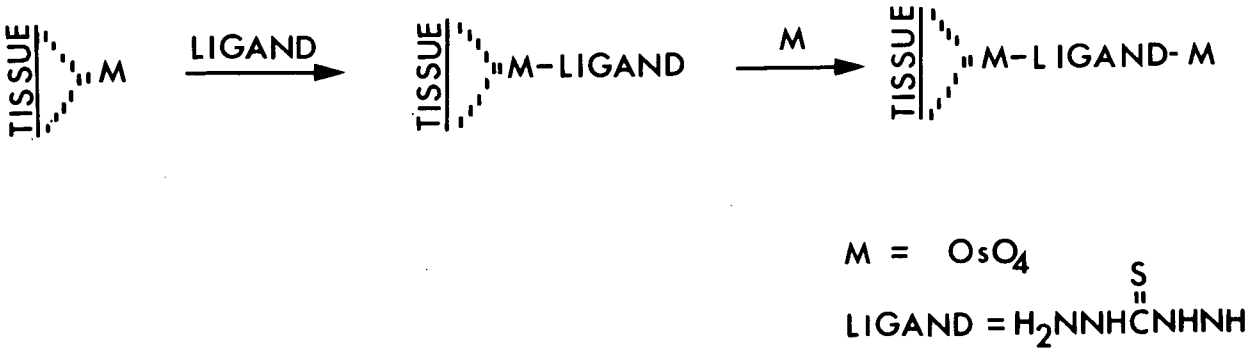
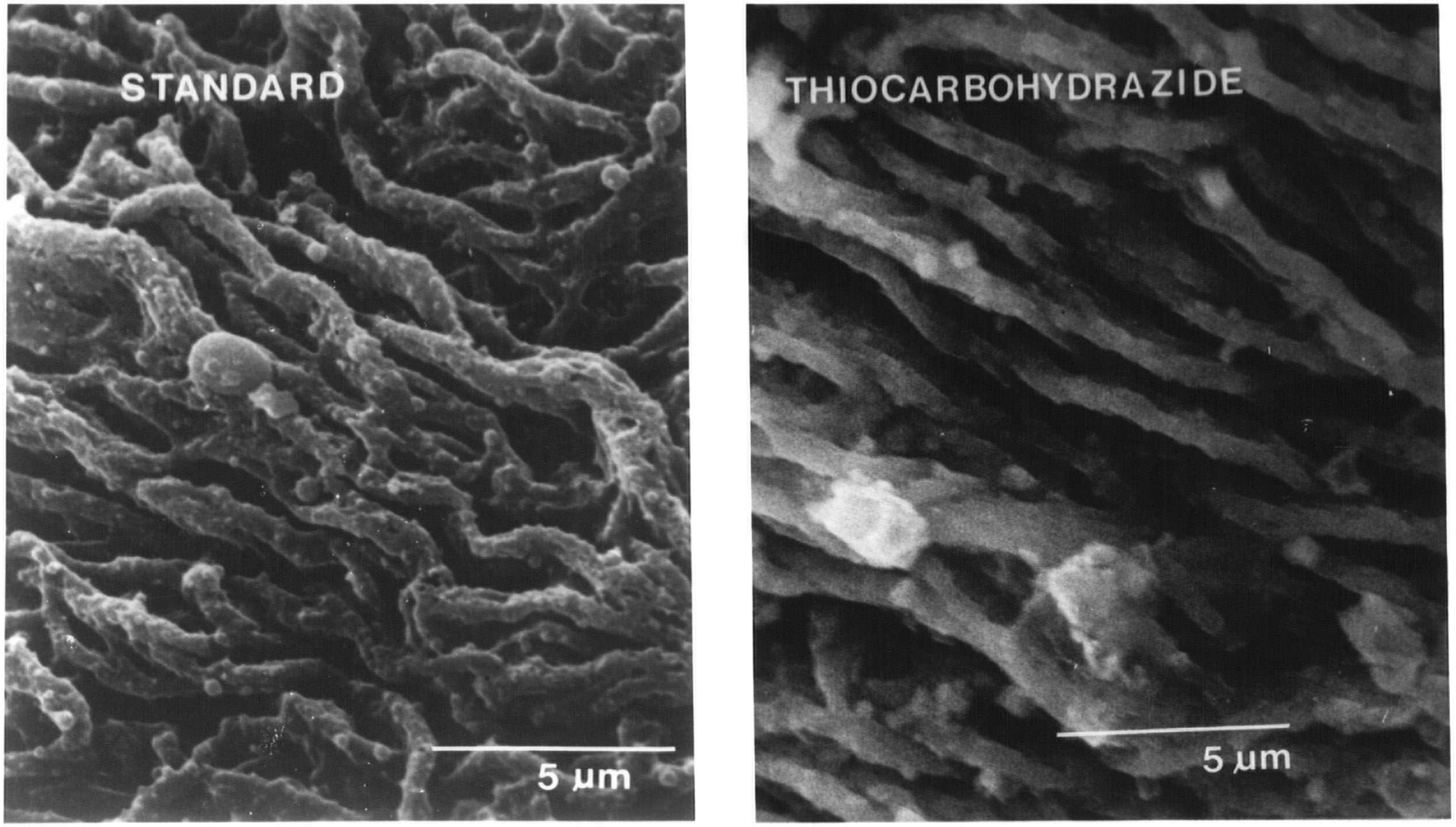


FIGURE 14: Bridging reaction between thiocarbohydrazide and osmium tetroxide for the enhancement of contrast in SEM.



(a) (b)

FIGURE 15: SEM micrographs of chicken breast skin prepared (a) by standard chemical fixation and (b) by the thiocarbohydrazide method.

In order to shorten the preparation time, 2,2-dimethoxypropane (DMP) was used for rapid dehydration. This step eliminated several hours of preparation time since ethanol dehydration and amyl acetate infiltration could be eliminated.

Acidified 2,2-dimethoxypropane combines with water to form methanol and acetone (Figure 16). This reaction is endothermic. The advantages of this technique have been listed by Maser and Trimble (1976). DMP is less expensive than either ethanol, acetone or amyl acetate and the procedure is rapid since it does not require physical exchange with water as does conventional dehydration. The products of the reaction, acetone and methanol, are solvents commonly used in critical point drying. DMP is miscible with liquid CO_2 and is therefore compatible with CO_2 exchange prior to critical point drying.

When one compares the micrographs of the specimens prepared by the two methods of dehydration (Figure 17), it is apparent that chemical dehydration did not induce alterations in the surface structure of poultry skin. The same cracks and crevices were again evident. The fibrous structures (Figure 17, arrow) tended to crop up in most of the sections, and similar structures have been reported by McMeekin *et al.* (1979).

This technique has been used with success by Kahn *et al.* (1977) in their study of cells in culture. They found that 2,2-dimethoxypropane is a useful alternative to ethanol in that *"it is fast, inexpensive, reduces the chance of air drying and insures complete removal of water"*.

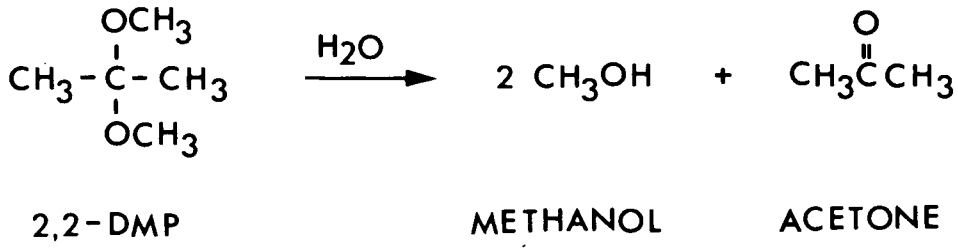
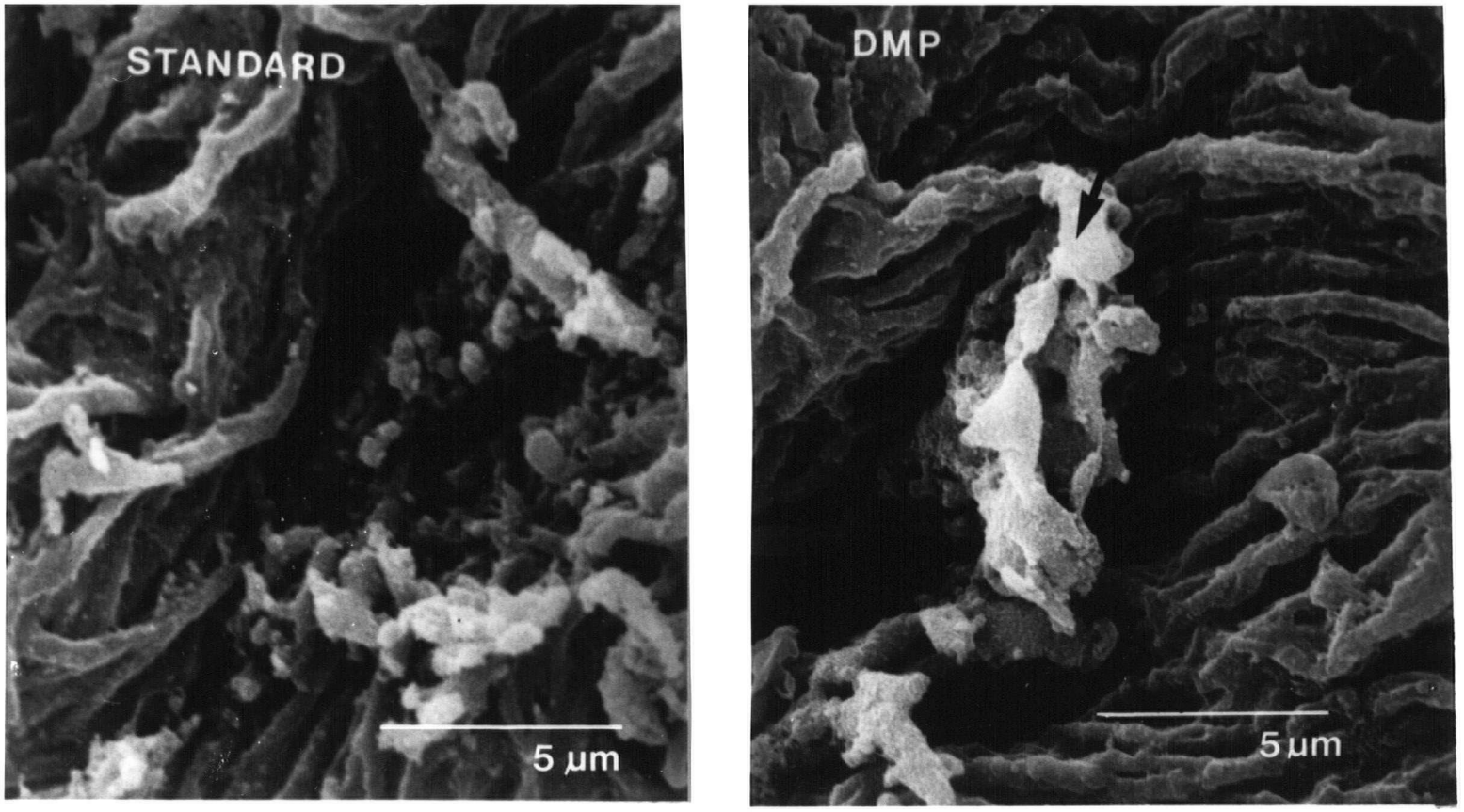


FIGURE 16: Reaction of 2,2 dimethoxypropane with water during dehydration of tissue samples for SEM.



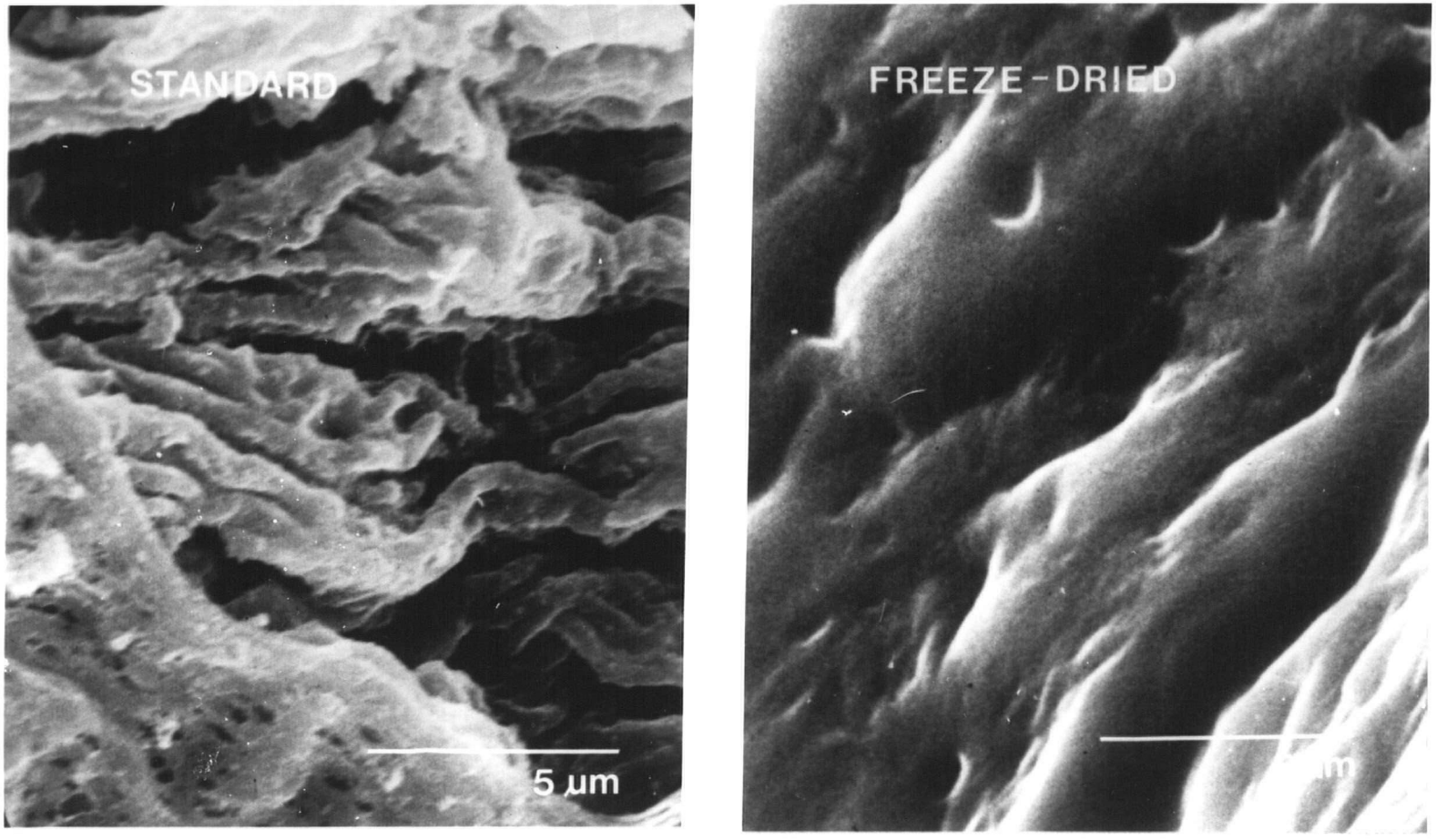
(a) (b)

FIGURE 17: SEM micrographs of chicken breast skin prepared by (a) conventional dehydration and (b) by chemical dehydration using 2,2 DMP.

The third method under investigation was freeze-drying. Freeze-drying would be relatively rapid and would bypass the *"washing"* effect of the buffers which are used in the other two methods. When comparing the micrographs of specimens prepared by the standard method and those prepared by freeze-drying (Figure 18) it is apparent that the standard method of fixation is better. It appears that the surface of the freeze-dried sample is obscured by a coating of what may be melted fat. This may be due to the use of isopentane, which could dissolve the lipid on initial contact and cause it to spread over the sample surface. Due to the rapid rate of freezing, the lipid may be frozen as an ice-like sheet on the skin surface.

Several problems were encountered in the process of freezing. Large samples of skin were required since the skin curled and cracked on contact with the cold isopentane. Other studies in the laboratory on beef muscle, did not yield positive results. A surface film was deposited on the samples during the freezing process (R. Yada, personal communication). Therefore, freeze-drying as a method of preparation of chicken skin for SEM was discarded.

When comparing these methods as to cost and time (Table I), as well as to resolution and clarity of detail, is apparent that the standard chemical method is approximately two-thirds the cost of the thiocarbohydrazide method. It is also much quicker (5 h compared with 13.5 h). Chemical dehydration using 2,2-dimethoxypropane is considerably faster than conventional methods and shortens the procedure by several hours.



(a) (b)

FIGURE 18: SEM micrographs of chicken breast skin prepared by (a) standard chemical fixation and (b) by freeze-drying.

TABLE I: Time and cost estimates for the preparation of ten samples for Scanning electron microscopy.

A. Fixation

Chemical	Standard Fixation	Thiocarbo-hydrazide Fixation	Freeze-drying
Glutaraldehyde	$4.20	$4.20	-
Osmium tetroxide	$2.85	$9.50	-
Thiocarbohydrazide	-	$0.95	-
Gold	$2.50	-	$2.50
Isopentane	-	-	$1.50
Liquid nitrogen	-	-	$2.00
Total cost	$9.55	$14.65	$6.00
Time	5h	13.5h	0.2h

B. Dehydration

Chemical	Standard Dehydration	Chemical Dehydration
Ethanol	$0.13	-
Amyl acetate	$1.00	-
2,2 DMP	-	$2.25
Total cost	$1.13	$2.25
Time	3.5h	0.25h

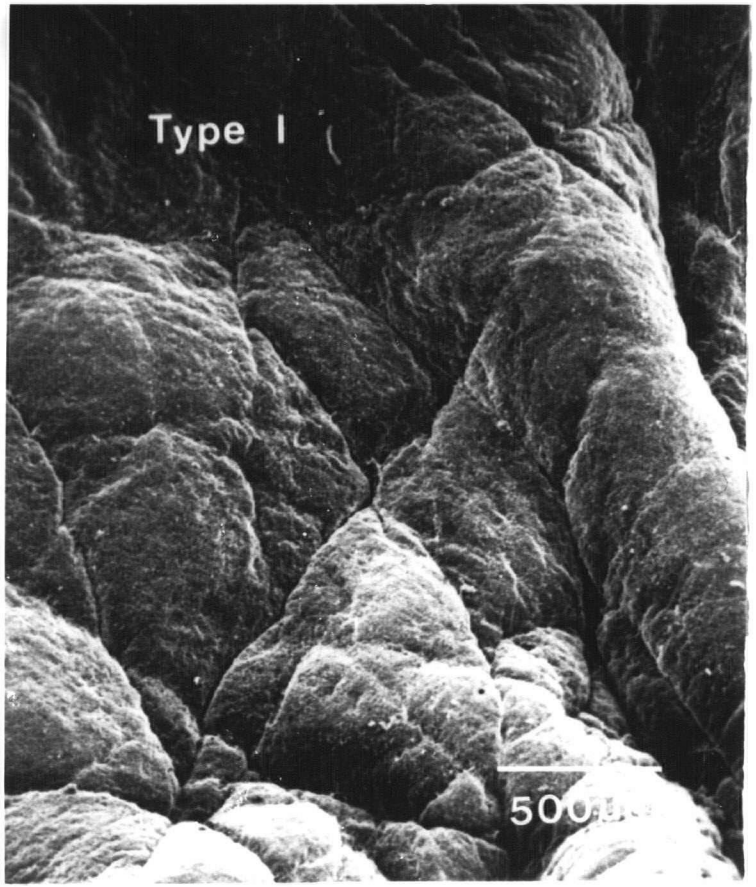
From these preliminary investigations, it was determined that the standard chemical fixation using glutaraldehyde and osmium tetroxide followed by chemical dehydration using 2,2-dimethoxypropane was the best method for preparing chicken skin for SEM. This combination gave reproducible results in a short period of time.

During the initial methodology trials, skin samples were taken from chicken breasts. Several types of microstructures were observed in the scanning electron micrographs. Two skin types could be clearly differentiated at higher magnifications (Figure 20) though at low magnifications (Figure 19), they were not discernable.

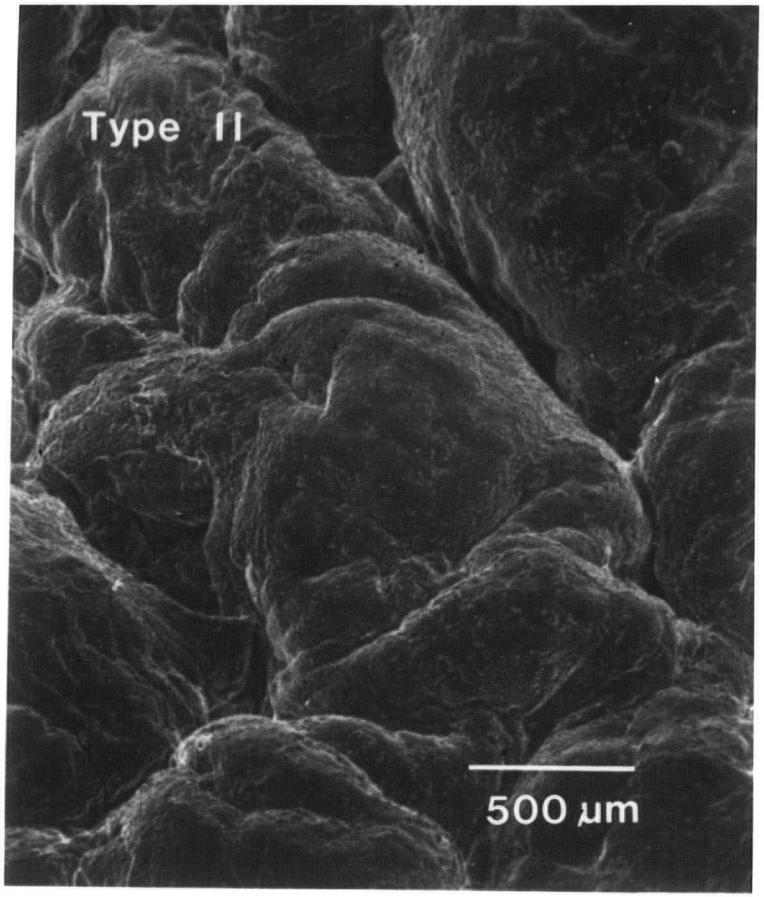
At a magnification of 7000x, breast skin falls into two categories. Micrographs (Figure 20) indicate Type I skin has the filamentous, convoluted structures discussed earlier. Type II skin has a smoother, globular surface appearance. There are also crevices in which bacteria may become entrapped. The existence of Type II skin has not been previously reported in the literature.

In order to determine whether Types I and II skin are present at other locations on the chicken carcass, samples were taken from the leg and back regions of the chicken carcass. The samples were prepared by the standard chemical method and chemical dehydration.

Sections from the leg (Figure 21) reveal once again, two clearly discernable types of chicken skin. Figure 21 is at a lower

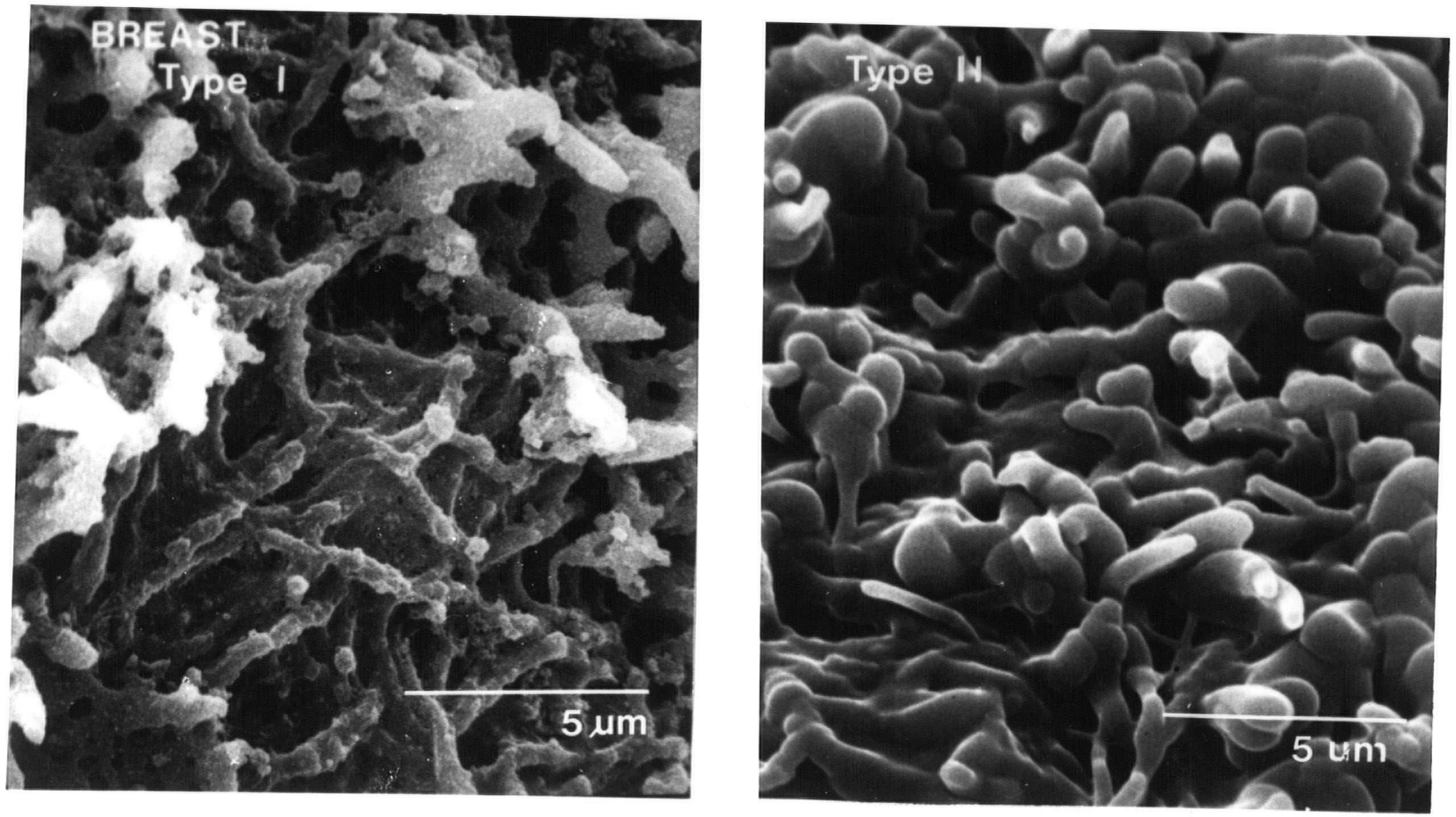


(a)



(b)

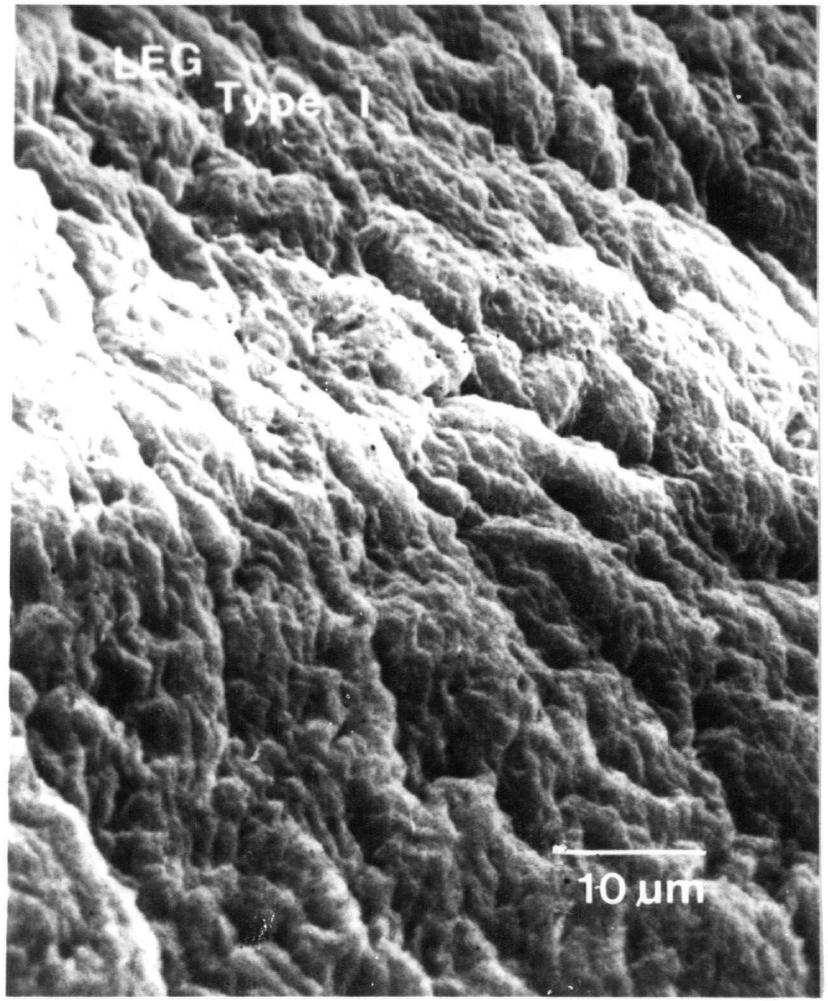
FIGURE 19: SEM micrographs of (a) Type I and (b) Type II skin obtained from chicken breasts.



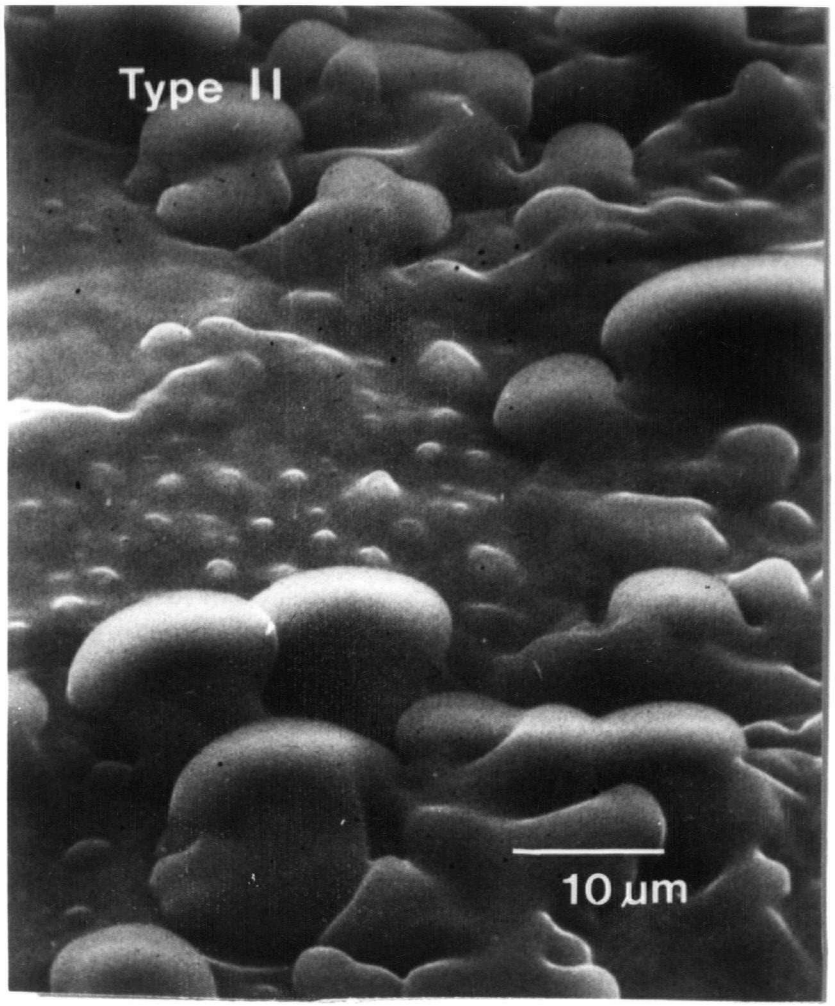
(a)

(b)

FIGURE 20: SEM micrographs of (a) Type I and (b) Type II skin obtained from chicken breasts.



(a)



(b)

/55

FIGURE 21: SEM micrographs of (a) Type I and (b) Type II skin obtained from chicken legs.

magnification than Figure 20 to demonstrate that the difference is observable at a somewhat lower magnification. Two skin types were also found on the back skin sections (Figure 22).

Comparison of the micrographs of Type I skin from leg, breast and back skin (Figure 23), indicate that leg and breast skin are quite similar in surface appearance. Skin from both of these locations display the filamentous, convoluted microstructure described earlier. The back skin, however, has a somewhat coarser topography, which is clearly different from either leg or breast skin.

Comparison of micrographs of Type II skin (Figure 24) reveals again, the congruence of leg and breast skin and a dissimilarity of back skin. The differences may be due to the fact that back skin has different subcutaneous components than the leg or the breast skin. Back skin is more firmly held to the bone, whereas leg and breast skin overlays muscle tissue and are easily removeable.

B. *Proximate Analysis*

Once it was established that there were at least two types of chicken skin, it was of interest to see what differences, other than appearance, existed between Type I and Type II chicken skin. Chickens were obtained before the scalding (site A) and after immersion chilling operations (site C) of the processing plant.

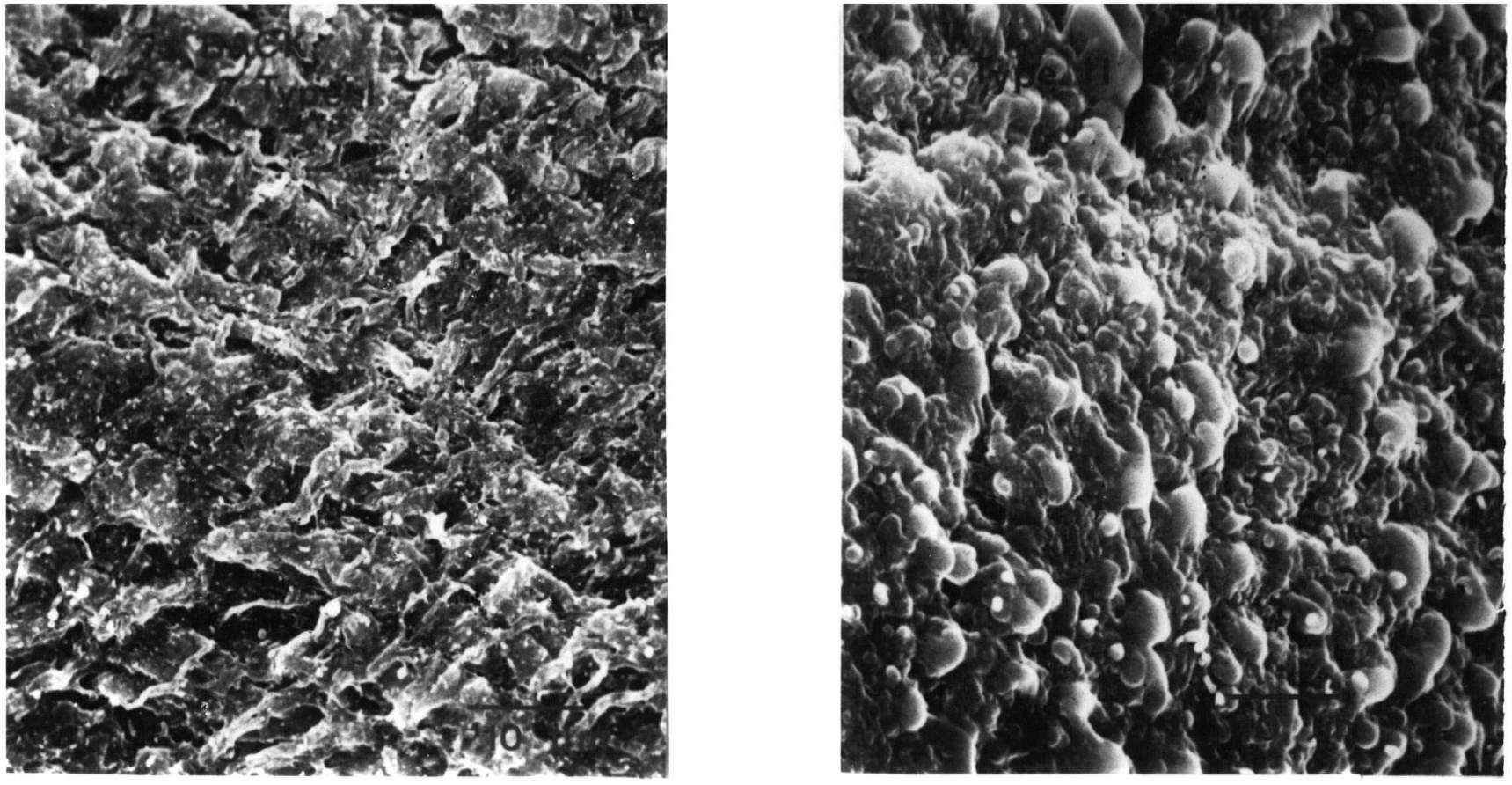
Proximate analysis (Table II) indicates that Type I chicken skin has 46% moisture before scalding and this increases to

TABLE II: Proximate analysis of Type I and Type II chicken skin before scalding (Site A) and after immersion chilling (Site C).

Site	Skin Type	Moisture, %	Fat, %	Protein, %	Carbohydrate, %
A	I	46.21* ± 1.92**[a]	22.70 ± 5.26[a]	16.80 ± 4.80[a]	0.308 ± 0.059[a]
	II	33.98 ± 2.63[b]	35.00 ± 4.09[b]	13.08 ± 4.19[a]	0.254 ± 0.098[a]
C	I	55.04 ± 5.06[c]	25.16 ± 5.68[a]	10.75 ± 4.47[a]	0.155 ± 0.046[a]
	II	33.64 ± 0.65[b]	52.14 ± 3.12[c]	8.63 ± 2.60[a]	0.148 ± 0.028[a]

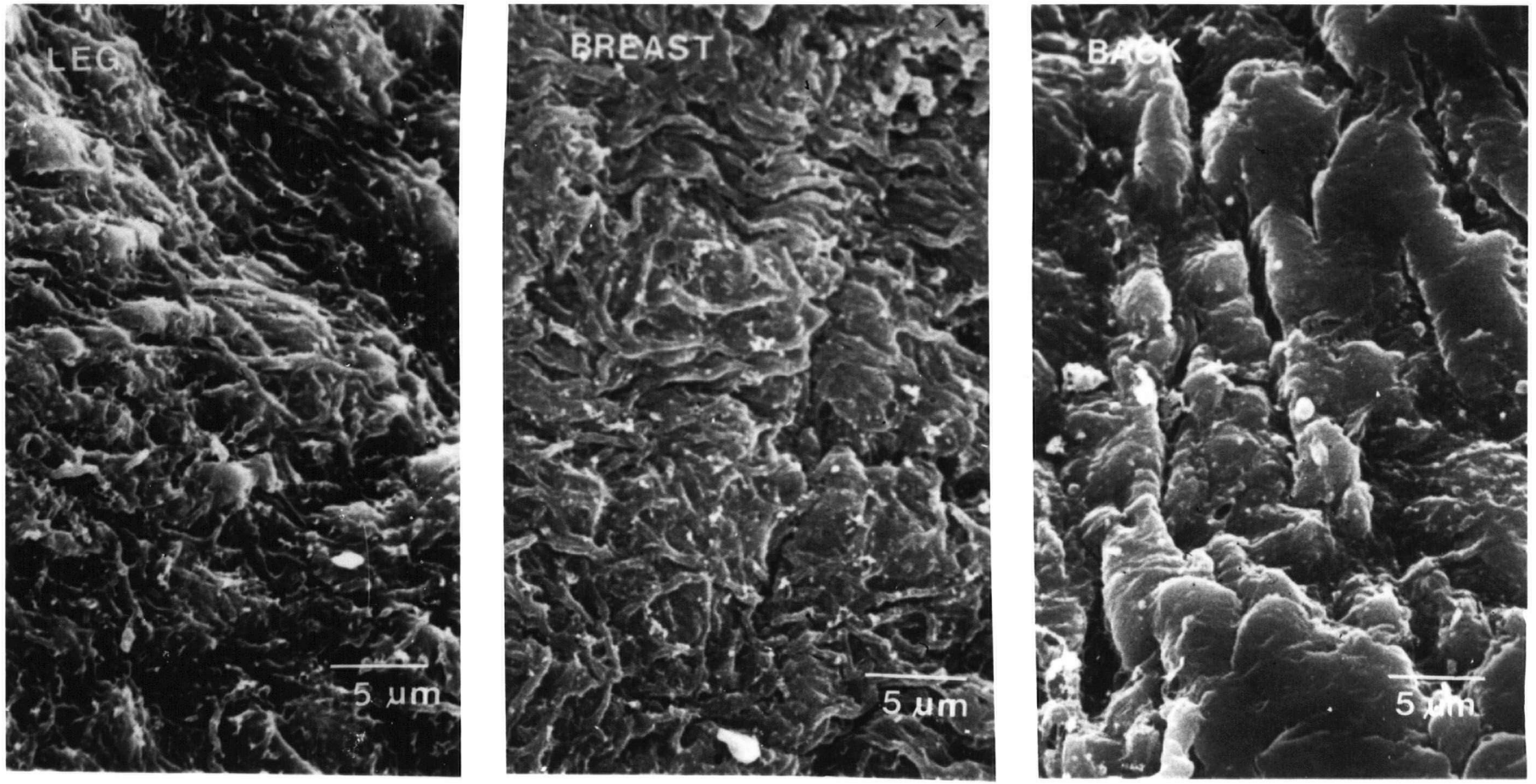
* mean of quintuplicate samples, means bearing the same superscript are not significantly different ($p < 0.01$)

** standard deviation



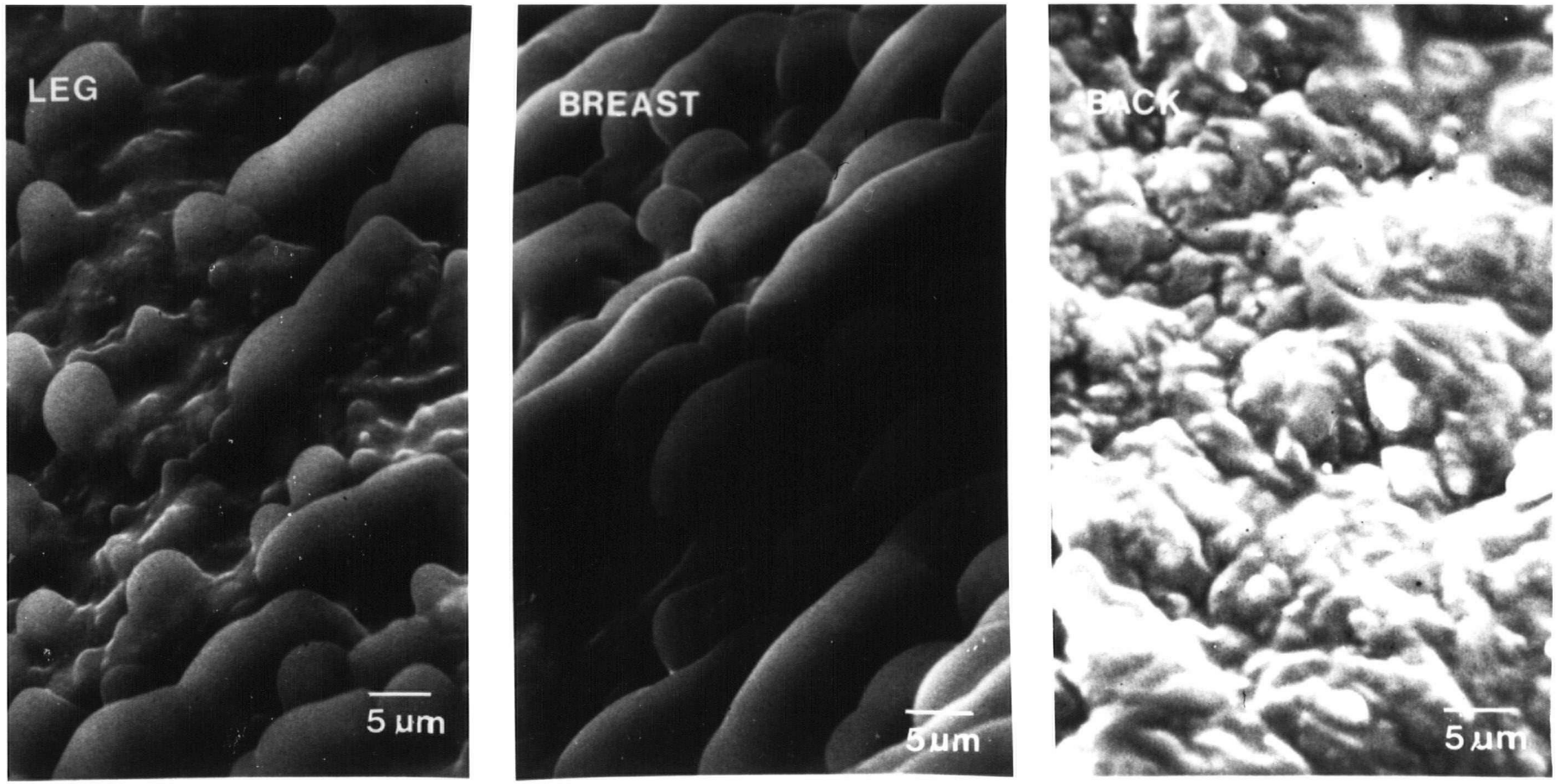
(a) (b)

FIGURE 22: SEM micrographs of (a) Type I and (b) Type II skin obtained from chicken backs.



(a) (b) (c)

FIGURE 23: SEM micrographs of Type I skin obtained from (a) chicken legs, (b) chicken breasts and (c) chicken backs.



(a) (b) (c)

FIGURE 24: SEM micrographs of Type II skin obtained from (a) chicken legs, (b) chicken breasts and (c) chicken backs.

55% after chilling. This high moisture content may be due to water uptake during processing (Mulder and Veerkamp, 1974; Notermans and Kampelmacher, 1975a). Type II skin absorbs very little water during processing. The moisture content of Type II is significantly ($p < 0.01$) lower than that of Type I skin.

There are no significant differences ($p > 0.01$) in protein or carbohydrate content between Type I and Type II skin. There is considerable difference ($p < 0.01$) in fat content between the two skin types. In Type I skin, there is little change in fat content during processing. Type II skin has approximately double the fat content of Type I skin after the chilling operation. Before scalding, Type II skin contains 35% fat, but after chilling, the fat content is 52% (Table II). Since Type II skin is located around the feather tracts, subcutaneous fat may be mobilized through the skin via pores and feather follicles during scalding, producing the higher fat content observed after processing.

Further moisture and fat analysis were carried out on two groups of five chickens obtained from Site C. The results, shown in Table III, display the same trend as that seen in Table II. The fat content of Type II chicken skin is approximately twice that of Type I chicken skin.

C. Fatty Acid Composition

The fatty acid composition of both types of chicken skin was determined to ascertain whether the differences in fat content were

TABLE III: Moisture and fat cantent of Type I and Type II chicken skin after immersion chilling. Trial 1 and 2.

Trial	Skin Type	Moisture, %	Fat, %
1	I	58.39* ± 6.54**a	27.13 ± 6.54[a]
	II	35.32 ± 2.28[b]	53.06 ± 3.91[b]
2	I	52.91 ± 10.32[a]	22.79 ± 5.21[a]
	II	31.86 ± 5.58[b]	59.67 ± 1.20[b]

* mean of quintuplicate samples, means bearing the same superscript are not significantly different ($p < 0.01$).

** standard deviation

just a difference in total amount of fat, or if there was a difference in the lipids themselves. Chickens were obtained from Site A and Site C. Samples of Types I and II skin were taken from the leg, breast and back.

The fatty acid profiles (Figures 25, 26 and 27) show that Type I and Type II skin display essentially no difference in fatty acid composition. The fatty acid compositions of leg, breast and back skin are also essentially the same.

The fatty acid composition (Tables IV and V) indicate that the major component is $C_{18:1}$ (oleate) at 46%, followed by $C_{14:0}$ (palmitate) at 28%. The amounts of short chain fatty acids ($C_{<14}$) are approximately equal, and are therefore combined into one value. These results are in agreement with the work by Pereira *et al.* (1976) on chicken tissue fat. They reported 38% of the fat was oleic acid and 28% was palmitic acid. They found that processing effects on the fatty acid composition of chicken fats was low, but dietary factors were found to be significant. Since this research involved chicken skin and not chicken tissue, the discrepancies are to be expected.

Since proximate analysis (Table II) suggested the mobilization of fat through Type II skin during processing, it was anticipated that lipids of Type II skin would have lower melting temperatures. Since short chain fatty acids and unsaturated fatty acids have lower melting temperatures (Babayan, 1974) than long chain saturated fatty acids, it was thought that Type II skin would

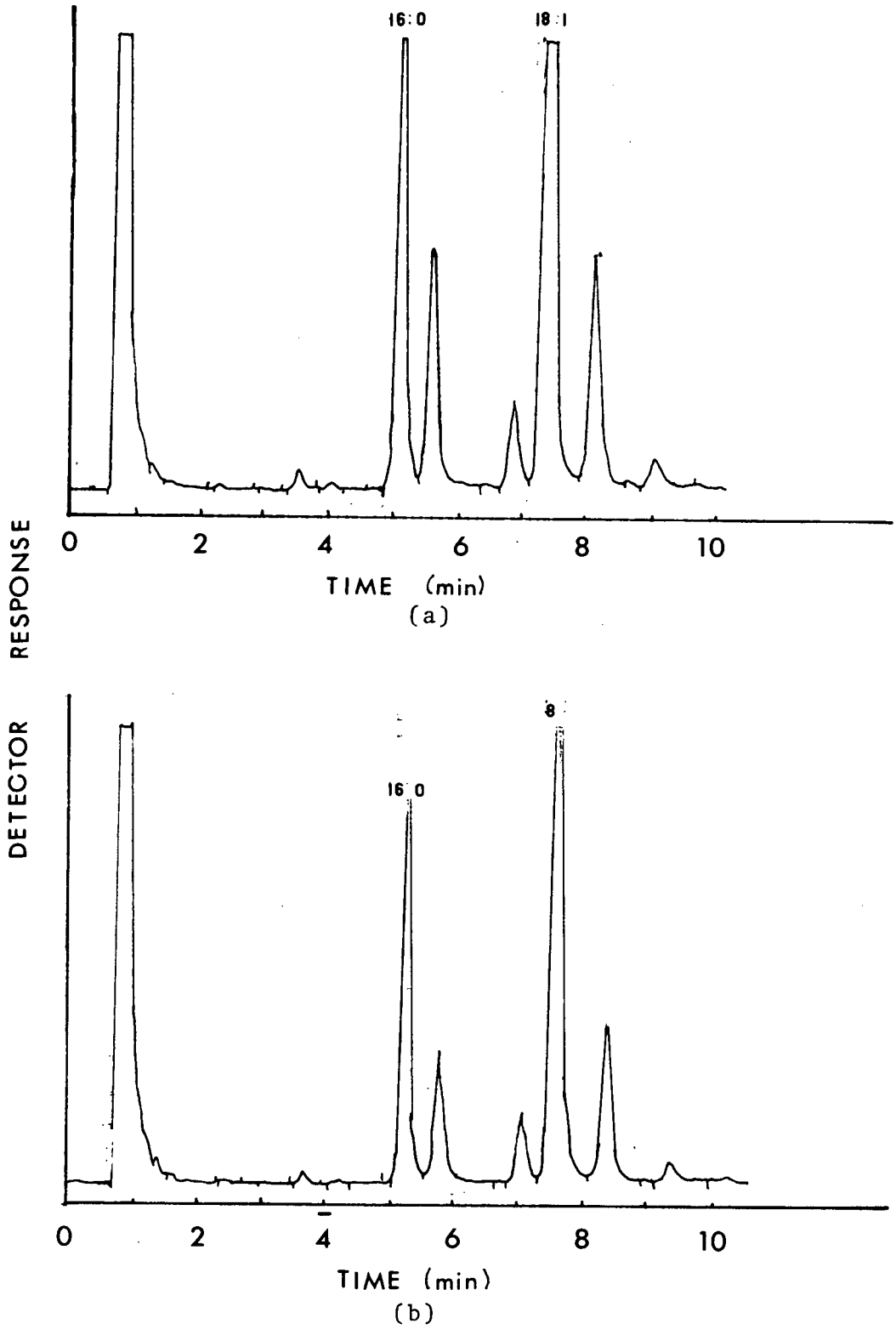


FIGURE 25: Fatty acid profile of (a) Type I and (b) Type II skin from chicken legs.

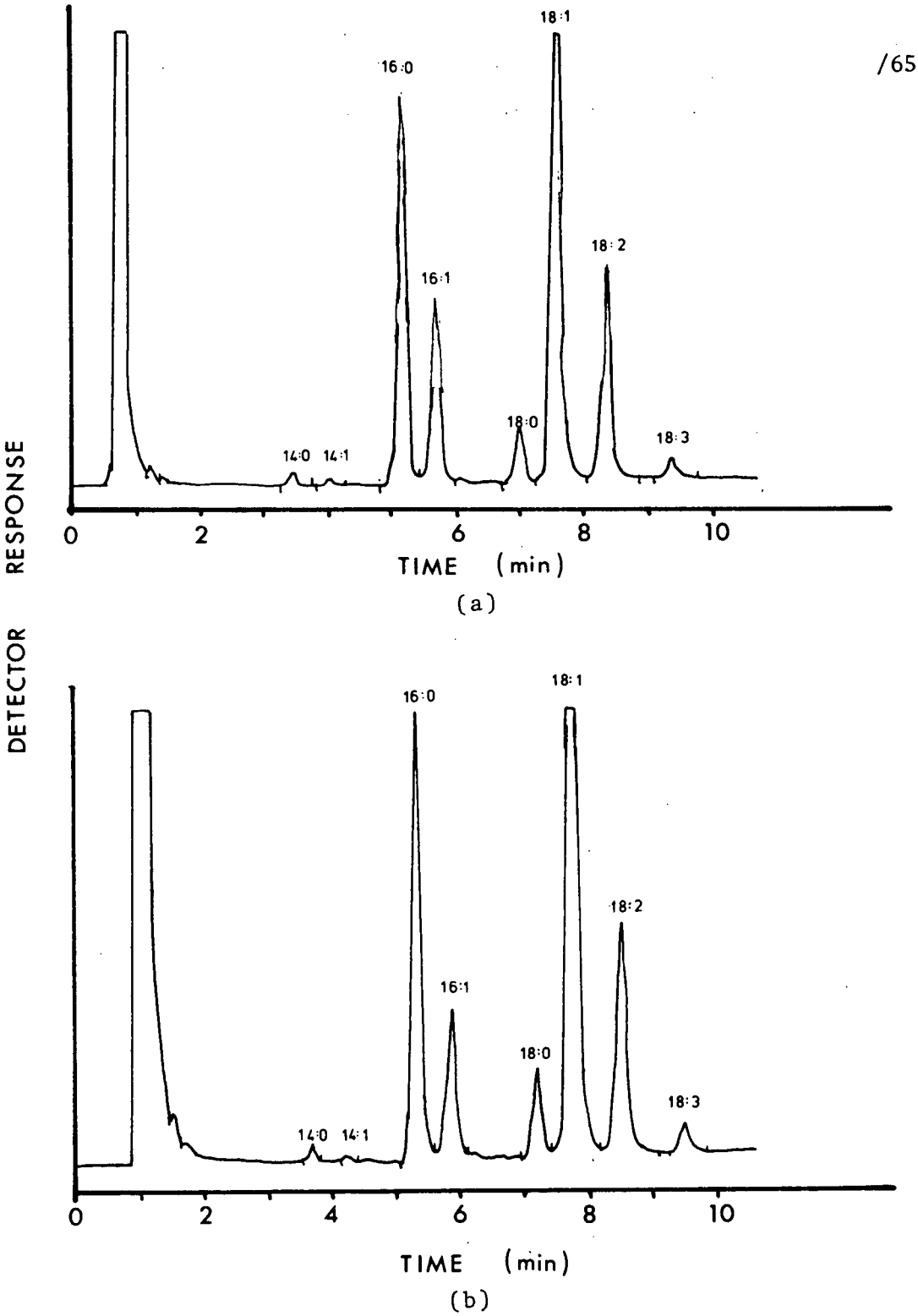


FIGURE 26: Fatty acid profile of (a) Type I and (b) Type II skin from chicken breasts.

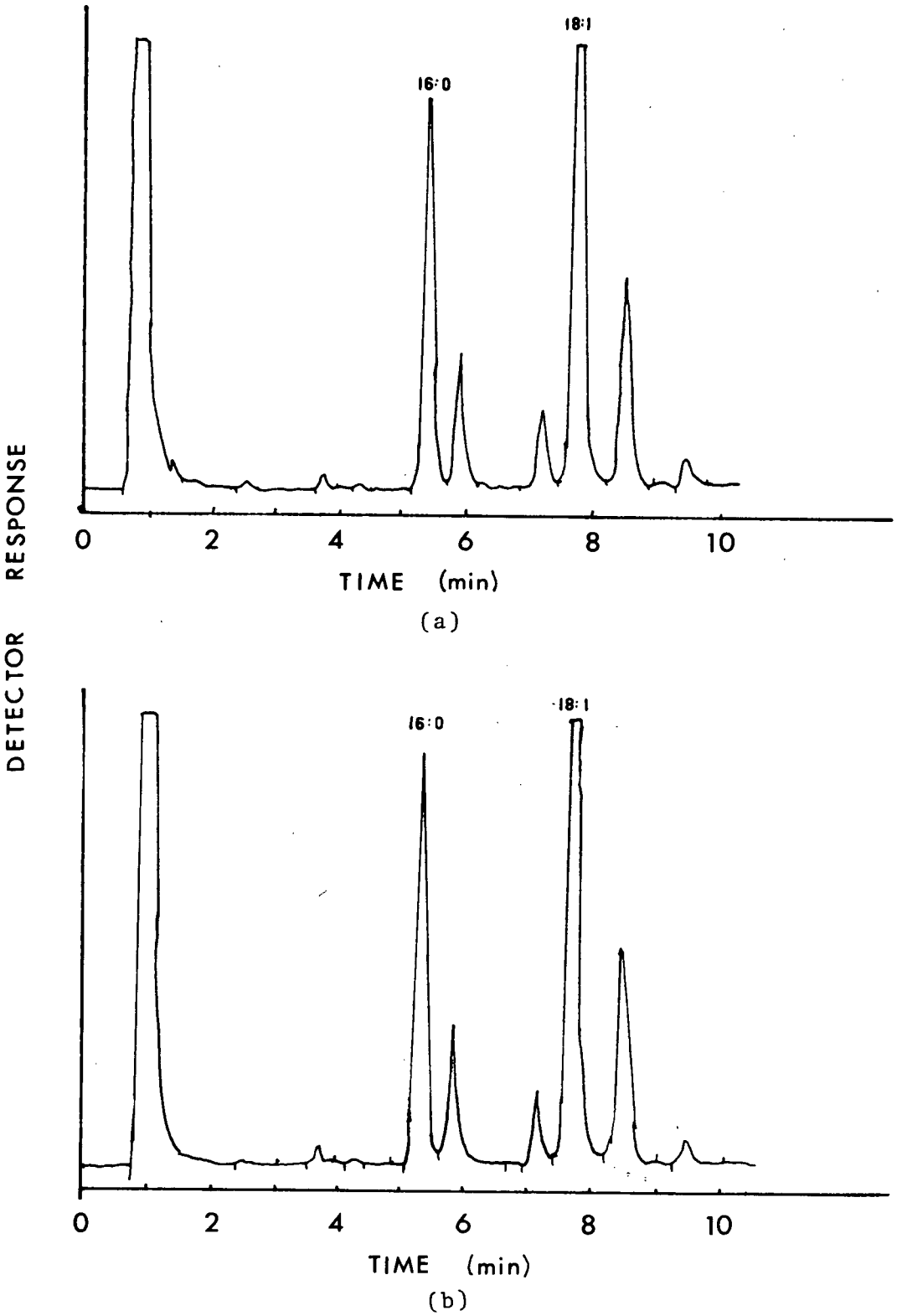


FIGURE 27: Fatty acid profile of (a) Type I and (b) Type II skin from chicken backs.

TABLE IV: Fatty acid composition of Type I and Type II chicken skin before scalding (Site A).*

Location	Skin Type	Fatty acid composition, %								
		$C_{<14}$	$C_{14:0}$	$C_{14:1}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Leg	I	0.173**a	0.706b	0.304a	22.09c	10.14a	4.54a	45.09c	14.14c	2.00d
	II	0.087	0.710	0.272	23.16	9.52	4.56	45.23	14.46	1.98
Breast	I	0.302	0.831	0.314	23.14	10.86	4.00	44.19	14.06	1.85
	II	0.429	0.770	0.303	22.55	11.00	4.07	45.05	14.32	2.01
Back	I	0.177	0.727	0.262	23.70	9.59	4.85	44.86	13.97	1.91
	II	0.091	0.738	0.245	22.95	10.43	4.68	45.51	14.06	1.86

* means of quintuplicate samples done in duplicate, row totals may not add up to exactly 100%, since the values are means.

** coefficients of variation for means in the same column range from a) 0.049-0.081; b)0.016-0.054; c) 0.009- 0.029 and d) 0.010-0.058

TABLE V: Fatty acid composition of Type I and Type II chicken skin after immersion chilling (Site C).

Location	Skin Type	Fatty acid composition, %								
		$C_{<14}$	$C_{14:0}$	$C_{14:1}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Leg	I	0.282**a	0.735[a]	0.275[a]	22.33[b]	9.79[c]	4.52[a]	46.60[d]	13.68[c]	2.88[c]
	II	0.040	0.609	0.264	22.91	10.26	4.39	46.80	12.86	1.84
Breast	I	0.297	0.707	0.269	22.78	10.99	4.22	45.76	13.19	1.80
	II	0.109	0.624	0.265	22.43	11.27	4.17	46.34	12.99	1.81
Back	I	0.159	0.897	0.276	23.10	10.38	4.39	45.77	13.03	1.86
	II	0.105	0.637	0.267	23.03	10.24	4.48	46.35	13.05	1.78

* means of quintuplicate samples done in duplicate, row totals may not add up to exactly 100% since the values are means

** coefficients of variation for means in the same column range from a) 0.022-0.083; b) 0.018-0.072; c) 0.063-0.101 and d) 0.0019- 0.031

have a higher proportion of short chain fatty acid and unsaturated fatty acids. This was not indicated in the fatty acid profile (Tables IV and V). Other considerations such as glyceride composition and skin permeability may be determinant factors.

D. Microbiological Sampling

Due to the large number of dilutions and samples required in some of the later experiments, the feasibility of using the drop plate technique (ICMSF, 1978) was evaluated. It saves time in both preparation of plates and in the actual plating. Six plates can be replaced by one plate having three sectors (Figure 8). Trials were conducted in order to determine whether the modified drop plate technique was reproducible, since 0.1 ml pipettes calibrated to 0.01 ml were used rather than special capillary tubes calibrated to 0.02 ml.

The drop plate technique was found to give reproducible results which compare favorably with those obtained by a more conventional method of plating (Table VI).

E. Bacterial Load

The bacterial load present on chicken legs at the processing plant was evaluated. Chicken legs were taken after defeathering (Site B) and after immersion chilling (Site C). They were examined for viable bacteria present on both Type I and Type II skin (Figures 28, 29).

TABLE VI: Comparison of the drop plate technique to a more conventional method of plating.

	concentration of *S. typhimurium* culture (cfu/cm^2)	
Trial	Standard method	Drop plate technique
1	$2.6^{a} \pm 0.2 \times 10^{8}$ [b]	$2.6 \pm 0.2 \times 10^{8}$
2	$4.7 \pm 0.1 \times 10^{5}$	$4.7 \pm 0.1 \times 10^{5}$
3	$7.5 \pm 0.1 \times 10^{3}$	$7.5 \pm 0.1 \times 10^{3}$

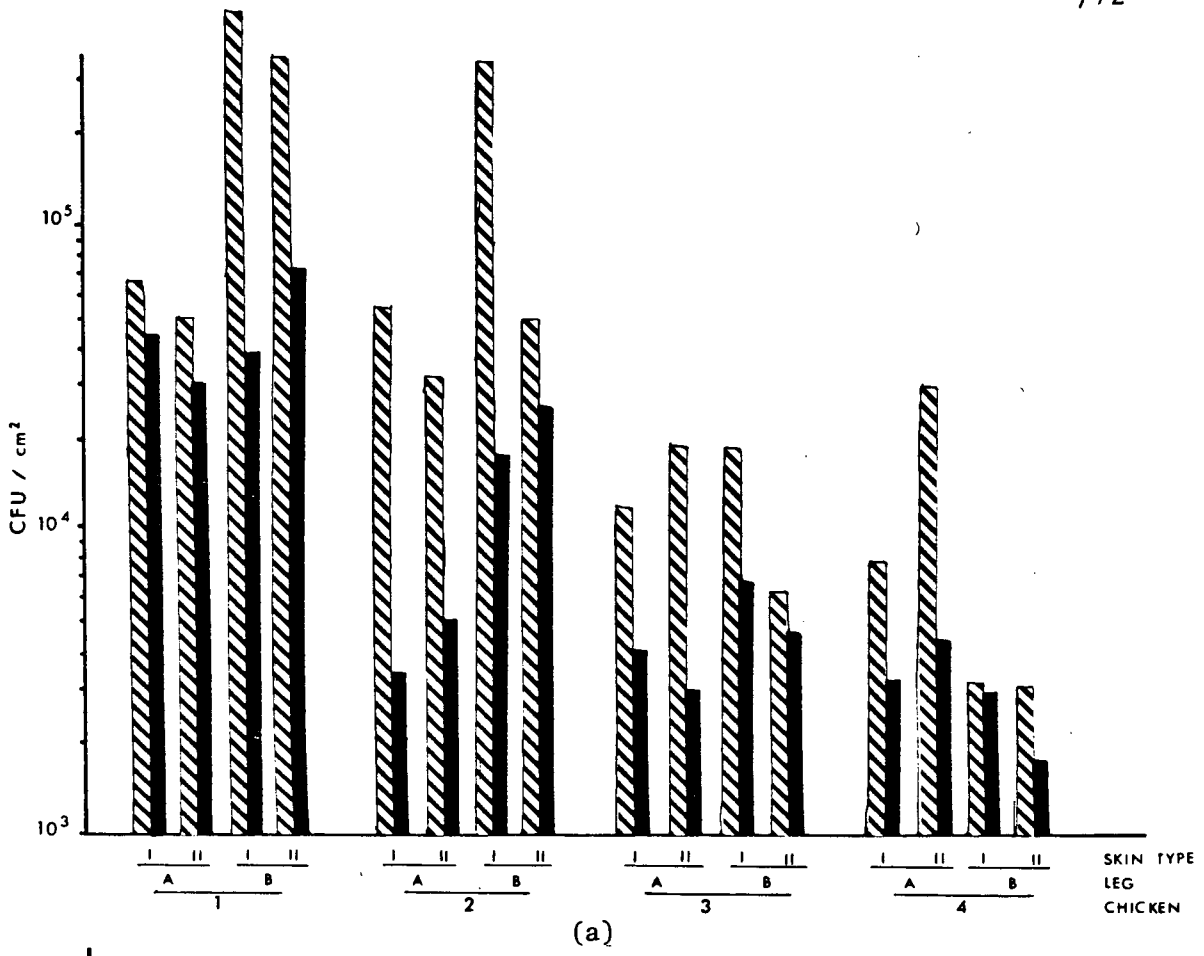
[a] mean of triplicate samples plated in duplicate

[b] standard deviation

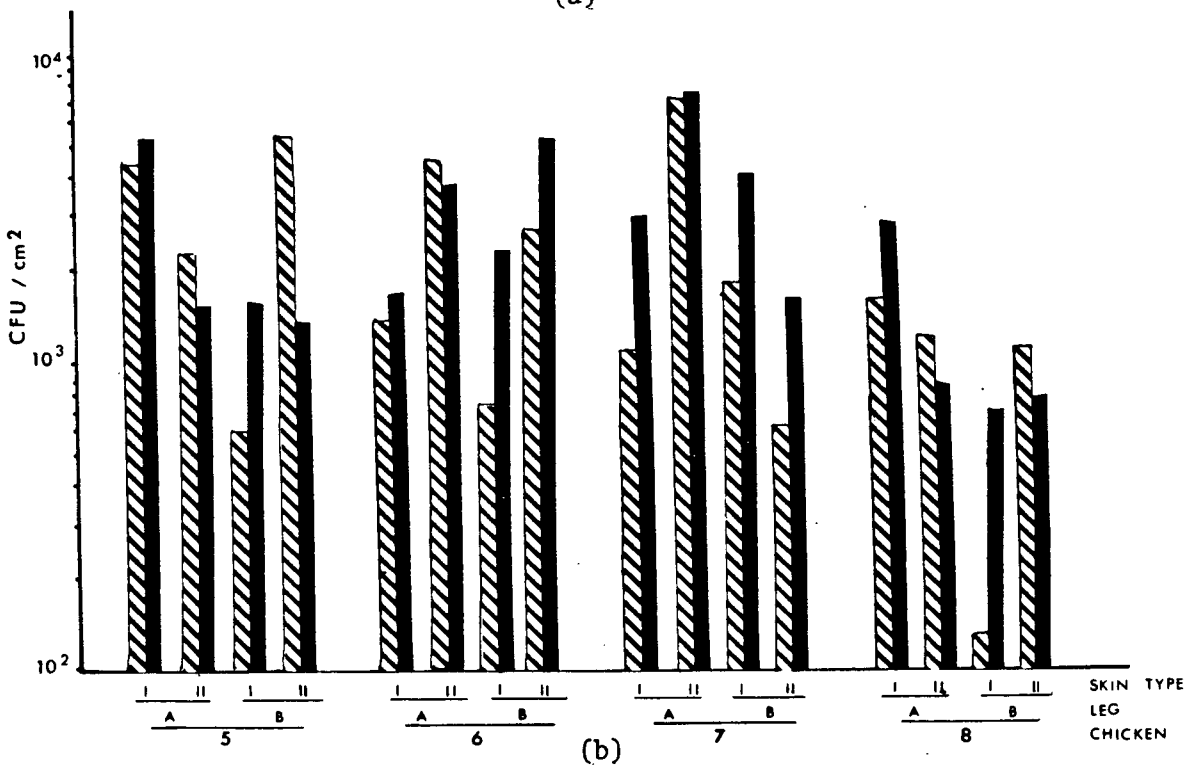
FIGURE 28: Bacterial load on chicken carcasses measured (a) after defeathering and (b) after immersion chilling.

Trial 1.

Aerobic (▬) and psychrotrophic (▨) bacteria were measured in duplicate.


CFU / cm²
10⁵
10⁴
10³
I II I II
A B
1
2
3
4
SKIN TYPE
LEG
CHICKEN

(a)

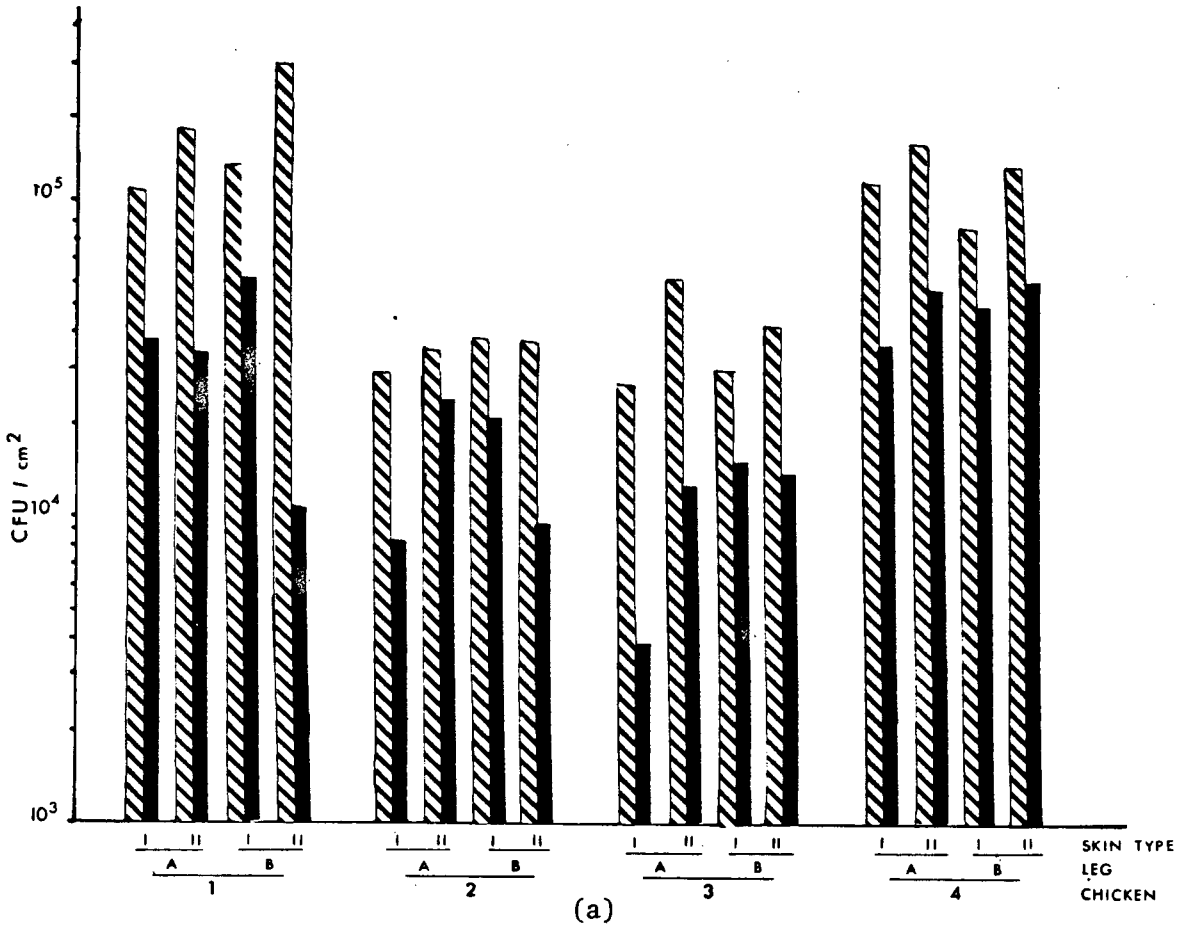

CFU / cm²
10⁴
10³
10²
I II I II
A B
5
6
7
8
SKIN TYPE
LEG
CHICKEN

(b)

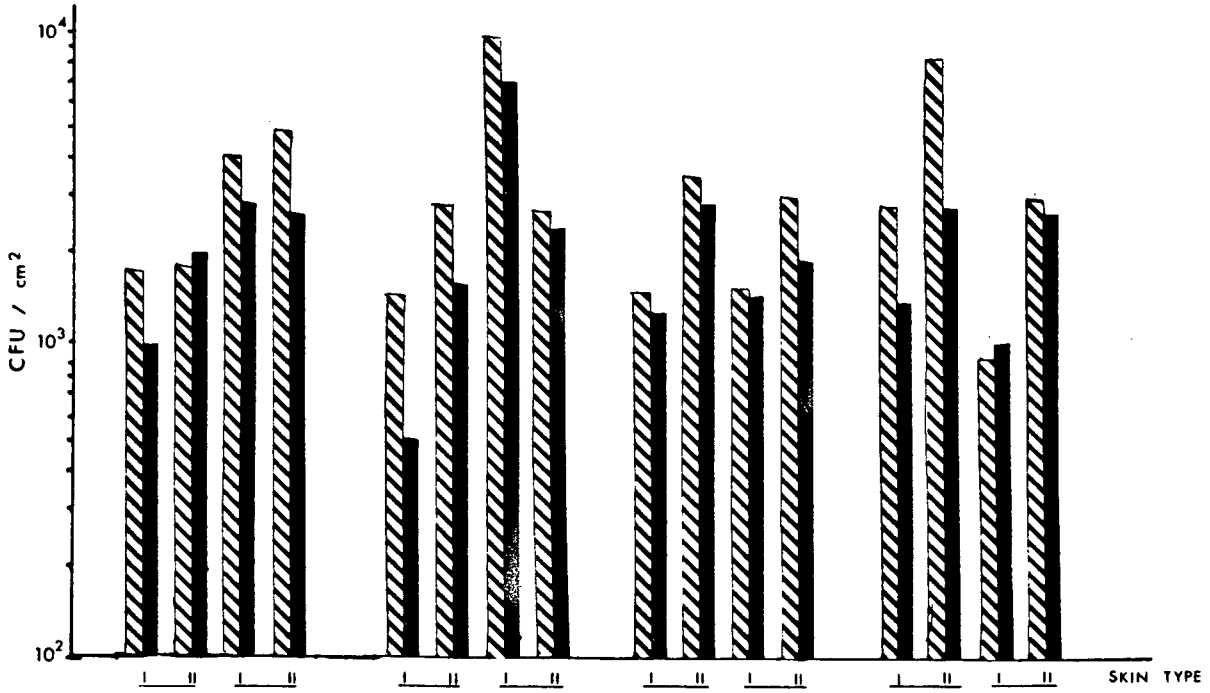
FIGURE 29: Bacterial load on chicken carcasses measured (a) after defeathering and (b) after immersion chilling.

Trial 2.

Aerobic (▬) and psychrotrophic (▨) bacteria were measured in duplicate.


CFU / cm²
10⁵
10⁴
10³
I II I II
A B
1
2
3
4
SKIN TYPE
LEG
CHICKEN

(a)


CFU / cm²
10⁴
10³
10²
I II I II
A B
5
6
7
8
SKIN TYPE
LEG
CHICKEN

(b)

There is considerable variation in the bacterial population between individual chickens as well as between legs from the same chicken. There are also differences in bacterial counts on Type I and Type II chicken skin from the same leg. In general, Type II skin has a higher bacterial load than does Type I skin.

The bacterial population seems to decrease ten fold between defeathering and the chill tank. The washing action of the chillers presumably reduced the number of coliforms, *E. coli*, *S. aureus*, and *Salmonella* and lowered the aerobic plate count (Surkiewicz *et al.*, 1969).

Psychrotrophic bacteria represent a greater proportion of the total bacterial population after chilling (Site C) than after defeathering (Site B). This is probably due to cross-contamination at the chill tank (Surkiewicz *et al.*, 1969).

Previous studies (Barnes, 1960; Mead and Impey, 1970; Mead and Thomas, 1973b; van Schothorst *et al.*, 1972; Notermans *et al.*, 1973 and McBride *et al.*, 1980) have demonstrated that the numbers of bacteria on carcass surfaces vary considerably at different stages of processing and both increases and decreases in numbers of bacteria may occur.

F. Attachment Studies

1. Bacteriological analysis

Inoculation of chicken legs was carried out by immersion in an attachment medium containing approximately 10^8 *Salmonella*

typhimurium/mL. After 15 min immersion in the attachment medium, the legs were removed and allowed to hang for the desired length of time. At each sampling period, two legs were removed for each test procedure..

The control samples, which were dipped in sterile attachment buffer, showed bacterial counts between 0 and 600 cell forming units (cfu)/cm^2. This indicates the extreme variability of the autochthonous population on chicken leg skin. This, in itself, leads to problems in interpreting the data.

Enumeration of the inoculated samples at time 0 (Figures 30 and 31) shows attachment in the range of 10^4 to 10^5 cfu/cm^2 for Type I skin and 10^5 to 10^6 cfu/cm^2 for Type II skin. As expected, this is lower than the concentration of *Salmonella typhimurium* in the attachment medium. Similar results have been reported by Notermans and Kampelmacher (1974) and McMeekin and Thomas (1978).

These bacteria are firmly attached almost immediately and cannot be easily removed by washing. Notermans and Kampelmacher (1975a) proposed the existence of a *"water film"* on the surface of chicken skin. Bacteria are present in this water film before physical attachment occurs. These bacteria were thought to be easily removable by replacing the water film with fresh uncontaminated water. Their results showed that a three minute wash removed approximately $2x10^3$ organisms per gram, regardless of the number of bacteria present in or on the skin. The

FIGURE 30; Bacterial population on inoculated, washed and surfactant treated chicken legs. Trial 1.

Inoculated (● , ○), washed (■ , □), and surfactant treated (▲ , △). Closed symbols indicate geometric means of two samples, each done in duplicate. Open symbols indicate range. Control samples had values between 0 and 965 cfu/cm^2.

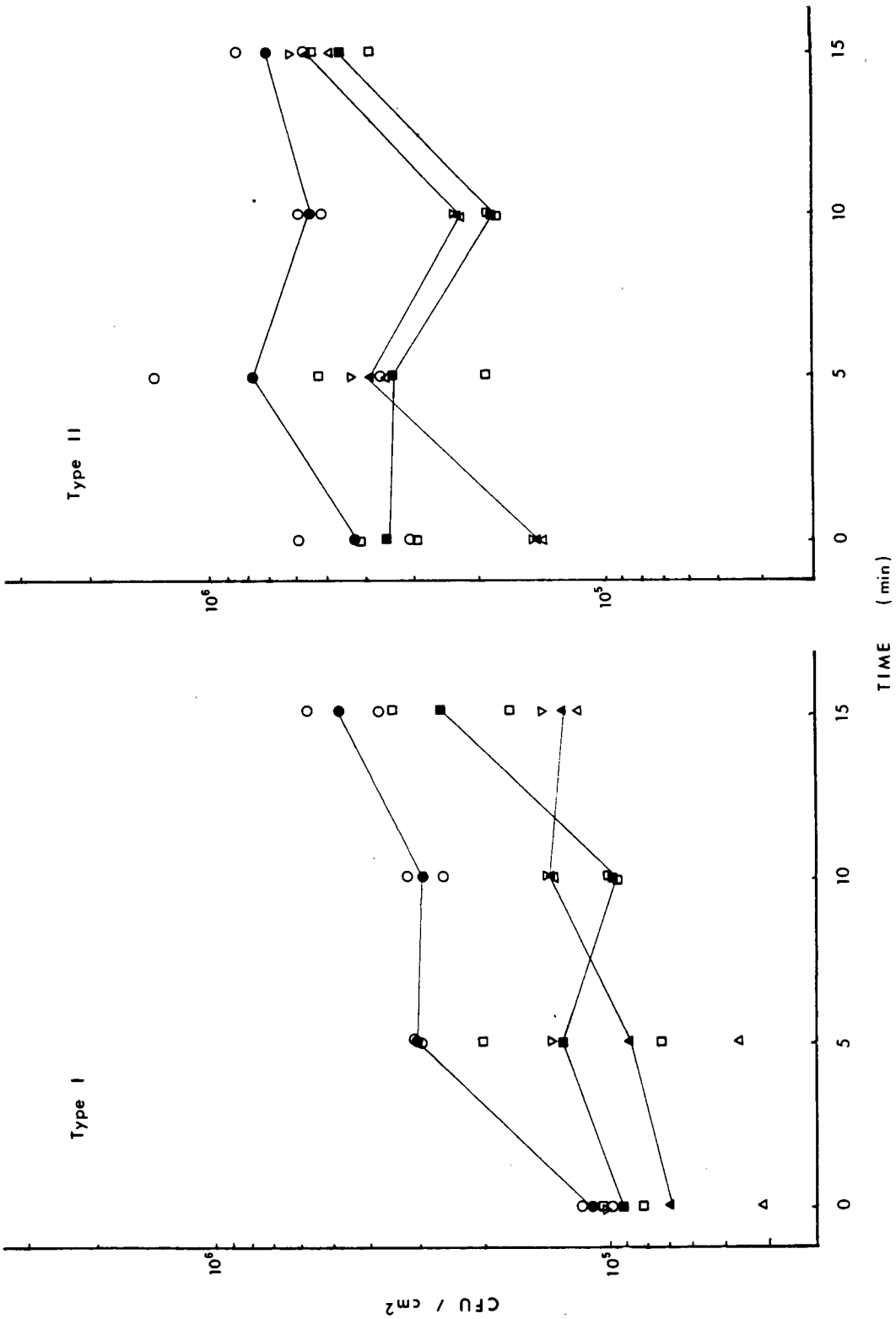
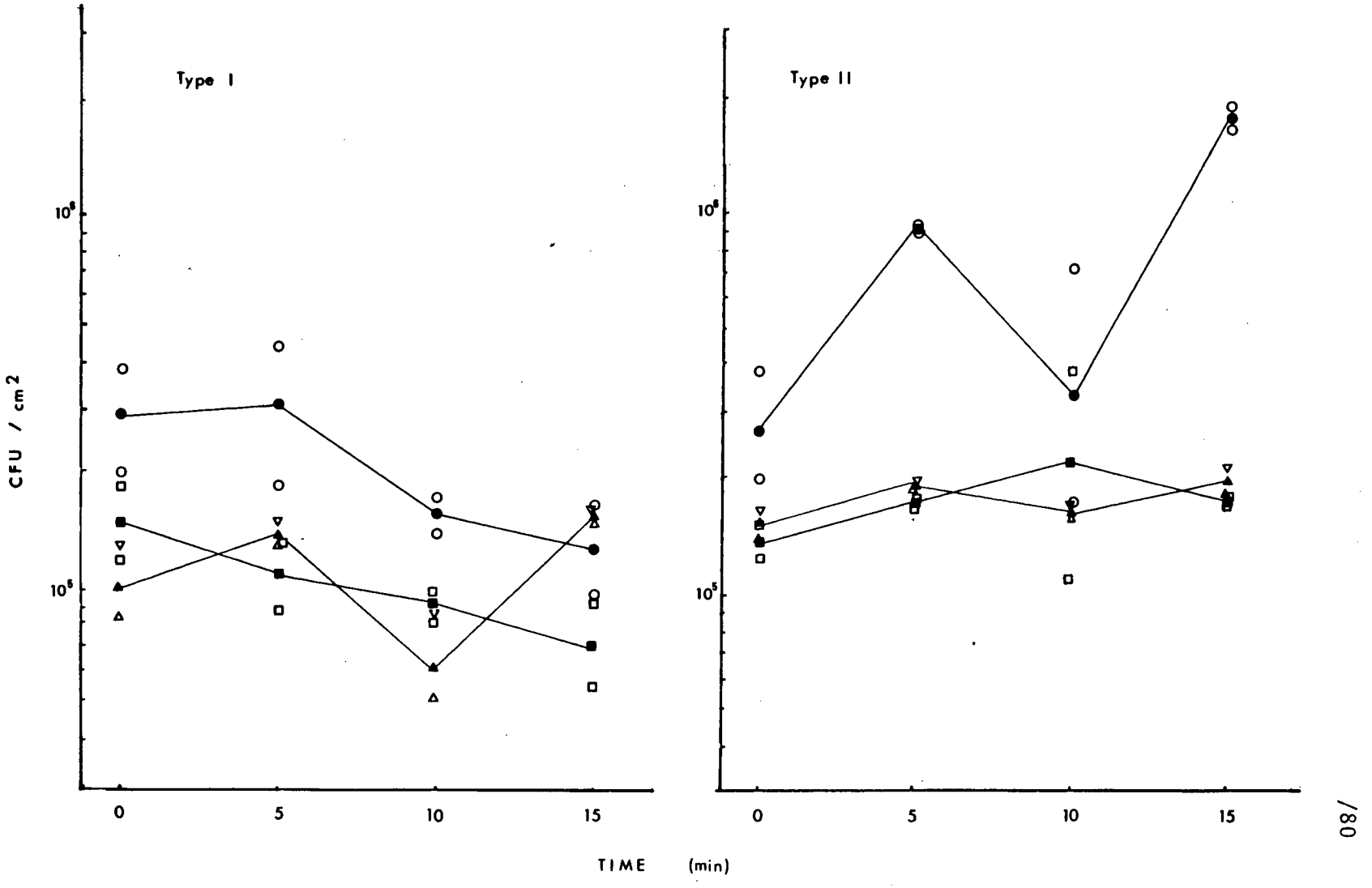

Type I
Type II
CFU / cm²
TIME (min)
10⁶
10⁵
0
5
10
15

FIGURE 31: Bacterial population on inoculated, washed and surfactant treated chicken legs. Trial 2.

Inoculated (●, ○), washed (■, □) and surfactant treated (▲, △). Closed symbols indicate geometric means of two samples, each done in duplicate. Open symbols indicate range. Control samples had values between 0 and 579 cfu/cm2.



/80

remainder of the bacteria were considered *"attached"*. The population of attached bacteria could be decreased by a maximum of one log unit with either immersion chilling or spray cooling. Notermans and Kampelmacher (1975a) believe that the bacterial flora in the water film is of great importance with regard to the number of bacteria in or on the skin, since the water film appears to play a key role as far as the *"attachment"* of bacteria to the skin is concerned.

If Notermans and Kampelmacher's hypothesis was to hold true, one would expect a dramatic decrease in the bacterial counts on the samples washed in buffer containing surfactant. The surfactant would act to reduce the surface tension, and the bacteria present in the water layer should be easily washed away. The data presented in Figures 30 and 31, however, does not support this theory.

Statistical analysis (Tables VII, VIII, IX) indicates that the two trials are not exact replicates. This is probably due to the different chickens used in each trial, differing cell densities in each of the two trials, and a host of other factors. Earlier data showed that the autochthonous population on chickens varies greatly.

Trial 1 (Table VII) indicates that treatment, time, skin type, and their interactions are significant ($p < 0.01$). The reason for the large F-value for treatments is the large difference between the counts for the control and the other treatments.

TABLE VII: Analysis of variance for the attachment study. Trial 1.

Source	DF	Sum Sq	F-Value	Prob
Treatment	3	427.65	1222.8	0.694E-16
Time	3	1.86	5.20	0.227E-02
Skin type	1	11.43	95.81	0.805E-15
Treatment x Time	9	5.76	5.36	0.623E-05
Treatment x Skin type	3	2.78	7.76	0.108E-03
Time x Skin type	3	2.36	6.61	0.417E-03
Treatment x Time x Skin type	9	3.51	3.27	0.166E-02
Error	96	11.45		
TOTAL	127	476.81		

TABLE VIII: Analysis of variance for the attachment study. Trial 2.

Source	DF	Sum Sq	F-Value	Prob
Treatment	3	173.52	166.39	0.139E-15
Time	3	0.90	0.86	0.462
Skin type	1	6.07	17.42	0.641E-04
Treatment x Time	9	8.11	2.59	0.101E-01
Treatment x Skin type	3	1.90	1.83	0.148
Time x Skin type	3	1.22	1.17	0.326
Treatment x Time x Skin type	9	3.84	1.23	0.287
Error	96	33.37		
TOTAL	127	228.94		

TABLE IX: Analysis of variance for the combined trials of the attachment study.

Source	DF	Sum Sq	F-Value	Prob
Trial	1	2.95	9.539	0.229E-02
Treatment	3	556.66	600.03	0.422E-70
Time	3	4.76	5.131	0.194E-02
Skin type	1	15.52	50.20	0.252E-10
Trial x Treatment	3	24.95	26.892	0.157E-13
Trial x Time	3	11.12	1.206	0.309
Trial x Skin type	1	0.05	0.171	0.680
Treatment x Time	9	9.64	3.463	0.548E-03
Treatment x Skin type	3	2.11	2.277	0.809E-01
Time x Skin type	3	0.74	0.794	0.498
Trial x Treatment x Time	9	9.33	3.351	0.773E-03
Trial x Treatment x Skin type	3	0.42	0.545	0.715
Trial x Time x Skin type	3	0.88	0.951	0.417
Treatment x Time x Skin type	9	1.41	0.506	0.870
Error	201	62.16		
TOTAL	255	692.69		

Trial 2 (Table VIII) indicates that only treatment and skin type are significant ($p < 0.01$). Using Neuman-Keuls' multiple range test, the treatments can be divided into three groups. The first includes the control samples; the second, inoculated samples; and the third, both washed and surfactant treated samples. Skin types are significantly different ($p < 0.01$), with Type II having more bacteria attached.

When the data from both trials are combined (Table IX), it is evident that the trials are significantly different, as are treatment, time, and skin type. Several interactions are also important. These are the trial-treatment interaction, treatment-time interaction, and the combined interaction between trial-treatment-time.

Since the sample size is small, and the bacterial counts may not represent a normally-distributed population, the null hypothesis that the parameters studied are the same, may be incorrect. Therefore these results cannot be taken as conclusive. However, in both of the trials, treatment with wash water, with and without the inclusion of a surfactant show no significant difference. In addition, Type II skin had significantly higher bacterial counts than did Type I skin.

In order to examine the data and separate out the interaction effects, it is possible to estimate the main effects. In Tables X, XI, XII, the interactions are examined, and the

TABLE X: Estimates of main effect and interaction means for trial and treatment from the analysis of variance.

	Interaction means (cfu/cm^2)				Trial means
Trial	Control	Inoculated	Washed	Surfactant	
1	$1.42x10^1$	$4.36x10^5$	$2.05x10^5$	$1.73x10^5$	$2.17x10^4$
2	$5.12x10^2$	$3.23x10^5$	$1.63x10^5$	$1.40x10^5$	$4.14x10^4$
Treatment means	$8.52x10^1$	$3.75x10^5$	$1.72x10^5$	$1.55x10^5$	$3.09x10^{4*}$

* grand mean

TABLE XI: Estimates of main effect and interaction means for time and treatment from the analysis of variance.

	Interaction means (cfu/cm^2)				Time means
Time	Control	Inoculated	Washed	Surfactant	
0	$3.08x10^1$	$2.78x10^5$	$2.39x10^5$	$1.13x10^5$	$2.19x10^4$
5	$1.64x10^2$	$5.82x10^5$	$1.68x10^5$	$1.69x10^5$	$4.05x10^4$
10	$2.27x10^2$	$3.05x10^5$	$1.37x10^5$	$1.37x10^5$	$3.34x10^4$
15	$4.60x10^1$	$4.03x10^5$	$2.10x10^5$	$2.14x10^5$	$2.98x10^4$
Treatment means	$8.52x10^1$	$3.75x10^5$	$1.84x10^5$	$1.55x10^5$	$3.09x10^{4*}$

* grand mean

TABLE XII: Estimates of main effect and interaction means for treatment and skin type from the analysis of variance.

Skin type	Interaction means (cfu/cm^2)				Skin type means
	Control	Inoculated	Washed	Surfactant	
I	$2.47x10^1$	$2.39x10^5$	$1.21x10^5$	$1.06x10^5$	$1.66x10^4$
II	$2.93x10^2$	$5.90x10^5$	$2.79x10^5$	$2.28x10^5$	$5.76x10^4$
Treatment means	$8.52x10^1$	$3.75x10^5$	$1.84x10^5$	$1.55x10^5$	$3.09x10^{4*}$

* grand mean

estimated means due to each factor are presented. The control samples have the greatest deviation from the overall mean. Inoculation raises the bacterial counts to 3.72×10^5 from the control level of 8.5×10^1. Washing with water decreases the bacterial numbers to 1.72×10^5 and the inclusion of Tween 80 further reduces the bacterial counts to 1.55×10^5. There is no significant difference ($p > 0.05$) between the water and surfactant treatments, however.

There is also a difference between Trial 1 and Trial 2. The average counts in Trial 2 are higher (4.14×10^4) than those in Trial 1 (2.17×10^4).

The bacterial counts on Type I skin are 1.65×10^4, whereas on Type II skin they are significantly higher ($p < 0.01$) at 5.75×10^4.

From these results it is evident that the attachment procedure is very complex and is further complicated by the inherent variability of biological systems.

2. *Scanning electron microscopy*

Samples of Type I and Type II skin from each of the treatments were prepared for SEM. Micrographs of skin, from all the treatments, were prepared. Since the micrographs of the controls were similar to those presented earlier, they are not repeated here.

Examination of the micrographs of washed and surfactant treated samples failed to reveal the presence of any bacteria,

even though enumeration on Brilliant green agar yielded bacterial numbers in the range of 10^4 to 10^5 cfu/cm^2 (Figures 30, 31). On a microscopic level, 10^5 cfu/cm^2 translates to 0.7 cfu/700 μm^2 assuming that the bacteria are evenly distributed. 700 μm^2 corresponds roughly to the skin surface area represented in Figure 32.

McMeekin *et al.* (1979), using SEM to study the attachment of microorganisms to chicken skin, found a ten-fold discrepancy between the counts obtained on nutrient agar and those calculated from the density of microorganisms on a micrograph. This could be due to the formation of a scum during fixation in glutaraldehyde. This scum is likely to have been unfixed lipid material or material washed from the surface of the skin by the fixative which presumably contains many organisms.

Micrographs from the inoculated skin samples (Figures 32, 33, 34) show the presence of bacteria on the surface of Type I and Type II chicken skin. The crevices and channels (arrow, Figure 32) in the surface of chicken skin, are in most cases, larger than the bacteria, thus favoring physical entrapment. Once trapped, the bacteria could secrete the exopolysaccarides necessary for attachment.

These observations may explain in part, the difficulties in decreasing bacterial populations on chicken carcasses at the processing plant (McMeekin *et al.*, 1979). It may also explain

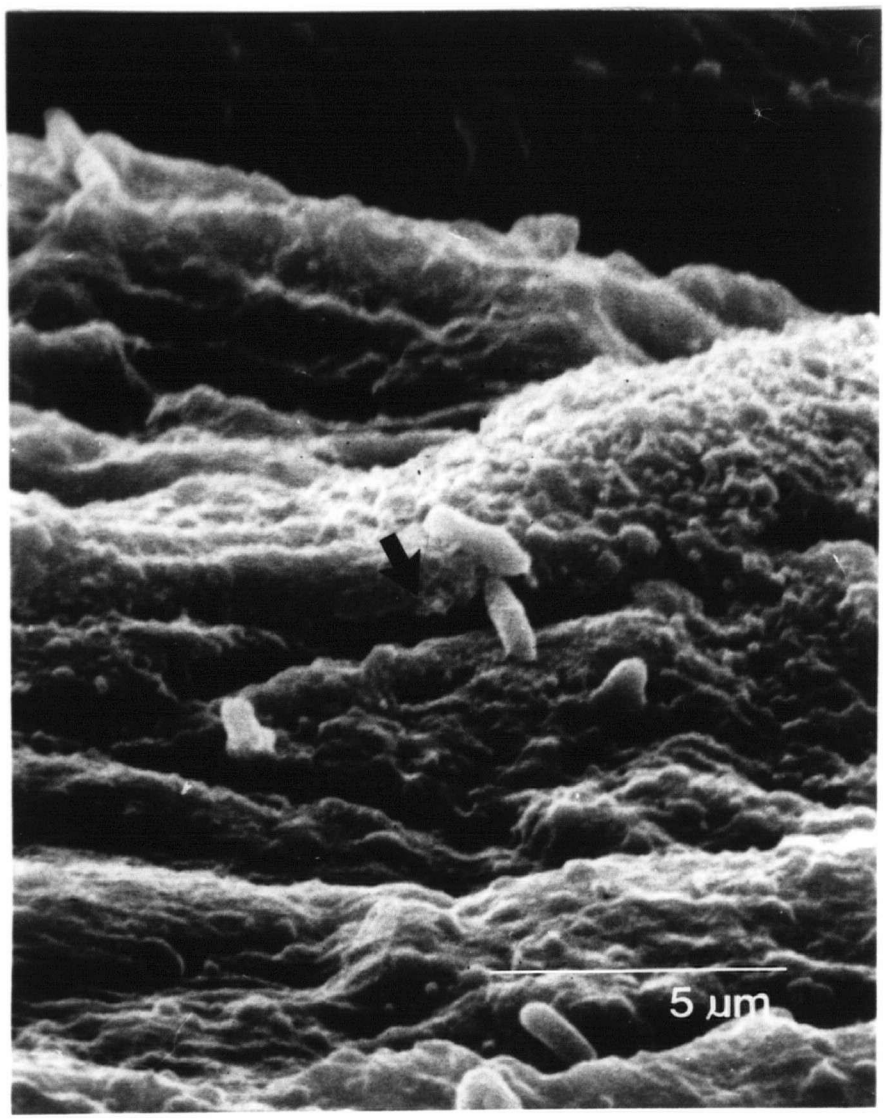


FIGURE 32: SEM micrograph of inoculated Type I chicken skin. Crevices and channels (arrow) are apparent on the skin surface.

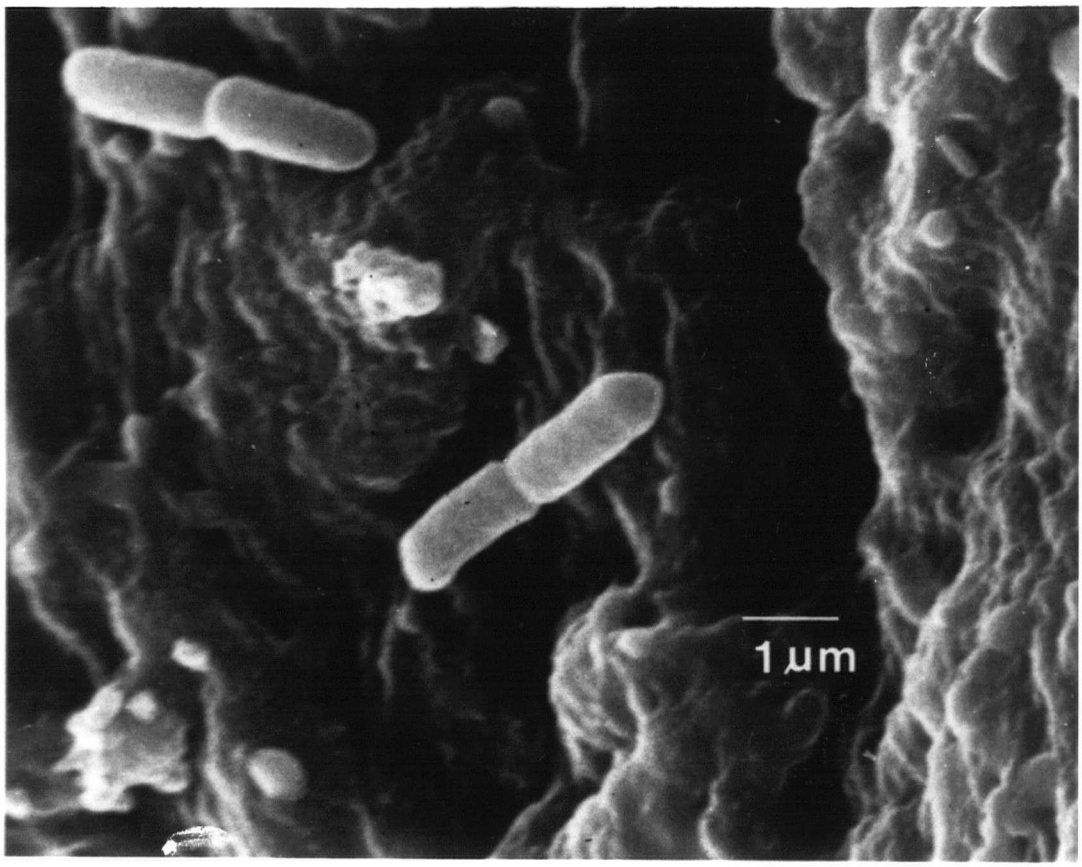


FIGURE 33: SEM micrograph of inoculated Type I chicken skin.

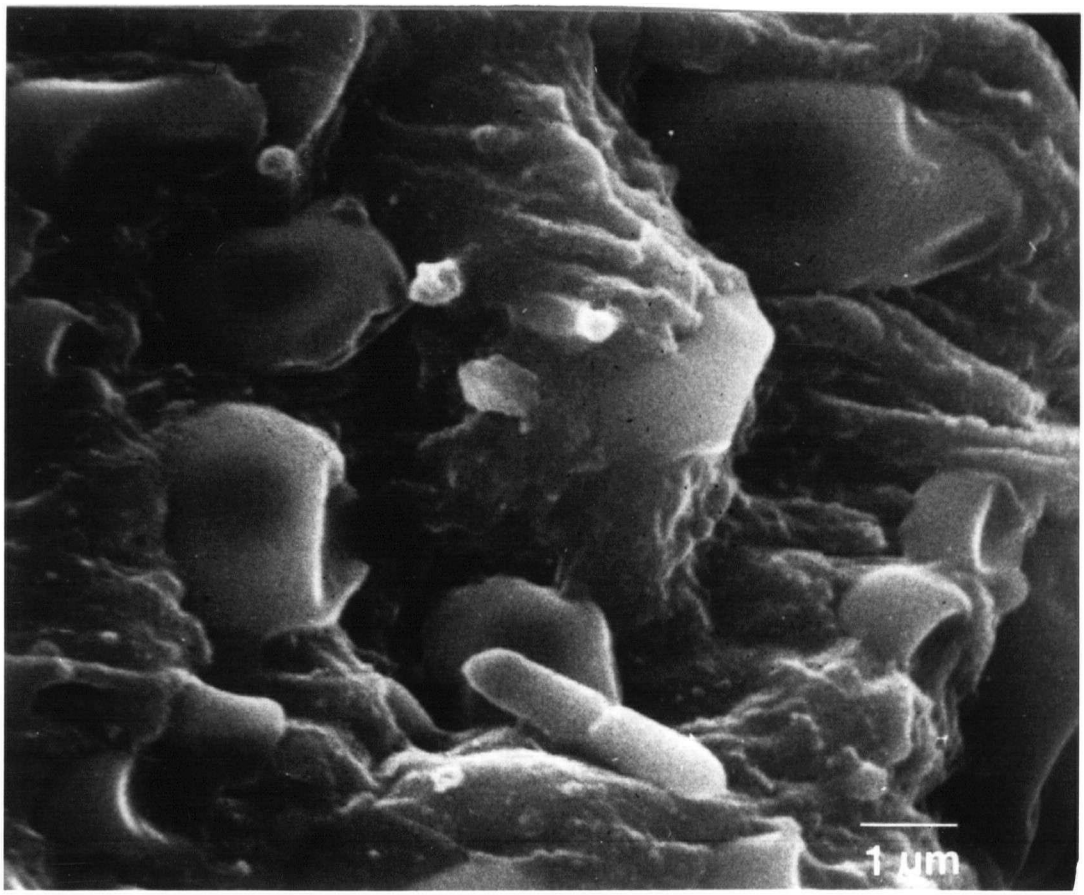


FIGURE 34: Sem micrograph of inoculated Type II chicken skin.

the observation that viable counts from macerated samples of skin are always greater than those obtained by swabbing or rinsing the skin (Avens and Miller, 1970; Patterson, 1971; Notermans *et al.*, 1975a).

GENERAL DISCUSSION

Several methods of fixing chicken skin for examination in the SEM were examined. The standard chemical method using fixation with 6.3% buffered glutaraldehyde at 4°C, and post fixation with osmium tetroxide was compared to the thiocarbohydrazide method, and to freeze-drying. The thiocarbohydrazide method involves fixation of the sample with glutaraldehyde, three post-fixations with osmium tetroxide interspersed with two treatments with thiocarbohydrazide (TCH). This method does not require the use of gold coating for conduction of electrons, since the OsO_4-TCH-OsO_4 bridging renders the tissues conductive. Micrographs of chicken skin fixed with TCH show poor detail and electron charging effects indicating that the TCH method is not suitable for the examination of chicken skin, although McCowan *et al.* (1978) recommended its use in the study of the rumen of cattle.

The third method involves freezing the chicken skin in isopentane cooled in liquid nitrogen. Little structural detail could be observed in the micrographs. The sample surface was obscured by a layer of what may be melted fat. This problem may be overcome if the isopentane was eliminated from the procedure and the sample frozen in liquid nitrogen. However, when skin samples are frozen in liquid nitrogen alone, the skin curls and cracks severely. Other studies in the laboratory showed that in freeze-dried samples of beef, the bacteria were easily lost onto other surfaces, such as tweezers, sides of

beakers, etc., due to electrostatic effects (R. Yada, personal communication). These results are in contrast to those of Suderman and Cunningham (1980) who found freeze-drying to be the method of choice. However, they fixed the chicken skin samples with glutaraldehyde prior to freezing.

The standard chemical fixation yielded micrographs with good clarity and resolution. Some samples, however, still showed some charging effects. This problem is quite common when examining samples with high lipid content (Suderman and Cunningham, 1980).

In addition, chemical dehydration using 2,2-dimethoxypropane was evaluated against the standard ethanol dehydration and amyl acetate infiltration. The rapid chemical dehydration was found to yield good quality micrographs with an appreciable time saving.

SEM indicated that chicken skin does not have a smooth surface, but is filamentous with many cracks and crevices. Other authors have reported similar results using different methods of fixation. McMeekin *et al.* (1979) used fixation above OsO_4 vapors to prepare chicken skin for SEM. Suderman and Cunningham (1980) used freeze-drying as well as several other methods in their study. Light microscopy also yields similar structures.

Scanning electron microscopy revealed the existence of at least two types of skin on the chicken carcass. The first type (Type I) has a filamentous surface structure, whereas the second type (Type II) has a more globular appearance. These two types of

chicken skin can be found at several locations on the chicken carcass. Leg and breast skin are very similar in appearance, but back skin is somewhat less differentiated. This could be due to the differing subcutaneous structures.. Breast and leg skin overlay muscle tissue, whereas little muscle tissue is found under back skin. Back skin is more firmly held to the underlying fat and bone. Type II skin seems to follow the feather tracts on the chicken carcass.

The two skin types differ in chemical composition. Type I chicken skin has approximately 55% moisture and 25% fat, whereas Type II skin has 52% fat and 33% moisture. Processing of chicken carcasses led to a significant increase ($p < 0.01$) in the fat content of Type II chicken skin.

There is no difference in fatty acid composition between the two types of chicken skin. Therefore, the difference between the two skin types may reside in the more complex lipids or lipoproteins.

The identification of the two types of chicken skin may help in the examination of the *"oily bird syndrome"* (OBS). Oily bird syndrome has recently received much attention. It is characterized by *"oily or greasy birds, water pockets under loose skin and broken skin"* (Garrett, 1975). OBS is thought to be caused by several factors such as environmental temperatures, fat deposition due to diet or processing plant stress. OBS appears most frequently in warmer months and almost completely disappears with the onset of cooler weather. Edwards *et al.* (1973) suggested that the degree of

saturation in carcass fat can be influenced by the type of fat in the diet. Energy level alone is not a causative factor in producing OBS since nutrient density could not be related to greasy appearance of a carcass. Female birds exhibit a greater tendency to be oily than males (Garrett, 1975). Females have a higher level of body fat than males of the same age (Edwards *et al.*, 1973). Garrett (1975) and Horvat (1978) were unable to detect significant differences in the fatty acid profile between birds classed as oily or non-oily. Similar observations were made between the fatty acid profiles of Type I and Type II skin.

Fletcher and Thomason (1980) and Jenson *et al.* (1980) investigated the effects of processing conditions on the incidence of OBS. Their results indicated that an increase in scald temperatures increases oily skin scores. This could be due to the mobilization of fat through the skin, onto the surface of the chicken carcass. In addition, Jensen *et al.* (1980) found that an increase in plucking stress increased water sorption and oily, loose and broken skin scores. This could be due to the mechanical action of the plucker, spreading the melted fat over the entire carcass. It would be of interest to examine chickens exhibiting OBS and determine whether they had a greater prevalence of Type II skin or just an elevated fat content.

The bacterial load on Type I and Type II skin was determined after defeathering (Site B) and after chilling (Site C). Type I

skin generally had a lower bacterial load than did Type II skin. However, there was considerable variability in bacterial counts between chickens from the same flock and between legs from each chicken.

The bacterial load decreases approximately ten-fold from Site B to C. Psychrotrophic bacteria form a greater proportion of the bacterial flora at Site C. These results are in agreement with Surkiewicz *et al.* (1969), who found that the aerobic count was lowered by the washing action of the chillers, whereas the psychrotrophic count increased due to cross-contamination in the chill tank.

Attachment studies also showed significantly higher ($p < 0.01$) bacterial counts on Type II skin, compared to Type I skin. The control samples, which were dipped into sterile attachment medium showed bacterial counts between 1 and 600 cfu/cm^2, indicating a wide variability in the autochthonous population on chicken leg skin.

Statistical analysis using Neuman-Keul's multiple range test indicated a wide variability in the autochthonous population on chicken leg skin.

Statistical analyses using Neuman-Keul's multiple range test indicated that there was a significant difference ($p < 0.01$) between inoculated and washed samples; inoculated and surfactant treated samples, but no significant difference ($p > 0.05$) between washed and surfactant treated samples. These results tend to refute Notermans and Kampelmacher's (1974) theory that bacteria are initially present

in a liquid film, since one would expect a dramatic decrease in bacterial counts when surfactant is incorporated in the wash water. Therefore, it would seem that Notermans and Kampelmacher's (1974) suggestion that replacement of the liquid film would reduce bacterial attachment may not adequately describe the situation.

Physical attachment of *Salmonella typhimurium* to chicken skin takes place more rapidly than originally suggested by Notermans and Kampelmacher (1974). These results indicate that simple washing techniques will not be effective in decreasing the *Salmonella* load on *Salmonella*-contamined chicken skin. McBride *et al.* (1980) and Campbell (1979) showed that in most cases, the incidence of *Salmonella*-contaminated carcasses did not decrease markedly after the spin-chilling operation. Incorporation of surface active agents in the chill water would probably not be effective in decreasing the bacterial load on the chicken skin since this study shows that the addition of Tween 80 to rinse water did not produce a significant decrease in the population of *S. typhmurium* on inoculated chicken skin.

The addition of chlorine to the chill-tank acts to kill the bacteria in the chill water (Sanders and Blackshear, 1971, Mead and Thomas, 1973b; Notermans *et al.*, 1973; Mulder and Veerkamp, 1974), and thus prevent cross-contamination. Those bacteria already attached to the skin surface, however, will not be affected. Kotula *et al.* (1967) found no practical advantage in spray-washing with 50 ppm chlorine immediately after chilling.

Results of the SEM study yielded little information on the attachment process. The bacteria were evident on the skin surface, and seemed to fit in the crevices and channels on the chicken skin surface.

CONCLUSIONS

The present study revealed that the standard chemical method of fixation using 6.3% glutaraldehyde and 1% osmium tetroxide, followed by chemical dehydration with 2,2-dimethoxypropane is the method of choice for preparation of chicken skin for scanning electron microscopy (SEM).

SEM revealed that the surface of chicken skin is not smooth. Rather, it is convoluted with many crevices and channels. Two types of chicken skin were discerned. Type I has a filamentous surface, whereas Type II chicken skin has a globular appearance. Skin samples from the leg and breast are similar in appearance but back skin is somewhat coarser.

Proximate analysis showed that the major chemical difference between Type I and Type II skin to be the moisture and fat contents. Type I skin had significantly higher ($p < 0.01$) moisture content and approximately half the fat content of Type II chicken skin. The fatty acid profiles of Type I and II skin were very similar.

Microbiological sampling of chicken carcasses showed that the bacterial load decreased approximately ten-fold between the scalding and the chilling operations. Psychrotrophic bacteria form a greater proportion of the microflora on chicken skin at the chill tank. There is considerable variation in the autochthonous bacterial population between individual chickens.

Attachment studies revealed that the physical attachment of bacteria occurs rapidly and these bacteria cannot be easily removed by washing with water, or with water containing a surfactant. Thus, it is important to decrease *Salmonella* contamination in poultry flocks since, once attachment occurs, it is difficult to remove the *Salmonella* from the poultry carcasses during processing.

Further chemical characterization of the chicken skin, particularly the complex lipids and glycoproteins, may be of interest to determine why bacteria have a greater affinity for Type II chicken skin than for Type I skin.

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