APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1980, p. 133-144
0099-2240/80/07-0133/12$02.00/0

Vol. 40, No. 1

Contamination of Broiler Carcass Skin During Commercial Processing Procedures: an Electron Microscopic Study

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Scanning and transmission electron microscopy were used in conjunction with normal microbiological cultural techniques to examine some aspects of contamination of broiler carcass skin by bacteria during processing. The autochthonous skin microflora of poultry, before processing, was mainly *Micrococcus* spp. which were located in accumulations of sebum-like substances on the surface of the stratum corneum. During scalding and plucking, the skin epidermis was removed, and exposed dermal tissue was contaminated by microorganisms from the mechanical plucker and subsequent stages of processing. Major sources of psychrotrophic contamination were the immersion washer and chiller water. Microbial contaminants were found within a fluid film on the skin surface and inside deep skin channels. Skin microtopography and the presence of the liquid film were implicated as major factors controlling contamination during processing.

During processing of poultry carcasses, microbial contamination inevitably occurs as a consequence of the processing procedures employed. At each stage of the process, ample opportunity exists for contamination of the carcass by microorganisms from the processing plant or by cross-contamination from other birds. Numbers of bacteria on carcass surfaces vary considerably at different stages of processing (4, 16, 23), and increases and decreases in numbers have been demonstrated (23, 25, 29, 36, 43).

The mechanism of contamination of various poultry tissues is not well understood. In particular, the relationship between the bacterium and the tissue substrate has been little studied, although this interaction is a major factor controlling contamination. Suggestions about the site and nature of skin contamination have been made on the premise of increased recovery of microorganisms from skin samples by maceration techniques as compared with swabs or rinses (1, 13, 25, 31, 34). However, there is no direct experimental evidence to confirm either the location of contaminant bacteria or the nature of the skin-bacterium interaction. Only Notermans and Kampelmacher (30-32) have provided data to help explain some aspects of the contamination process, although their interpretations and conclusions are a matter of dispute (19).

Initial work published by McMeekin et al. (20) suggested that scanning electron microscopy (SEM) could well provide information about the ecology of food-borne bacteria and emphasized the possible role of the skin microtopography as a determinant of contamination of poultry carcasses during processing. In this study, the course of contamination of poultry carcasses during processing was studied, and the results were related to SEM and transmission electron microscopy (TEM) examinations of skin structural changes caused by various processing procedures.

MATERIALS AND METHODS

Samples. All broiler carcasses used in this study were obtained from a commercial poultry processing plant. Freshly killed and bled poultry with feathers intact, and other carcasses from various stages of processing, were packed in sterile polythene bags and transported under ice to the laboratory. Carcasses which had passed through stages of processing had been subjected to a hard scald treatment (58°C, 2.5 min) before mechanical plucking. Feathers from nonprocessed or scalded, unplucked carcasses were carefully removed by hand from the leg and breast areas at the laboratory.

Origin and isolation of the skin microflora. Pieces of skin (16 cm^2 in area) were aseptically excised from the leg and breast areas of both the hand-plucked and the immersion-chilled carcasses. Each piece was homogenized in 100 ml of saline (0.8% [wt/vol] NaCl) with a model 400 Colworth Stomacher (A. J. Seward and Co. Ltd., London), and 0.1-ml aliquots of appropriate serial decimal dilutions of the homogenates were surface spread on nutrient agar plates. All inoculated plates were incubated aerobically at 22°C for 3 days. Total viable counts per 16 cm^2 of skin were recorded for each sampling site.

Colonies from appropriate dilution plates were isolated and purified on nutrient agar. All strains were maintained on nutrient agar slopes.

Characterization of isolates. Bacteria isolated from skin samples were identified by a modified

scheme based on that proposed by Shewan et al. (39) (Table 1). Twenty-four-hour nutrient agar slope cultures were used to assess Gram reaction and morphology oxidase reaction (15). These cultures were also used to determine the mode of utilization of glucose (11) and production of fluorescent pigment (12). Motility was examined by microscopy of overnight broth cultures (Difco nutrient broth; 0.8%, wt/vol). Flagellum arrangement was determined by electron microscopy: Formvar-coated copper grids were placed on drops of nutrient broth cultures (10 to 30 s), removed, and immediately placed on a drop of 0.5% (wt/vol) aqueous uranyl acetate (10 s), and excess stain was removed with filter paper. The grids were air dried and examined with a Hitachi H-300 transmission electron microscope.

Gram-positive aerobic cocci isolated from the hand-plucked carcasses were further classified by the scheme of Baird-Parker (3). Twenty-four-hour nutrient agar slope cultures were used to assess pigment and catalase production. Mode of utilization of glucose; acid production from arabinose, lactose, maltose, and mannitol; and phosphatase activity were tested with media described by Baird-Parker (2). Acetoin production from glucose-phosphate-peptone-water (glucose, 0.5%, wt/vol; K_2HPO_4, 0.5%, wt/vol; and Oxoid Peptone Bacteriological Code L37, 0.5%, wt/vol) was tested with Barritt's modification (7).

Sources of psychrotrophic microorganisms contaminating carcasses during processing. Pieces of breast skin (16 cm^2 in area) were aseptically excised from nonprocessed carcasses, plucked uneviscerated carcasses, eviscerated carcasses, immersion-cooled carcasses, and immersion-chilled carcasses. Each piece was stomached in 100 ml of saline, and serial decimal dilutions of the homogenate were prepared. Where expected numbers of psychrotrophs were greater than 10^3/16 cm^2 of skin, 0.1-ml aliquots of appropriate dilutions of the homogenates were surface spread on nutrient agar plates. Inoculated plates were incubated aerobically at 4°C for 14 days to determine numbers of psychrotrophic organisms or at 22°C for 3 days to determine the total viable counts. A most probable number technique was used to enumerate numbers of psychrotrophs less than 10^3/16 cm^3 of skin; 10-, 1-, and 0.1-ml portions of the undiluted skin homogenate were used to inoculate a five-tube glucose-tryptone broth most probable number system (Oxoid tryptone, 1%, wt/vol; glucose, 0.5%, wt/vol; plus 5 ml of a 0.4% [wt/vol] aqueous solution of bromociesol purple per liter of broth). All tubes were incubated at 4°C for 14 days and examined for growth and acid production. All total viable counts and psychrotroph counts were expressed as numbers per square centimeter of breast skin.

The total viable counts and numbers of psychrotrophic organisms present in scald water, fresh tap water used for the scald input, immersion cooler, and chiller water and ice were determined by the surface spread technique. The most probable number method was used to enumerate numbers of psychrotrophs less than 10/ml of water. All counts were expressed as numbers per milliliter of water.

Microscopic examination of broiler carcass skin after various stages of processing. Pieces of skin (≃1 cm^2) excised from the breast and outside leg areas of nonprocessed poultry, plucked uneviscerated carcasses, and immersion-chilled broiler carcasses were pinned to dental wax.

Skin pieces to be prepared for examination by SEM were fixed overnight at 4°C in either tetroxide vapor or a glutaraldehyde solution (5% [vol/vol] in 0.1 M sodium phosphate buffer, pH 7.2). Fixed tissue was rinsed in cold phosphate buffer, dehydrated in a graded ethanol series (30 → 50 → 60 → 70 → 80 → 90 → 95 → 100% × 3, with distilled water as the diluent) and sliced into small pieces with a razor blade. These pieces were rinsed in absolute ethanol, infiltrated with amyl acetate (25 → 50 → 75 → 100% × 2, with absolute ethanol as the diluent) and critical point dried with carbon dioxide with a Polaron E-3000 critical point dryer (Polaron Equipment Pty. Ltd., Watford, England). The dried skin pieces were glued on SEM stubs and coated with about 27.0 nm of gold in a Dynavac SC150 sputter coating unit (Dynavac High Vacuum Ltd., Victoria, Australia) and examined in a JEOL JXA 50-A SEM operated with an accelerating voltage of 15 kV. Micrographs were recorded on Polaroid type 52 or 107 Polaplan film.

Skin to be prepared for examination by TEM was fixed as described above, except that skin treated with glutaraldehyde was postfixed at 4°C with 1% (wt/vol) osmium tetroxide in sodium phosphate buffer (0.1 M, pH 7.2). Alcian blue 8GX (1%, wt/vol) was added to glutaraldehyde solutions used to fix some skin specimens. All skin pieces were rinsed in cold phosphate buffer and dehydrated in a graded ethanol series (as described above). Dehydrated tissue was cut into small pieces, rinsed with absolute ethanol followed by two 30-min changes in 1,2-epoxy propane and embedded in Araldite (10) or Spurr medium (40). Silver or gold sections of embedded tissue were cut and placed on Formvar-coated copper electron microscope grids. Mounted sections were stained with 1% (wt/vol) aqueous uranyl acetate followed by lead citrate (38) and examined with either a Phillips EM201 or a Hitachi H-300 TEM operated at 60 or 72 kV, respectively. Micrographs were recorded on Ilford electron microscope film.

RESULTS

Numbers and incidence of different bacteria present on nonprocessed and immersion-chilled broiler carcass skin. The numbers and types of bacteria found on breast and leg skin of nonprocessed and immersion-chilled broiler carcass skin are presented in Table 1. Greater numbers of bacteria were found on both leg and breast skin of nonprocessed carcasses compared with the skin of immersion-chilled carcasses.

Micrococcus spp. were almost the only bacteria isolated from breast and leg skin of nonprocessed poultry carcasses. All strains of *Micrococcus* isolated were assigned to either subgroup 5 or subgroup 6 of Baird-Parker's classification scheme (3). Subgroup 6 predominated on both leg and breast skin. Only one other type of

TABLE 1. *Differentiation of some bacteria isolated from poultry carcasses*[a]

Organism	Gram reaction	Morphology	Motility	Flagella arrangement	Oxygen relations	Metabolism of glucose	Oxidase reaction	Pigment production	Fluorescent pigment
Pseudomonas									
Group I	−	R	+	P	AERO	O	+	−	+
Group II	−	R	+	P	AERO	O	+	−	−
Group III/IV	−	R	+	P	AERO	O	+	V	−
Micrococcus	+	C	−	−	AERO	O	NT	−	−
Enterobacteriaceae	−	R	(+)	PERI	FAC	F	−	−	−
Moraxella/Acinetobacter	−	R	−	−	AERO	O	+/−	−	−
Flavobacterium/Cytophaga	−	R	−	−	AERO	O	+	+	−
Coryneforms	+	R*	(−)	−	V	V	NT	V	−

[a] *, Displaying club shapes, Chinese characters and "V" pairs; R, rod shaped; C, coccus; P, polar; PERI, peritrichous; AERO, aerobic; FAC, facultatively anaerobic; O, oxidative; F, fermentative; V, variable; NT, not tested; (+), most strains positive; (−), most strains negative.

bacterium, an actinomycete, was isolated from nonprocessed poultry skin.

The flora of immersion chilled carcass skin was composed of a variety of types of bacteria (Table 2). Gram-negative *Pseudomonas* spp., *Moraxella/Acinetobacter, Flavobacterium/Cytophaga,* and enteric types were the predominant types present on both leg and breast skin. The remainder of the flora isolated were *Micrococcus* spp. and coryneform bacteria.

Sources of psychrotrophic microorganisms contaminating poultry carcasses during processing. Table 3 shows the incidence of psychrotrophs and related total viable counts present in processing waters and on the skin of broiler carcasses sampled from various stages of processing. In general, although poultry processing procedures brought about a reduction in the total viable flora present on broiler carcass breast skin, a large increase in the number of psychrotrophic contaminants occurred.

Before scalding, small numbers of psychrotrophs were found on breast skin of poultry carcasses ($\simeq$10/cm^2 of skin). However, during plucking and subsequent stages of processing, large increases in numbers of psychrotrophic organisms contaminating carcass surfaces occurred. Scald water and tap water used for plucking, spray washing, and supply of the immersion washer and the immersion chiller contributed only small numbers of psychrotrophic microorganisms (i.e., <10/ml). The major sources of contamination were the immersion washer water, the immersion chiller water, and ice used to cool immersion chiller water. Generally the higher the numbers present in the immersion chiller water, the higher the numbers of psychrotrophs recovered from the skin of broilers.

Microscopic examination of broiler carcass skin at various stages of processing. SEM was used to examine the skin surface of nonprocessed poultry carcasses. The rough and folded surface (Fig. 1a) was covered with thin, flattened skin cells in various stages of exfoliation (Fig. 1b). Parts of the surface of these cells were often covered by dense clumps of particulate matter (Fig. 1c). The size range of individual particulates was 0.5 to 3.0 μm.

Three morphologically different microbial cell types were located on the surface of the corneous layer of skin from nonprocessed carcasses. Low numbers of yeasts and rod-shaped bacteria were found lying on the skin among dust and other

TABLE 2. *Aerobic microbial flora of breast and leg skin from nonprocessed poultry and immersion-chilled carcasses*

Group	% of population			
	Nonprocessed poultry skin		Immersion-chilled carcass skin	
	Breast	Leg	Breast	Leg
Micrococcus				
Subgroup 5	29	9	25	3
Subgroup 6	71	87		
Coryneforms				3
Moraxella/Acinetobacter				74
Flavobacterium/Cytophaga			11	8
Pseudomonas				
Group I			50	
Group II				
Group III/IV			7	5
Enterobacteriaceae			4	8
Yeasts/others		4		
Total no. of isolates	24	23	28	37
Total viable count/16 cm^2 of skin	3.55×10^6	6.25×10^6	2.5×10^4	4.95×10^4

TABLE 3. *Mean psychrotroph and total viable counts of carcass skin and processing waters sampled at various stages of processing*

Sample site	Mean[a] psychrotroph count	Mean[a] total viable count
Nonprocessed poultry skin	9.18/cm^2	2.03×10^4/cm^2
Scald water	0.45/ml	2.40×10^4/ml
Fresh scald input water	8.00/ml	2.14×10^3/ml
Scalded carcass	0.78/cm^2	1.78×10^3/cm^2
Fresh plucker input water	9.60/ml	1.02×10^3/ml
Plucked carcass skin	14.9/cm^2	1.93×10^4/cm^2
Eviscerated carcass skin	31.8/cm^2	9.00×10^3/cm^2
Ice	770/ml	6.80×10^3/ml
Immersion washer water	2,293/ml	4.07×10^5/ml
Washed carcass skin	60.5/cm^2	1.64×10^4/cm^2
Immersion chiller water	6,596/ml	1.97×10^5/ml
Immersion-chilled carcass skin	347/cm^2	4.74×10^4/cm^2

[a] Each value represents the mean of eight separate determinations.

debris (Fig. 1d and e). Gram-positive cocci were regularly found within the clumps of particulate material (Fig. 1c and 3a and b) found on the skin surface, and hence were not often located on specimens examined by SEM. These cocci, because of their regular occurrence, probably correspond to the *Micrococcus* spp. isolated from nonprocessed carcass skin. Microorganisms were not found within feather follicle shafts or within normal skin tissue.

Sections of the material enclosing the gram-positive cocci on the skin surface (Fig. 3a and b) suggest that this material is composed of substances stained by osmium tetroxide, uranyl acetate, or lead citrate as well as compounds soluble in 1,2-epoxy propane (used during the embedding process). Compounds soluble in this solvent are represented as clear unstained zones within the clumps in Fig. 3a and b.

The general histology of the skin of nonprocessed carcasses examined by TEM of thin sections of Araldite-embedded tissue was similar in detail to that already described by Matoltsy (18) and Lucas and Stettenheim (17). Briefly, the skin of poultry may be divided into the epidermal and dermal layers which are separated by a basal membrane (or lamina) attached to the dermis by anchor fibrils (Fig. 2a and b). The epidermis is composed of a superficial corneous layer (stratum corneum) and a deeper germinative layer (stratum germinativum). Germinative cells are all living and produce new cells or become cornified. The corneous layer is composed of highly flattened cells joined mainly at their edges, thus forming thin sheets of lamellae. Corneous cells usually contain only keratin and lipid material. Keratinization is largely completed in the deepest part of the stratum corneum. The dermis, by comparison, is mainly composed of connective tissue. Superficial layers of the dermis are characterized by several plies of orthogonally oriented collagen fibrils.

Comparison of TEMs of thin sections of skin from nonprocessed carcasses and mechanically plucked, uneviscerated carcasses demonstrated that plucking and scalding resulted in the removal of the epidermal skin layer and exposed underlying dermal tissue (cf. Fig. 2a and 4a). However, small epidermal cell fragments attached to intact basal lamina were found on skin from carcasses sampled at all post-plucking stages of processing examined (Fig. 7a and c). These fragments corresponded in size and shape to particulates observed on processed skin examined in the SEM (Fig. 6b). The newly exposed dermal surface was relatively smooth compared to the surface of skin from nonprocessed poultry, but was deeply channelled where the basal lamina conformed to channels and folds in the epidermal surface or dermal intercellular spaces (Fig. 4a and d).

The skin surface of plucked carcasses, sampled before evisceration and after immersion chilling, was covered by a liquid film derived from plucker water or water from the immersion chiller. Organic components present in this film were fixed in situ by treatment of skin samples in osmium tetroxide vapor. Glutaraldehyde-alcian blue solutions also preserved these materials present on skin from carcasses sampled immediately after plucking. These preserved materials were observed as a layer which covered the skin surface of whole specimens examined with the SEM (Fig. 4c and 5a and b), or thin sections of skin examined by TEM (Fig. 4a and b; 7a and b). Most of the layer material was present in folds and channels in the skin, but was also found as a thin film over the remainder of the surface. By contrast, fluid film components were not observed on skin fixed by immersion in glutaraldehyde solutions (Fig. 4d, 6a, and 7c). The organic components of the fluid film were rinsed from the skin before adequate fixation could occur. This rinse effect was also noted for skin from immersion-chilled carcasses treated with glutaraldehyde-alcian blue solutions and some parts of skin from carcasses sampled immediately after plucking. The intensity of stained material in sections of the film on the skin surface of plucked carcasses (sampled after plucking) was greater than that observed for immersion-chilled carcass skin (cf. Fig. 4a and 7a and b).

In some thin sections of skin from carcasses sampled immediately after plucking, lipid droplets and bacteria (Fig. 4b) were found within the layer of preserved fluid film components. How-

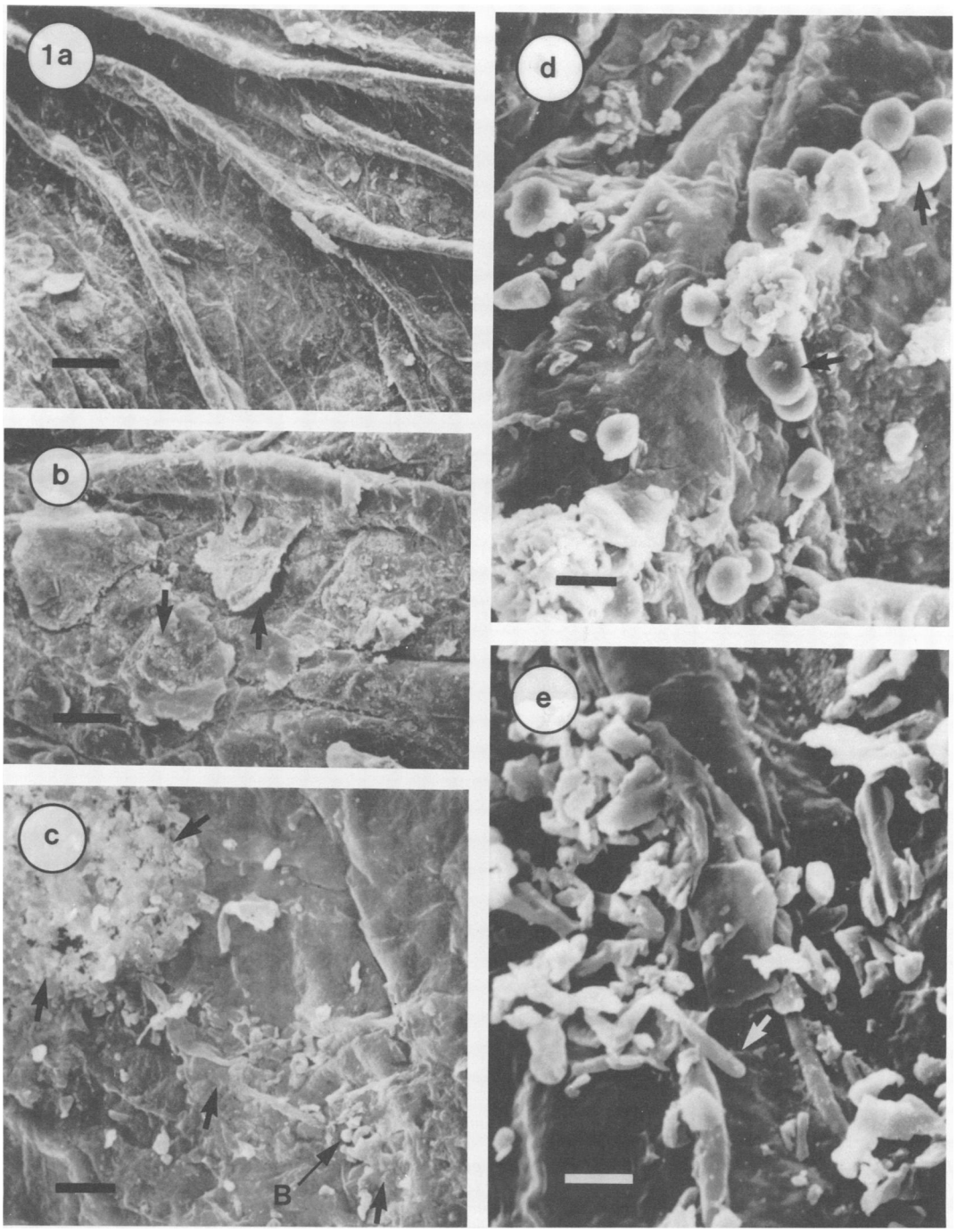

FIG. 1. *Scanning micrographs of the surface of skin of poultry sampled before processing. Specimens fixed in osmium tetroxide vapor. (a) Low-magnification micrograph of the skin surface showing the rough, folded nature of this tissue. Bar = 250 μm. (b) Cells at the skin surface are thin and flattened and commonly in various stages of exfoliation (arrows). Bar = 80 μm. (c) Dense clumps of particulate material (arrows) on the skin surface. Coccoid bacteria (B) were often found associated with these clumps. Bar = 3 μm. (d) Yeast cells (arrows) on the edge of a corneous skin cell. Bar = 10 μm. (e) A rod-shaped bacterium (arrow) among debris on the skin surface. Bar = 3 μm.*

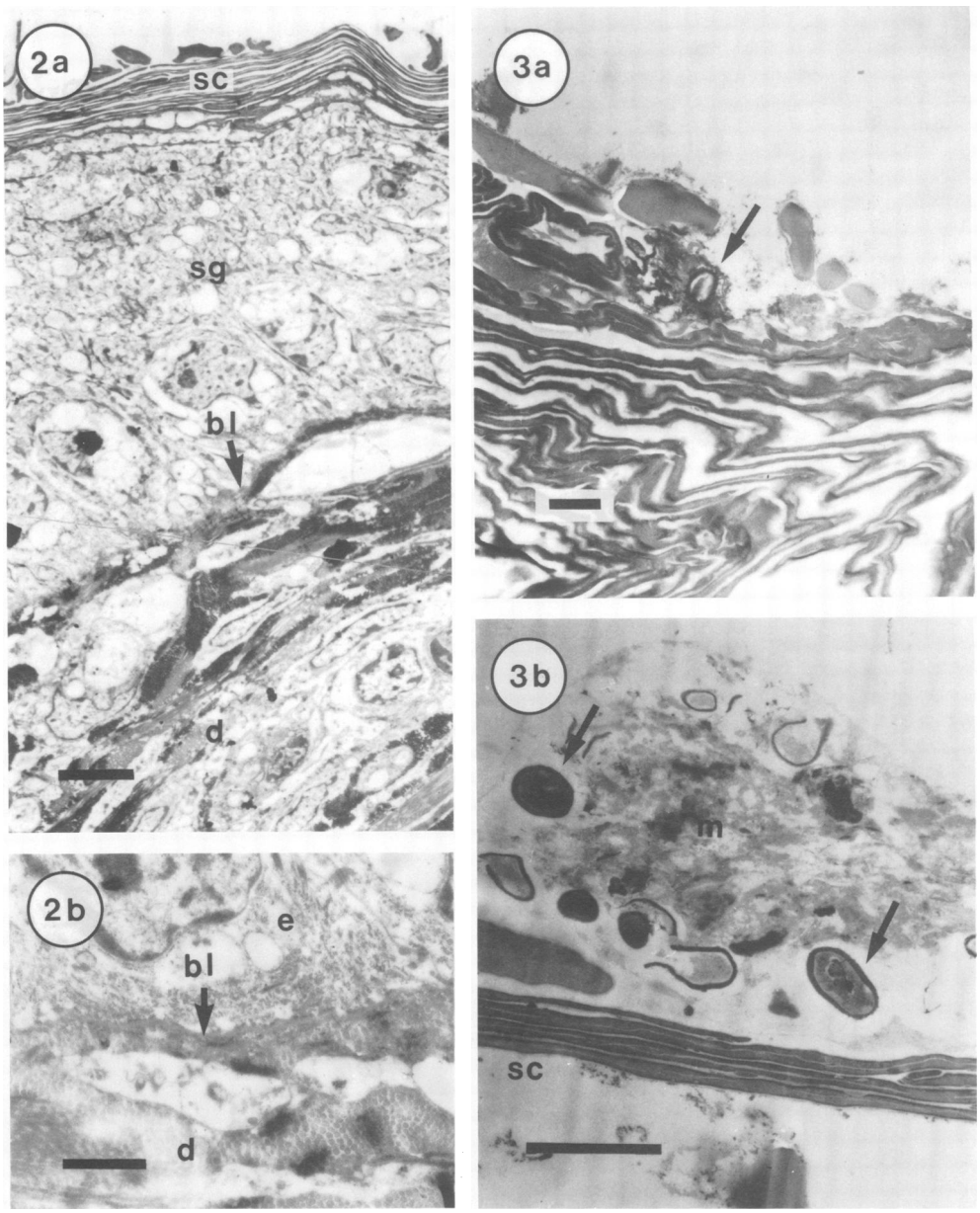

FIG. 2. *Electron micrographs of sectioned skin from poultry sampled before processing. (a) Transverse section through epidermal and dermal tissue. The superficial stratum corneum (Sc) composed of thin, flattened, highly cornified cells, overlays the stratum germinativum (Sg). These layers, which constitute the epidermis, are separated from the dermis by the basal lamina (bl). Dermal tissue (d) is characterized by plies of orthogonally oriented collagen fibrils and is predominantly a layer of connective tissue. Bar = 3 μm. (b) Micrograph of the basal lamina (bl) separating epidermal (e) and dermal (d) tissue. Bar = 1 μm.*

FIG. 3. *Electron micrographs of sectioned skin from unprocessed poultry carcasses, showing the location of skin-borne microorganisms. (a) Gram-positive coccoid bacterium (arrow) associated with particulate matter on the surface of the stratum corneum. Bar = 1 μm. (b) Several gram-positive bacteria (arrows) within a clump of particulate matter on the stratum corneum. Bar = 1 μm.*

ever, bacteria were not observed in any thin sections of the preserved film from immersion-chilled carcasses, although some yeasts were found on the skin surface of specimens examined in the SEM. Bacteria within preserved film materials could not be examined by SEM, and

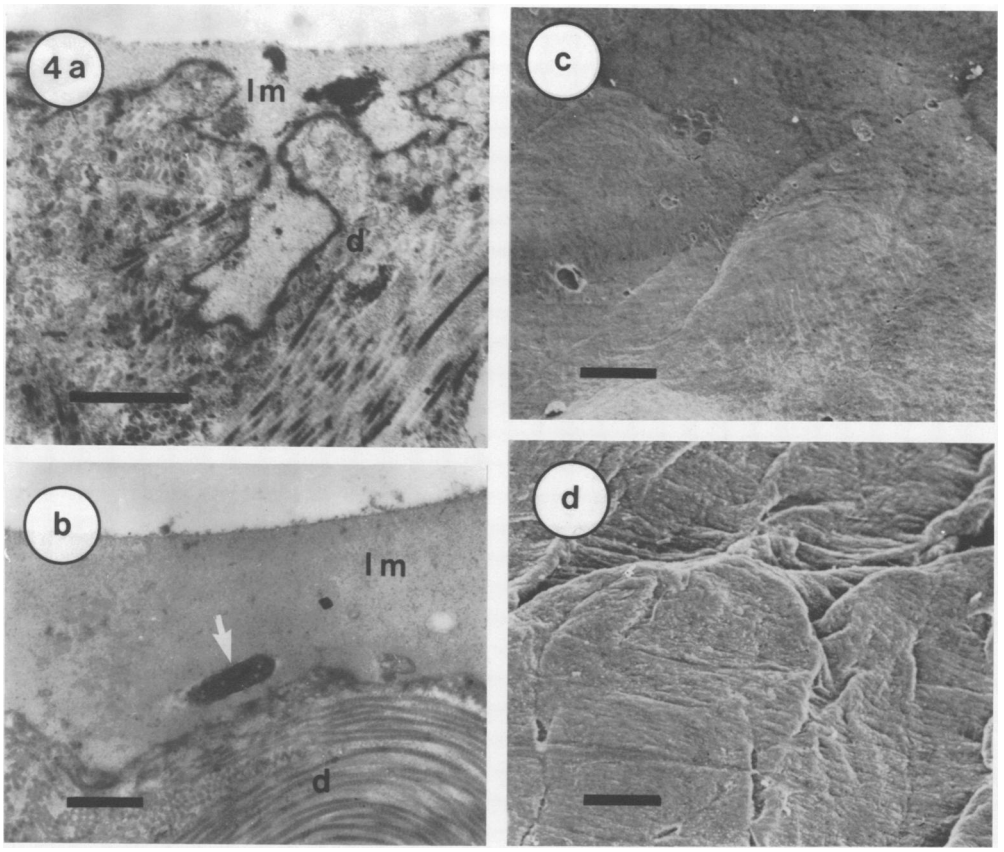

FIG. 4. *Electron micrographs of skin from scalded and plucked broiler carcasses sampled before evisceration. (a) Transverse section of skin fixed in osmium tetroxide vapour. Scalding and plucking processes have removed the epidermis and exposed dermal tissue. The basal lamina may or may not be left intact. The channel in the dermal surface (d) is filled with a material (lm) associated with the liquid film covering carcass surfaces. Bar = 1 μm. (b) Transverse section of skin fixed in the presence of alcian blue. Note the bacterium present in the layer of material filling a large channel in the dermal (d) surface. Bar = 1 μm. (c) and (d) Scanning micrographs of skin fixed in osmium tetroxide vapor (c) or glutaraldehyde (d). The skin surface detail on vapor-fixed tissue is obscured by a material not present on glutaraldehyde-treated tissue. Bar = 100 μm.*

microorganisms were not found on skin rinsed free of fluid film components. No microorganisms were found in feather follicle shafts or normal skin tissue from carcasses sampled from either site.

Significant changes in the appearance of surface features of skin occurred during immersion chilling. Figure 7a shows the swollen appearance of skin from immersion-chilled carcasses as compared with skin from fresh plucked carcasses shown in Fig. 4d.

DISCUSSION

Throughout this study, microscopic evidence has been combined, where possible, with counts of bacteria and flora analyses to allow a more reasoned interpretation of the mechanism(s) of contamination of broiler carcass skin during processing. This is a novel approach for examination of these aspects of the microbial ecology of microorganisms associated with flesh foods.

Before processing, broilers carry up to 10^6 microorganisms per 16 cm^2 of skin. The majority of these bacteria are *Micrococcus* spp. belonging to Baird-Parker's subgroups 5 and 6 (3) and most are located within clumps of particulate material lying on the skin surface. Yeasts and rod-shaped bacteria were also found, but much less frequently than gram-positive cocci. These organisms were only located on specimens examined by SEM and occupied superficial sites on the skin surface, and probably represent part of a transitional skin microflora. The gram-positive cocci, however, must be regarded as a more permanent or normal skin microflora because

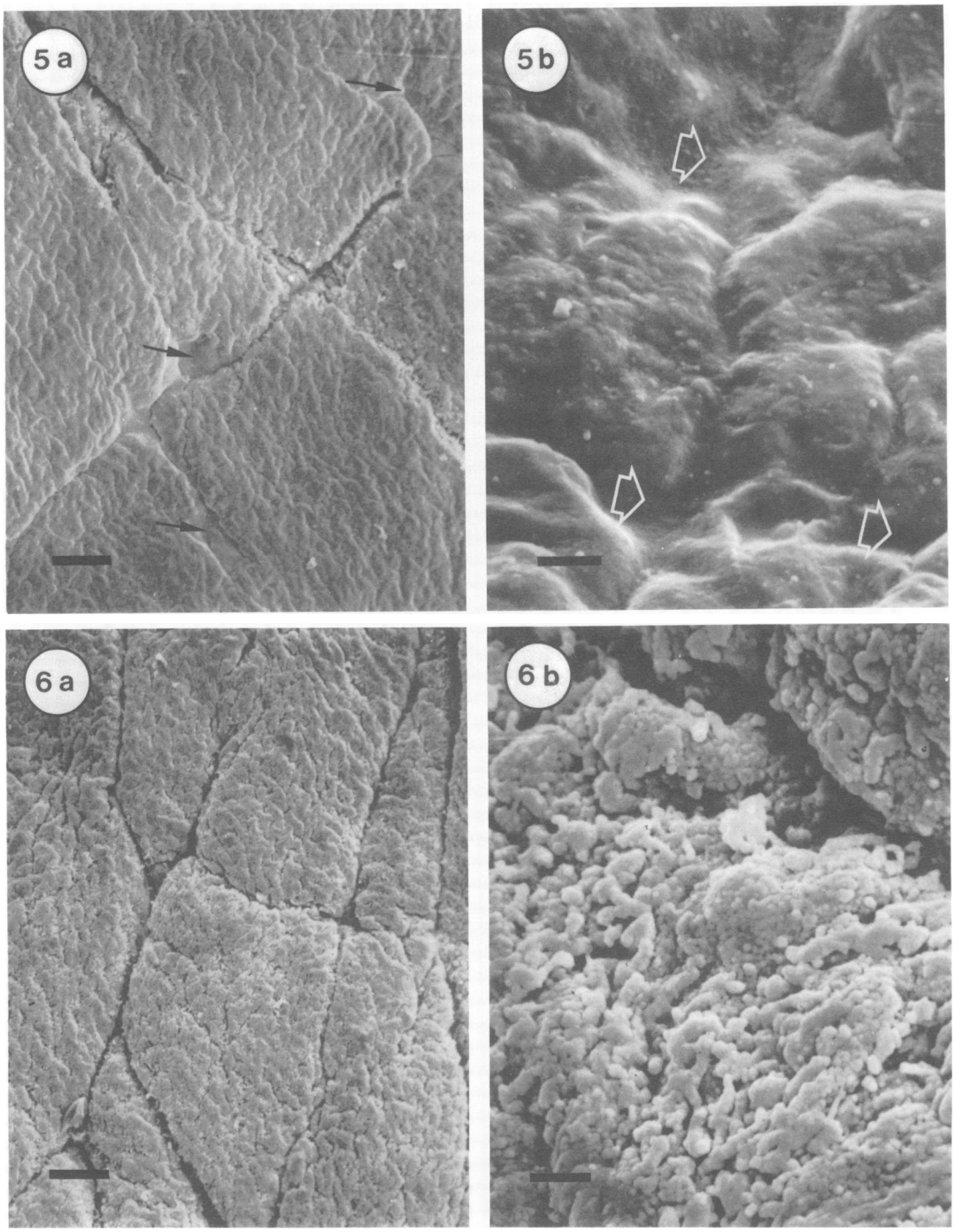

FIG. 5. *Scanning micrographs of skin from immersion-chilled broiler carcasses fixed in osmium tetroxide vapor. (a) Low-magnification micrograph showing material filling large channels in the skin surface (arrows). Bar = 30 μm. (b) Higher magnification micrograph showing the material filling smaller crevices (arrows) and the obscured surface detail. Bar = 3 μm.*

FIG. 6. *Scanning micrographs of skin from immersion-chilled broiler carcasses fixed in glutaraldehyde. (a) Low-magnification micrograph. Note the absence of any materials filling the large channels in the skin surface. Bar = 30 μm. (b) Higher magnification micrograph showing the unobscured surface detail and epidermal fragments. Bar = 3 μm.*

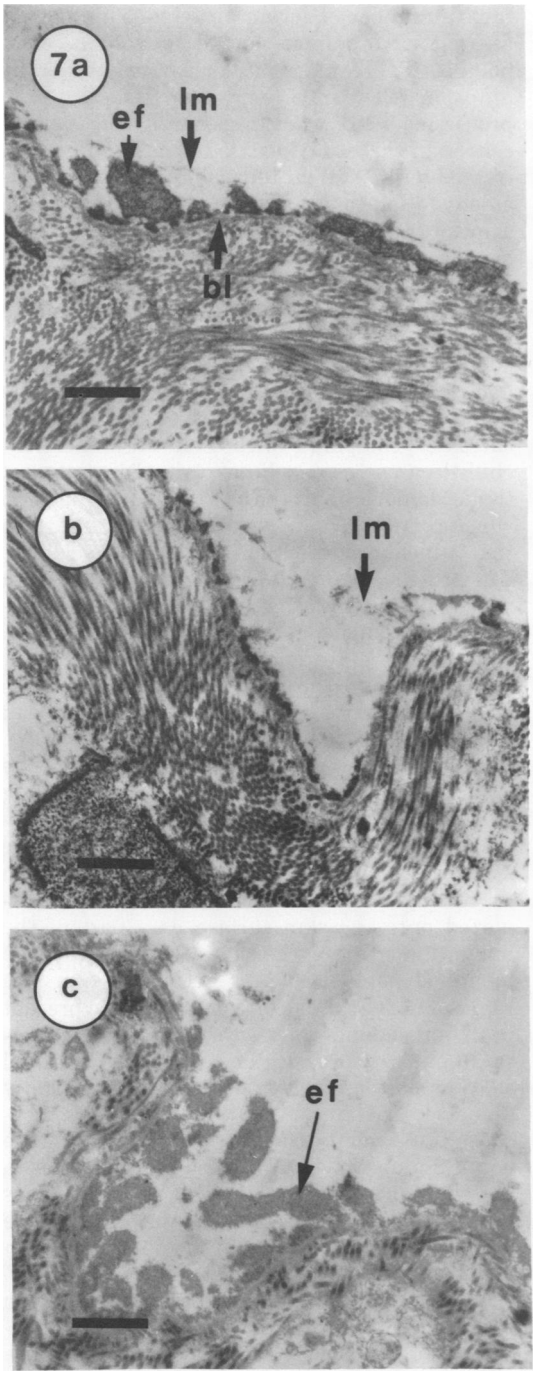

FIG. 7. *Micrographs of sectioned skin from fresh immersion-chilled broiler carcasses. All bar markers represent 1 μm. (a) and (b) Skin fixed in osmium vapor. Note the diffuse layer of material filling skin channels and covering epidermal fragments (ef) attached to intact basal lamina (bl). (c) Skin fixed by immersion in glutaraldehyde. Note the absence of the layer of material on the skin surface.*

they are always present in high numbers and are located in specific sites on the skin surface.

The nature and origin of the clumps of particulate material enclosing the micrococci are unknown, but may represent accumulations of lipid and other materials exuded from lysed corneous cells. Matoltsy (18) has established the presence of lipid droplets in cells of the transitional layers of the epidermis and shown that the vacuolar spaces of the stratum corneum contain mainly lipid. Lucas and Stettenheim (17) noted that the skin of the chicken secretes a lipoid or sebaceous material similar to that produced by the oil gland. These workers also demonstrated lipid granules on the skin surface of poultry and on the basis of their results suggested that the entire avian skin is a secretory organ providing its own requirement for sebaceous material. Indirect microscopic evidence from the present study suggested a lipid component within the clumps of particulate matter (Fig. 3a and b). Thus, it is possible that the clumps of material on the skin surface represent accumulations of skin lipoid secretions as well as skin cell fragments and dust from the environment. Micrococci growing within this material may therefore enjoy a unique ecological niche.

During processing, the predominantly gram-positive microflora of the skin of nonprocessed poultry carcasses is removed and replaced by a heterogeneous population largely composed of gram-negative bacteria. Several authors have reported similar types of bacteria contaminating the skin of processed broiler carcasses (5, 6, 9), but in each study the proportion of each particular type present on the skin was different. Previous results reported by McMeekin and Thomas (19) suggest that these differences probably represent changes in the proportion of each type present in chiller water which may occur on a seasonal, day-to-day, or locational basis. It is interesting that micrococci (presumably those normally removed from the skin of nonprocessed carcasses during plucking) were found on the skin of processed carcasses. Similar observations have been reported by Barnes and Thornley (6) and Daud et al. (9), whereas van Schothorst et al. (43) and Mulder et al. (27) have reported that enteric marker strains used to inoculate the skin of carcasses before plucking have been recovered from the skin of immersion-chilled carcasses. Therefore, it seems the microflora of nonprocessed poultry, which survive scalding, contribute to the microflora of the processed carcass.

Overall, processing procedures caused a significant reduction in numbers of viable, aerobic microorganisms present on the skin of broiler carcasses, although both increases and decreases in the number of contaminant organisms oc-

curred at several stages during processing. Similar trends have been reported previously (22, 24, 26, 27, 41). However, numbers of psychrotrophic contaminants increased at all stages of processing examined, especially during the immersion washing and chilling stages. Knoop et al. (14) and Lahellec et al. (16) reported similar increases in psychrotrophic contaminants, and, as in the present study, immersion washer and chiller water was implicated as the major source of these organisms. The skin of freshly plucked carcasses was found to be relatively free of psychrotrophic microorganisms, a result in agreement with published data (4, 8, 33). Plucking, however, was the first stage of processing to contribute to psychrotrophic contamination of carcasses (Table 3). Since water inputs were virtually free of these organisms, plucking machine surfaces must have acted as a source of contamination. Mead (21) has mentioned that the flexible rubber fingers used in plucking machines, to flail the carcasses, are not easy to clean and may carry organisms from one working period to the next unless special attention is given to cleaning and disinfection at the end of a working day. Therefore, systems which incorporate both scalding and plucking in one process may help to reduce this source of contamination (28, 44).

Clark (8) and Thomson et al. (42) noted that scalding and plucking processes caused removal of the outer skin layers, but did not provide any microscopic data to confirm their observations or determine the extent of skin damage. Microscopic data presented in this study showed that these processes caused removal of the skin epidermis and as a consequence also removed microorganisms colonizing the stratum corneum. Exposed dermal skin tissue provides a new surface available for colonization by microbial contaminants which arise during plucking and subsequent processing procedures. This new surface is smoother and less hydrophobic than that of the stratum corneum, but is covered with capillary-sized channels and crevices associated with dermal intercellular spaces and epidermal fragments. Additional significant changes in the microtopography of the skin surface also occur during immersion chilling and cleaning. Skin swelling, apparently associated with uptake of water by skin tissue, opens and exposes channels and crevices to contaminants present in waters used during these processing procedures.

As an added consequence of transient immersion of carcasses in processing waters, the skin surface becomes covered by a fluid film. Thomas (Ph.D. thesis, University of Tasmania, Hobart, Tasmania, 1979) has shown the fluid film contains a number of serum proteins, amino acids, as well as other suspended or soluble compounds. These materials originated either by diffusion from underlying skin tissue or from processing water used to clean or chill the carcasses. In this study, excised skin samples were treated with osmium tetroxide vapor or glutaraldehyde solutions as part of preparation of the skin for microscopic examination with or without the organic components present in the liquid film. Vapor fixation stabilized these components in situ, whereas immersion in glutaraldehyde rinsed these materials from the surface before adequate fixation could occur. The difference in density of stainable materials present within the liquid film present on the skin of freshly plucked carcasses compared with that of immersion-chilled carcasses can be explained in terms of tissue damage and limited component dilution which occurs during plucking as compared with that which occurs during chilling procedures. The presence of organic matter in the liquid film may explain why all skin bacteria are not destroyed by chlorine or other bacterial agents. Chlorination of water supplies used by plucking machines and other processing equipment is usually ineffective in reducing the bacterial load of carcasses as well as cross-contamination (22).

Skin microtopography and the presence of the liquid film on the skin of carcasses after plucking and other washing procedures are major determinants of the mechanism of contamination. As a result of transient immersion of carcasses in processing water, microorganisms are transported onto the skin surface as a part of the liquid film covering the skin. Consequently, the microbial population present in this film is a representative sample of that population present in the processing water (19). Therefore, the water film microflora may be changed or modified as often as the carcass surfaces are transiently immersed in different situations. However, because some microorganisms present within the liquid film may occupy capillary-sized spaces in the skin surface, these bacteria will not be as easily removed by cleaning practices as those more superficially located in the film on the skin surface.

Notermans and Kampelmacher (31) have noted that bacteria which become firmly attached to the skin during plucking are more difficult to remove than those added subsequently and display increased heat resistance compared with unattached bacteria. These workers suggested the location of attached bacteria within the skin surface may afford protection from serious heat damage. Microscopic data presented in this study lend support to this

argument, and it seems reasonable to suggest that bacteria located deep in channels will be protected from both heat and chemical damage and physical removal. Deep location of bacteria also explains the reasons for only a partial reduction in contamination effected by various cleaning procedures (25, 32) and the fact that viable counts obtained by maceration of skin samples are always greater than those obtained by swabs or rinses (1, 31, 32, 35).

On the basis of microscopic evidence presented, suggested roles of acidic mucopolysaccharides in attachment of bacteria to the skin surface (30–32; C. Vanderzant, Z. L. Carpenter, and G. C. Smith, 29th Annu. Reciprocal Meat Conf. Amer. Meat Sci. Assoc., 1976) do not seem appropriate, but more detailed microscopic data are needed before an accurate assessment of any possible role of these bridging substances can be made. Attached bacteria may simply represent organisms lodged in channels and crevices in the skin surface, whereas bacteria more superficially located in the liquid film correspond to the water microflora described by Notermans and Kampelmacher (32).

The role of changes in the microtopography of the skin, which occur as a result of immersion in water, on contamination is not obvious. Skin swelling may trap bacteria already located in deep channels and crevices and render them even less accessible to physical removal. Alternatively, skin swelling may provide additional access to deep skin contamination. This aspect therefore warrants further investigation. An examination of the effects of the soft scald procedures and air chilling on the structure and microtopography of the skin would also be of interest, especially in relation to the effects of these treatments on contamination and subsequent microbial development.

ACKNOWLEDGMENTS

The generous financial assistance provided by the Australian Chicken Meat Research Committee is gratefully acknowledged.

We also thank Glenila Poultry Service, Sorell, Tasmania, for provision of broiler carcasses.

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