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Attachment of *Salmonella* spp. to Chicken Muscle Surfaces

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Immersion of chicken muscle fascia in water or physiological saline caused collagen associated with the connective tissue to expand and form a dense network of fibers on the surface. Similar changes were noted for muscle perimysium. Two test strains of *Salmonella* spp. attached to the collagen fibers only when muscle was immersed for extended times in water. Bacteria did not attach to the fascia or perimysium of muscle that was transiently immersed in suspensions. The presence of sodium chloride in the suspension media prevented firm attachment, whereas saline rinses removed many attached cells.

Attachment of bacteria to a wide variety of biological and nonbiological surfaces is well known. However, it has not been firmly established whether microbial contaminants of meat surfaces are attached to or merely retained on these surfaces. Studies based solely on counts of bacteria have suggested that microorganisms may adhere to chicken and pork skins (1, 9), chicken, beef, and lamb meat surfaces (1, 4), and cow teats (3, 8), but definitive microscopic data have not been provided to support these conclusions. Other work on chicken skin (7, 11) has shown that contamination by bacteria is a function of the concentration of organisms suspended in processing water and the microtopography of the skin surface. Bacteria which lodge deep in channels and crevices in the skin surface would be difficult to remove and therefore may represent "attached bacteria" described by Notermans and Kampelmacher (9) and others. Conversely, microorganisms which remain in the liquid film covering the skin surface after processing would be easily removed by conventional cleaning practices.

Changes in the microtopography of tissue surfaces caused by various processing procedures may also have an important effect on the mechanism of contamination (11). This communication describes the effects of water-induced changes on the microtopography of chicken muscle fascia and their significance for contamination by *Salmonella* spp. and subsequent decontamination.

MATERIALS AND METHODS

Organisms. *Salmonella typhimurium* (strain M48, University of Tasmania, Hobart) and a strain of *Salmonella singapore* (obtained from the Salmonella Reference Laboratory, Adelaide, South Australia) were used. Each strain was grown in nutrient broth incubated at 37°C for 18 h. Cells were harvested by centrifugation and suspended in a small volume of saline (0.9%, wt/vol) immediately before inoculation of test media.

The motility status of both strains of bacteria when suspended in water or physiological saline was assessed by examination of wet-mount preparations with a light microscope. Nutrient broth cultures of each strain were also examined for production of fimbriae by a negative staining technique (11).

Contamination experiments. Skin from the breast area of fresh chicken carcasses (scalded at 58°C for 2.5 min and mechanically plucked) was carefully removed to expose the thin connective tissue layer, or fascia, which covers the pectoral muscles. Slabs of muscle with intact fascia (ca. 1 cm^2 by 0.5 cm deep) were excised and vigorously agitated in 500-ml volumes of water or physiological saline which contained ca. 10^8 cells of either strains of *Salmonella* per ml. Control muscle pieces were agitated in sterile water or physiological saline. All test media were agitated by magnetic stirring and were maintained at 20°C. Muscle pieces were immersed for periods of up to 30 min, rinsed with distilled water or physiological saline, and prepared for examination by scanning electron microscopy.

Scanning electron microscopy preparation. Treated muscle pieces were placed in glutaraldehyde fixative solutions (2%, wt/vol, in 0.1 M sodium phosphate buffer, pH 7.2) overnight at 4°C. Fixed muscle was then rinsed in cold phosphate buffer, sliced into small pieces (ca. 0.5 cm^2), dehydrated in a graded acetone series, and critical-point dried from carbon dioxide. Dried tissue was mounted on stubs, coated with about 40 nm of gold by sputtering, and examined in a JEOL JXA-50A scanning electron microscope unit operated with an accelerating voltage of 15 kV.

RESULTS AND DISCUSSION

The microtopography of the muscle fascia surface before immersion in either water or physiological saline (Fig. 1) was significantly different from that of muscle immersed in these fluids for 30 min (Fig. 2 and 3). The initially flat surface became covered by a dense mat of collagen fibers originally contained within the fas-

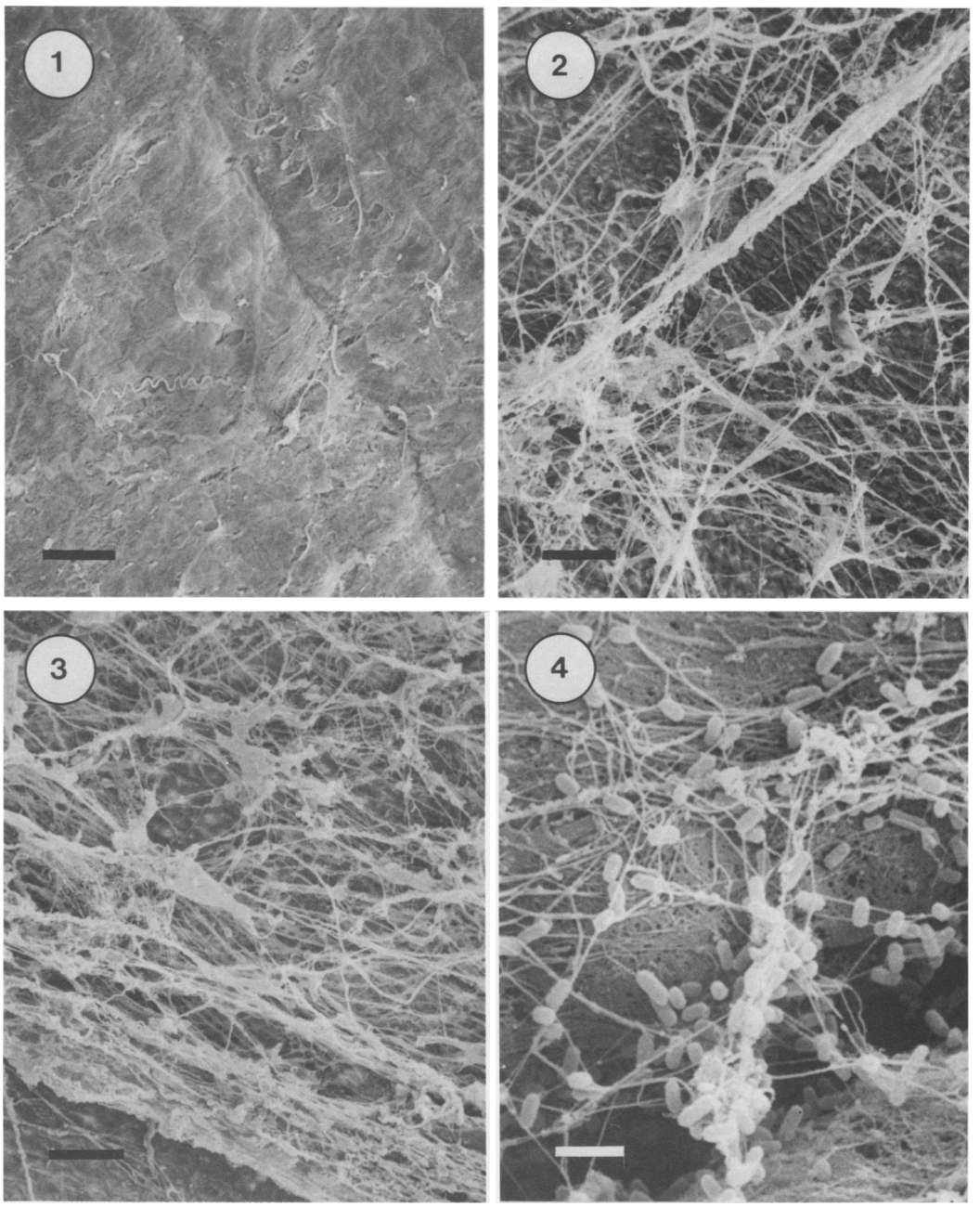

FIG. 1. *Scanning electron micrograph of the fascia surface of chicken muscle. Note the smooth appearance of this surface with only a few exposed connective fibers. Bar, 100 μm.*

FIG. 2. *Scanning electron micrograph of the fascia surface of muscle that was immersed in water for 30 min. Exposure of this surface to water allowed the collagen fibers within the fascia to expand and form the dense network of fibers shown in this micrograph. Bar, 100 μm.*

FIG. 3. *Scanning electron micrograph of the fascia surface of muscle that was immersed in physiological saline for 30 min. Notice the similar network of collagen fibers on this surface to that shown in Fig. 2. Bar, 100 μm.*

FIG. 4. *Scanning electron micrograph of cells of S. singapore attached to expanded collagen fibers of muscle perimysium. The muscle tissue was immersed in water containing these bacteria (ca. 10^8 cells per ml) for 30 min before fixation. Bar, 3 μm.*

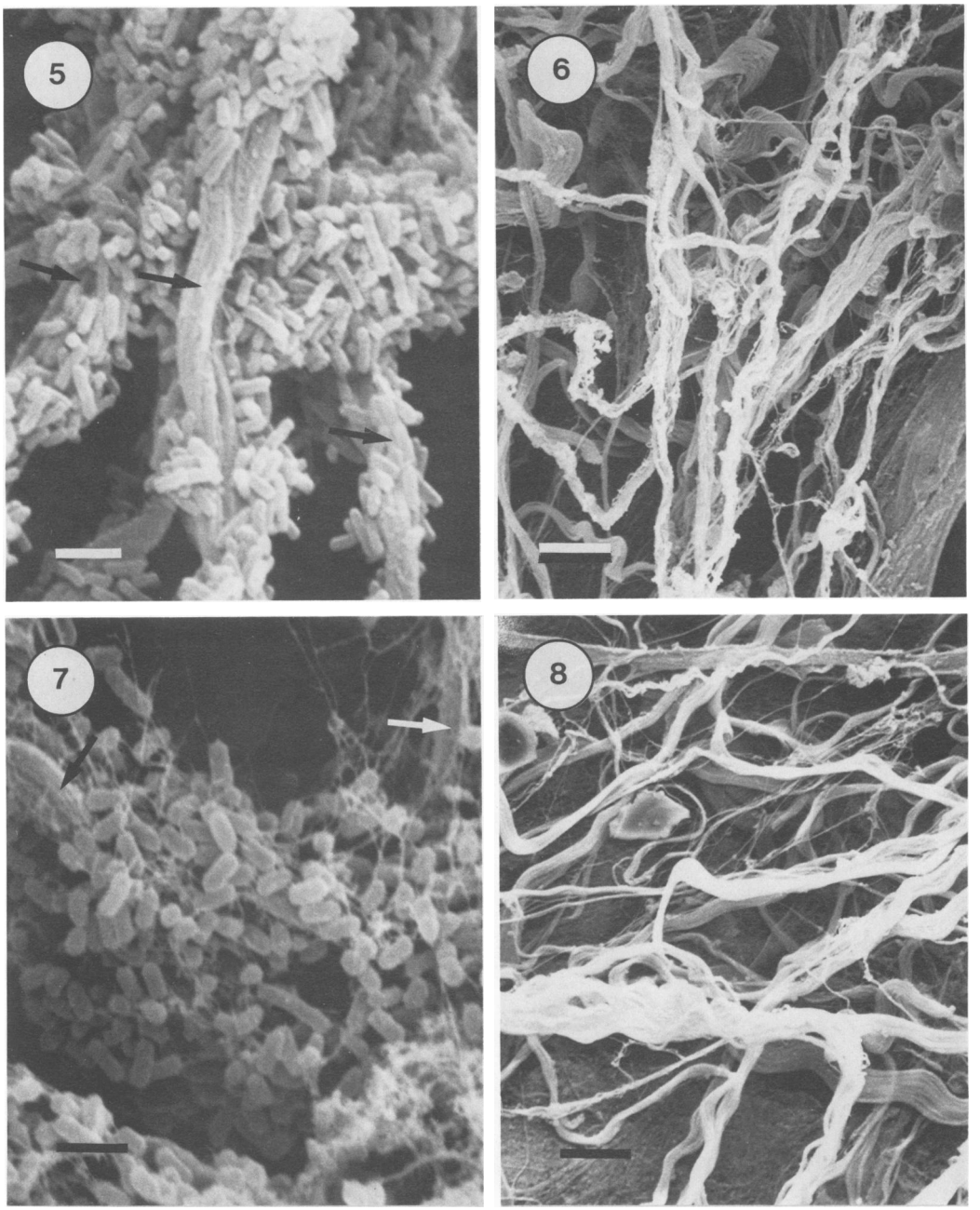

FIG. 5. *Scanning electron micrograph of S. typhimurium attached to the expanded collagen fibers (arrows) on the fascia surface of muscle that was immersed in water containing these bacteria (ca. 10^8 cells per ml) for 30 min before fixation. Bar, 3 μm.*

FIG. 6. *Scanning electron micrograph of the fascia surface of muscle immersed in a physiological saline suspension of S. typhimurium (ca. 10^8 cells per ml) for 30 min before fixation. Notice that bacteria have not attached to the collagen fibers. Bar, 10 μm.*

FIG. 7. *Same as Fig. 5, except S. singapore cells were suspended in water (ca. 10^8 cells per ml). Bar, 2 μm.*

FIG. 8. *Same as Fig. 6, except S. singapore cells were suspended in physiological saline (ca. 10^8 cells per ml). Bar, 10 μm.*

cia. The degree of cover depended upon the time of immersion but formed equally well for tissue immersed in either fluid. A similar change was noted for exposed perimysium connective tissue. These changes in microtopography occurred as a result of water uptake by collagen, which caused these fibers within the connective tissue framework to swell and expand. Similar changes are well known for isolated and intact collagen fiber systems immersed in aqueous media, with swelling and fiber expansion being relatively constant for water and neutral salt solutions (5).

Cells of both strains of *Salmonella* spp. examined were found to attach to the expanded collagen fiber network of muscle fascia and muscle perimysium. Attachment occurred only when muscle tissue was immersed in water inoculated with bacteria and allowed to swell (Fig. 4, 5, and 7). Bacteria did not attach to the fascia surface of fresh muscle that was only transiently immersed in test suspensions. The presence of sodium chloride in the suspension fluid prevented firm attachment of cells to collagen fibers (Fig. 6 and 8).

Alteration of the ionic environment of attached bacteria allowed removal of the majority of the organisms from collagen fibers. Physiological saline rinses effectively detached a large majority of both strains of bacteria, whereas even extended rinses in water removed comparatively few organisms.

Absence of fimbriae (or pili) from test bacteria suggested that these structures, which have been implicated in attachment of *Salmonella* spp. to animal tissues (2), were not involved in attachment to collagen fibers. Also, the motility status of both strains did not significantly affect attachment since *S. typhimurium* was nonmotile, whereas *S. singapore* remained motile when suspended in either of the suspension fluids tested. The experimental evidence therefore indicates that the bacterium-collagen fiber interaction is a function of the ionic environment of the muscle tissue. The fact that sodium chloride can prevent attachment and cause detachment suggests that the attachment process is mediated by a physicochemical relationship with either the collagen or the mucopolysaccharide cementing matrix between individual collagen fibrils. Indeed, it is possible that sodium chloride may prevent attachment to collagen fibers by reducing the viscosity of the mucopolysaccharide matrix, thereby allowing elution of this material before attachment could take place. In this respect, low concentrations of sodium chloride (ca. 0.05 M) have been shown to dramatically reduce the viscosity of hyaluronic acid, which is a common component of connective tissues (6). Similarly, saline rinses may elute bacteria already attached to collagen fibers.

Although the formation of the network of collagen fibers on the fascia surface of muscle agitated in water is a necessary prerequisite for attachment of salmonellae, physical entrapment of microorganisms will also play an important role in contamination. Consequently, although attachment may be prevented by the addition of sodium chloride to water in which muscle surfaces may be exposed, totally effective decontamination seems improbable, unless the water-induced changes in fascia microtopography can be avoided.

These results have important implications for the chicken and other carcass meat industries where meat surfaces are washed with water. In particular, the underside of the chicken carcass neck flap undergoes similar changes in microtopography of muscle fascia during immersion chilling procedures (Thomas and McMeekin, unpublished data). Since this tissue is the complementary surface of muscle fascia, salmonellae and other bacteria may attach to and contaminate the expanded network of collagen on this loose connective tissue surface. This mechanism of contamination may therefore explain the greater contamination of neck flap skin as compared with that of breast and leg skins (10).

Detailed bacteriological and electron microscopic studies are currently in progress to examine the effect of modification of the ionic environment on attachment of salmonellae and other bacteria to collagen fibers on muscle fascia and neck flap tissue. The mechanism of attachment is also under investigation.

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