

SOME ASPECTS OF THE MICROBIAL ECOLOGY OF POULTRY
PROCESSING AND STORAGE WITH PARTICULAR REFERENCE
TO MICROBIOLOGICAL/HISTOLOGICAL RELATIONSHIPS OF
BROILER CARCASS SKIN

by

C.J. THOMAS, B.Agr.Sc.(Hons.), Tas.

Submitted in fulfilment of the requirements for the Degree of
Doctor of Philosophy

University of Tasmania

Hobart

August 1979

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and to the best of my knowledge contains no copy or paraphrase of any material previously published or written by any other person, except where due reference is made in the text of the thesis.

A handwritten signature in cursive script, reading "C. J. Thomas". The signature is written in black ink on a white background.

C. J. THOMAS

University of Tasmania

Hobart

August 1979

NOTES

Symbols, units and their abbreviations, text reference citations and the layout of the bibliography have been written in accordance with Board, R.G. and Carr, J.G. (1976). A guide to contributors. Journal of Applied Bacteriology 40, 1, wherever possible.

In this text, the term psychrotroph has been used to describe microorganisms which form colonies on nutrient agar after aerobic incubation at 2⁰C for 14 days and which grow well at 22⁰C. Psychrophile has been used only where cited original work has specified the use of this terminology.

The abbreviations SEM and TEM, used commonly in this text, have been used in the following contexts:-

SEM : scanning electron microscop(y) or (ic)

TEM : transmission electron microscop(y) or (ic)

TABLE OF CONTENTS

	<u>Page</u>
Acknowledgements	i
Summary	ii
I. INTRODUCTION	1
II. LITERATURE REVIEW	4
a. Microflora of the Poultry Carcass Prior to Processing	5
1. The microflora of the chicken alimentary canal	6
2. The microflora of the external surfaces of the chicken	6
b. Microbial Contamination of Carcasses During Processing	8
c. Methods to Assess Carcass Contamination	21
d. Development of Microorganisms on Carcass Surfaces During Storage at Chill Temperature	29
e. Histological/Microbiological Relationships	45
III. EXPERIMENTAL	48
Section A. Microbial Contamination of Carcasses During Processing	49
Materials and Methods	50
a. Origin and isolation of strains	50
b. Characterization of isolates	50
c. Sources of psychrotrophic microorganisms contaminating carcasses during processing	53

TABLE OF CONTENTS (Continued)

	<u>Page</u>
d. Evaluation of fixation and dehydration techniques for electron microscopic examination of processed broiler skin	55
e. Microscopic examination of broiler carcass skin at various stages of processing	56
f. Recovery of microorganisms from broiler carcass skin by the stomacher	57
g. Characterization of some water soluble components present in immersion chiller water and on the skin of fresh and stored immersion chilled broiler carcasses	59
Results	61
a. Numbers and incidence of different bacteria present on non-processed and immersion chilled broiler carcass skin	62
b. Sources of psychrotrophic microorganisms in a poultry processing plant	62
c. Evaluation of fixation and dehydration techniques used for examination of processed broiler skin	64
d. Microscopic examination of the skin from broiler carcasses at various stages of processing	67
e. Recovery of microorganisms from broiler skin by the stomacher	82
f. Water soluble compounds present in immersion chiller water and on the skin of fresh and stored broiler carcasses	84

TABLE OF CONTENTS (Continued)

	<u>Page</u>
Section B. Development of Microorganisms on Broiler Carcass Skin During Storage at Chill Temperatures	91
Materials and Methods	92
a. Origin and isolation of strains	92
b. Characterization of isolates	93
c. Production of off odours by isolates grown on sterile excised leg muscle	93
d. Production of off odours by isolates grown on broiler skin preparations	94
e. Production of sulphur volatiles by isolates grown in methionine or cystine supplemented peptone broth	94
f. Analysis of sulphide odours produced by isolates grown on muscle, skin or artificial substrates	95
g. Detection thresholds of DTNB and lead acetate papers	95
h. Microscopic examination of microorganisms on skin from broiler carcasses stored at various temperatures	96
Results	99
a. Numbers and incidence of different bacteria during spoilage of naturally contaminated skin	100
b. Production of off odours by isolates grown on sterile leg muscle and skin preparations	105

TABLE OF CONTENTS (Continued)

	<u>Page</u>
c. Production of sulphide volatiles from methionine and cystine supplemented peptone broth	113
d. Types and identity of off odours produced by psychrotrophic isolates grown on various substrates	114
e. Detection thresholds of DTNB and lead acetate papers	116
f. Microscopic examination of microorganisms on skin from broiler carcasses stored at various temperatures	120
1. Broiler carcasses stored at 2 ⁰ C under high humidity conditions	120
2. Broiler carcasses stored under high humidity conditions at 4, 10, 15 or 22 ⁰ C until onset of spoilage.	135
3. Carcasses stored at 2 ⁰ C under drying conditions	145
IV. DISCUSSION	148
a. Contamination of broiler carcass skin during processing	149
b. Growth of microorganisms on the skin of broiler carcasses	160
c. Metabolism of microorganisms on the skin of chill stored broiler carcasses	166
d. Application of microscopy to other areas of food microbiology.	177

TABLE OF CONTENTS (Continued)

	<u>Page</u>
V. CONCLUSIONS	183
VI. BIBLIOGRAPHY	187
VII. APPENDICES	227
Appendix 1	228
Appendix 2	230
Appendix 3	232
Appendix 4	235
Appendix 5	237

ACKNOWLEDGEMENTS

I wish to thank my supervisor, Dr. T.A. McMeekin (Senior Lecturer in Agricultural Microbiology, Faculty of Agricultural Science) for advice, criticism and encouragement during this project.

I thank Mr. Ralph Cruickshank (Faculty of Agricultural Science, University of Tasmania), Mr. D. Monroe (New Town Research Laboratories, State Department of Agriculture, Hobart), Dr. A.N. McKee (Telecom Australia Research Laboratories, Melbourne, previously Manager, Central Science Laboratory, University of Tasmania) and Mr. A. Eastgate (Central Science Laboratory) for help with some aspects of Electron Microscopy.

I also wish to acknowledge Dr. June Olley (C.S.I.R.O., Food Research Unit, Hobart) for helpful discussion and criticism; the assistance of Mr. W. Peterson (Laboratory Manager, Faculty of Agricultural Science); and Mrs. S. Jones for typing this thesis.

The generous financial assistance of the Australian Chicken Meat Research Committee, the help from the Executive Director of the Australian Chicken Meat Research Committee, Dr. J.G. Fairbrother and cooperation of Glenila Poultry Service, Hobart, Tasmania, are gratefully acknowledged.

Finally, I thank my wife, Angela, for her support and encouragement during this project.

SUMMARY

Scanning and transmission electron microscopy were used in conjunction with standard microbiological procedures to examine some aspects of contamination of broiler carcass skin by bacteria during processing as well as the subsequent growth of these microorganisms during storage.

The autochthonous skin microflora of the skin of poultry prior to 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 the exposed dermal tissue was contaminated by microorganisms from the mechanical plucker and subsequent stages of processing. The major sources of contamination of psychrotrophic bacteria were immersion washer and chiller water. Microorganisms contaminating the skin were mainly found within a fluid film covering this surface, but also deep inside skin channels. The fluid film contained serum proteins and some amino acids and the quantity of fluid and concentration of the components increased during storage of broiler carcasses. Skin microtopography and the presence of the liquid film were implicated as major factors controlling contamination of carcasses during processing.

Fixation of skin in osmium tetroxide vapour allowed preservation of the liquid film components *in situ*, whereas fixation by immersion in solutions of glutaraldehyde or osmium tetroxide rinsed these materials from the skin and allowed unobstructed observation of the surface. Addition of alcian

blue 8GX to glutaraldehyde solutions improved preservation of the fluid film components, especially when high numbers of spoilage bacteria were present.

During storage of carcasses under humid conditions, bacteria grew within the fluid film but were also found in deep skin channels and in feather follicle shafts, whereas under drying conditions, microorganisms grew in discrete colonies. Spoilage bacteria were not attached by acidic mucopolysaccharide fibrils to the skin of carcasses stored at 2⁰, 4⁰, and 10⁰C, although bridging substances were associated with bacteria on carcasses stored at 15⁰ and 22⁰C.

Skin invasion or gross degradation of this substrate by the spoilage microflora was not observed for carcasses stored at 2⁰C. However, bacteria did degrade proteinaceous material present within the liquid film even before onset of spoilage. Strains of *Pseudomonas* groups I and II were the predominant types producing off odour on the skin of carcasses stored at 2⁰C. Sulphide-like or fruity-type off odours were produced by pure cultures of these bacteria when grown on either leg muscle pieces or skin. The major sulphur volatile produced was methanethiol. This volatile was also produced by these isolates when grown in a methionine supplemented medium, although the presence of glucose inhibited production of methanethiol.

The significance of the results is discussed in relation to the mechanisms of contamination of broiler carcasses during processing and the growth and metabolism of microorganisms during storage.

INTRODUCTION

Microbial ecosystems are characterised by the presence of a diversity of microbial species engaged in various activities in association with a particular niche. In foods, microbial activity may lead to spoilage, disease or production of a characteristic product. However, in examining food ecosystems, many microbiologists have simply concentrated on the effect of pure cultures of bacteria on suitable substrates, or enumerated microorganisms from particular habitats. Consequently, an adequate understanding of many aspects of the ecology of food-borne microorganisms has not been developed.

In general, the relationship between the organism of interest and its substrate has been little studied. Although Ingram (1971) emphasised only a small part of a food may be involved in critical changes associated with spoilage, few foods have been studied in sufficient histological and microbiological detail to illustrate this point. Lack of definitive data in this regard is especially evident in published studies of aspects of poultry processing and storage.

The nature of the bacterium-chicken skin interaction is an important determinant of contamination and subsequent growth of microorganisms on this substrate. Available data is confined almost solely to counts of bacteria and flora analyses at various stages of processing and several authors have attempted to indirectly suggest the site of skin contamination and growth on the basis of greater recovery of organisms by skin maceration compared to swabs or rinses. Thus Avens and Miller (1970a) described contaminant bacteria as "in or on the skin". Patterson (1971a) suggested shaking skin with rough sand to

remove bacteria from the skin and Barnes *et al.* (1973) indicated bacteria grew down in feather follicle shafts rather than on the surface of the skin. Apart from triphenyl tetrazolium chloride reduction and ultraviolet fluorescence studies described by Barnes *et al.* (1973), there is no direct experimental evidence to confirm the location of microorganisms on chicken skin or to indicate the nature of the bacterium-skin interaction.

In this study, scanning and transmission electron microscopy have been used in conjunction with normal microbiological procedures to provide information about the microbiology/histology relationships of broiler carcass skin. This novel approach encompasses an examination of contamination of broiler carcass skin at various key stages of processing and includes a study of the growth and metabolism of microorganisms on this substrate during chill storage of broiler carcasses.

LITERATURE REVIEW

Introduction: This review examines several aspects of the microbial ecology of poultry processing and storage. These include:-

- (a) microflora of the carcass prior to processing;
- (b) microbial contamination during carcass processing;
- (c) the methods used to assess contamination;
- (d) the development of microorganisms on carcass surfaces during storage at chill temperatures; and
- (e) histological/microbiological relationships.

Attention is drawn to the bacterium-substrate relationship where possible, since it may be suggested that this is an important determinant in both contamination and subsequent growth of microorganisms on chicken carcasses. Ingram (1971) emphasised the lack of definitive data in this regard, and indicated that only in a few foods has the histology/microbiology relationship been examined.

(a) The Microflora of Poultry Prior to Processing

The live bird carries large numbers of microorganisms associated with both interior and exterior surfaces (Mead, 1976). This microflora may be considered to consist of both a resident (or autochthonous) and a transient population. Woodroffe and Shaw (1974) define a resident population as one which multiplies and not merely survives in the habitat under consideration and although this may not be readily demonstrable it may be inferred that an organism is resident if it is repeatedly recovered in large numbers. However, discrimination between minor residents and transients is more difficult, if at all possible, by methods based on viable counts. The determination of status of a population

is further complicated by the fact that various sampling techniques may yield widely differing results. Hence the sampling technique must be designed to account for the complex terrain of various tissue surfaces, specific locations of various microorganisms and specialised cultural requirements of the organism(s) of interest.

(1) The microflora of the chicken alimentary canal

The normal microflora of the chicken alimentary canal has been extensively examined (Huhtanen and Pensack 1964; Ochi *et al.* 1964; Smith 1965; Barnes and Impey 1968; Timms 1968; Barnes and Impey 1970; Fuller and Turvey 1971; Barnes *et al.* 1972; Fuller 1973; Salanitro, Blake and Muirhead 1974; Salanitro, Fairchilds and Zgnornicki 1974; Salanitro *et al.* 1978) and aspects of the microbiology histology relationships reported by Fuller and Turvey (1971), Fuller (1973), Brooker and Fuller (1975), Fuller (1975), Glick *et al.* (1978) and Salanitro *et al.* (1978).

Microorganisms commonly associated with food-borne diseases are found in the contents of the alimentary tract of broiler chickens. Organisms such as *Clostridium perfringens*, *Staphylococcus* spp. and *Echerichia coli* are found as part of the normal gut flora (Huhtanen and Pensack 1964; Ochi *et al.* 1964; Barnes and Impey 1968, 1970; Timms 1968; Barnes *et al.* 1972; Salanitro, Blake and Muirhead 1974; Salanitro, Fairchilds and Zgnornicki 1974; Salanitro *et al.* 1978). Also, salmonellae and other enteric pathogens are often isolated as a transient gut population and are present in only a fraction of a normal flock (Tamura *et al.* 1971; Simmons and Byrnes 1972; Dougherty 1974; Mead 1976; Mossel 1977; and others).

(2) The microflora of the external surfaces of the chicken

No information is available about the autochthonous microflora of commercial poultry skin. Experiments to date have

concentrated on characterization and enumeration of microorganisms of economic and public health significance such as *Staphylococcus aureus*, dermatophytic fungi and yeasts and organisms associated with food-borne disease.

The incidence of *Staphylococcus aureus* biotypes on the skin and feet of healthy commercial poultry is well documented (Hagburg *et al.* 1973; Devriese *et al.* 1975; Cooper and Needham 1976; Götze 1976; Gibbs *et al.* 1978). Numbers recovered from the skin varied from 100 to more than 10^5 per cm^2 of skin and usually more than 80% of a sample of birds were carriers. Devriese *et al.* (1975) showed that a large proportion of these organisms could be rinsed from the skin surface and concluded that *S. aureus* was located superficially on the skin. However, *S. aureus* has also been isolated from subcutaneous bruises (Hamdy *et al.* 1964; Roskey and Hamdy 1975) and breast blisters (Dobson 1966). Evidence from bacteriophage typing (Smith and Crabb 1960; Harry 1967; Kusch and Götze 1974) have suggested that *S. aureus* strains isolated from the skin of poultry are mainly of human origin and hence are probably not components of a normal flora. However, more recent evidence (Gibbs *et al.* 1978) has suggested some strains may be specific for poultry.

The outside of the bird is also contaminated with various types of bacteria, fungi and yeasts largely derived from the litter, soil, air and other components of the poultry environment. These organisms can only be described as transient populations. *Clostridium perfringens*, *Staphylococcus* spp., salmonellae, *Escherichia coli*, other enteric microorganisms and psychrophilic bacteria have all been isolated from the exterior surfaces of the bird and from its environment (Hamdy *et al.* 1964; Fannelli *et al.* 1970; Mead

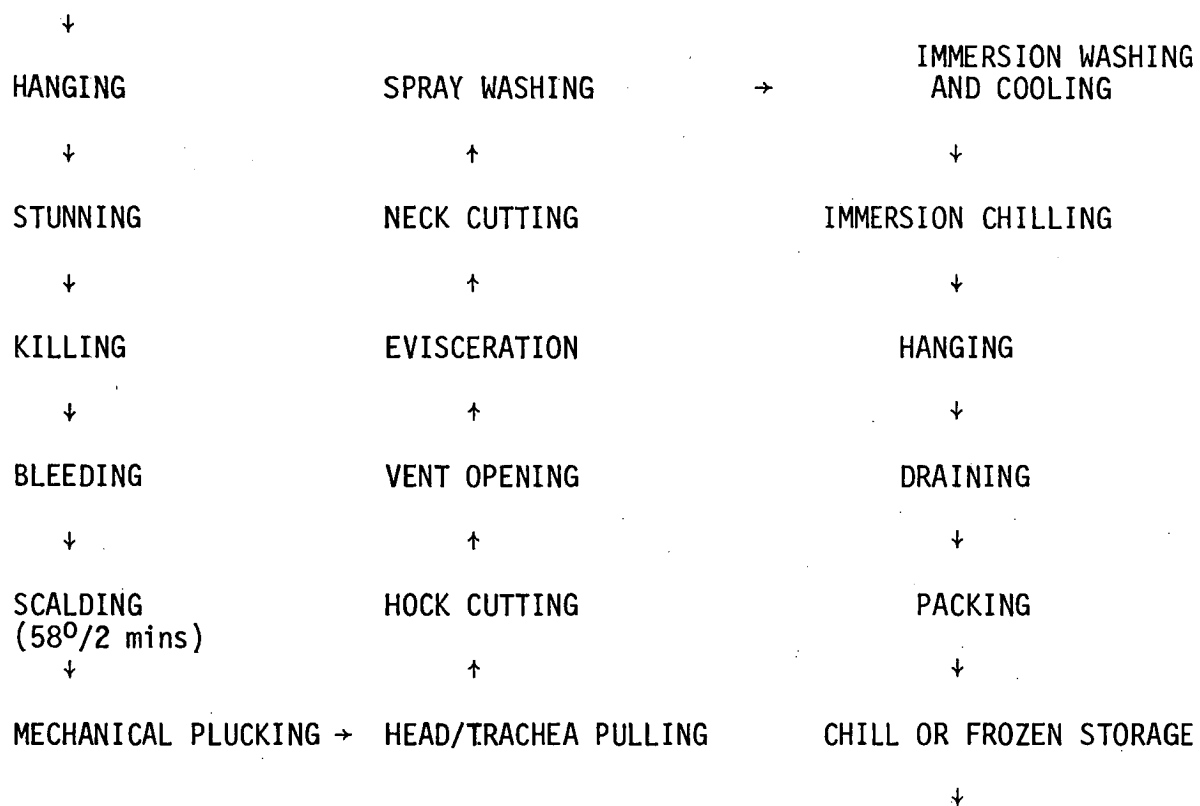
and Impey 1970; Lillard 1971; Lovett *et al.* 1971; Srivastava *et al.* 1972; Stott *et al.* 1975). *Penicillium* spp., *Aspergillus* spp., *Mucor*, *Phycomycetes*, *Cephalosporium* spp., *Trichoderma* spp., *Paecilomyces*, *Scopulariopsis*, *Candida* spp., and other unidentified yeasts have all been associated as transients of the respiratory tract, noses, skin and other external surfaces as well as commonly being isolated from the environment (Jordan 1954; Chute *et al.* 1956; Bacle and Bigland 1962; Chute *et al.* 1962; Garg and Sethi 1969; and others).

(b) Microbial Contamination During Carcass Processing

Contamination with microorganisms is an inevitable consequence of modern processing methods and may occur at any stage of carcass preparation. The various stages of poultry processing are outlined in Fig. 1. Following slaughter poultry carcasses are scalded and plucked, eviscerated, chilled and packaged. At each stage of the process ample opportunity exists for the contamination of the carcass with microorganisms from the environment of the processing plant or by cross contamination from other birds. Barnes (1960) showed that the number of bacteria on poultry carcasses varied considerably at different stages of processing, and both increases and decreases in numbers have been reported (Peric *et al.* 1971a; van Schothorst *et al.* 1972; Mead and Thomas 1973a, b).

Since the live bird carries large numbers of organisms, both on its outer surfaces and in the intestine, plant design should ensure physical separation of live birds from processing areas in order to minimise transfer of contaminant organisms (Patterson 1971a; Mead 1976). Following slaughter, the carcasses are scalded in a bath of heated water to wash dirt from the carcass exterior and allow easy removal of feathers. Clark (1968) and

FIGURE 1. FLOWCHART OF POULTRY PROCESSING



Thompson *et al.* (1974) have noted that scalding and plucking removed a layer of skin from the carcass surface. Soft scalds (50-52°C) allowed less complete removal of the yellowed outer skin layers than did hard scalds (58-63°C). Consequently many organisms found on the skin of the live bird are removed and may be damaged by the scald treatment. However, the extent of damage and removal of the outer epidermal layer has not been critically examined. Detailed microscopy would elucidate this aspect and allow a more reasoned interpretation of decontamination effected by the scalding process.

Water temperature has been shown to have an important influence on cross contamination at the scalding stage. Essary *et al.* (1958), Mead and Impey (1970), Lahellec *et al.* (1972), and Mulder and Dorresteyn (1977) have reported reductions in numbers of viable microorganisms present on the skin of carcasses following scalding. The highest reductions (up to 3 log₁₀ cycles) were achieved by hard scald treatments. The ability of microorganisms to survive scalding is dependent upon the heat sensitivity of the organism under examination and the time-temperature variables of the treatment. Psychrotrophic bacteria which represent the potential spoilage flora of chill stored carcasses, display low heat resistance and scalded carcasses are free of psychrotrophic contaminants (Clark 1968; Barnes 1960; Notermans *et al.* 1977). *Clostridium perfringens* spores remain viable after either hard or soft scalds and since large numbers of these organisms can be found on live poultry, cross contamination during scalding cannot be prevented. *Enterobacteriaceae* also tend to be heat resistant and as might be expected, salmonellae have been isolated from both scald water and carcasses after scalding at 51-52°C (Lahellec 1975). Büchli *et al.* (1966) showed counts of *Enterobacteriaceae* in scald water were

reduced to undetectable levels by raising the water temperature from 50°C to 62.5°C and Mulder and Dorresteyn (1977) did not find salmonellae in scald water above 56°C. The latter authors also found that fewer salmonellae contaminating the skin of carcasses survived scalding at 60°C compared to carcasses scalded at 51.5°C. However, Notermans and Kampelmacher (1975a) have reported that microorganisms remaining viable on carcasses after scalding were more difficult to remove than those added subsequently, and mesophilic bacteria attached to the skin showed increased heat resistance compared to "unattached" bacteria. Klose *et al.* (1971) have also noted increased heat resistance of bacteria on carcass surfaces following a heat treatment. Both groups of workers concluded that these heat resistant bacteria were protected by their location in the skin, but neither group produced any direct microscopic evidence to support this inference.

The mechanism of scalding influences contamination of carcasses by microorganisms. Lillard (1973) showed carcasses scalded by immersion in hot water were contaminated internally by *Clostridium perfringens*. This organism was found in lungs, heart, liver and edible parts of the carcass. Using tracer organisms he was able to demonstrate the lungs and the digestive tract as the portals of entry. Subsequent studies by Lillard *et al.* (1973) compared steam and immersion scalding procedures and concluded that the steam treatment virtually eliminated contamination of carcasses by *Clostridium perfringens*.

Immediately following scalding, carcasses are mechanically plucked. This process significantly increases the level of contamination of carcass surfaces (Walker and Ayres 1956, 1959; Clark and Lentz 1969a; Lahellec *et al.* 1972; van Schothorst *et al.* 1972). This increase in contamination is in part due to

faecal material pressed out of carcasses by the rotating flexible rubber fingers used in plucking machines (Elliot 1969; Notermans, van Leusden and van Schothorst and Kampelmacher 1975). Mead (1976) noted that these fingers are not easy to clean and hence may carry organisms from one working period to another. Plucking processes are known to be a major cause of cross contamination and this problem has been studied by van Schothorst *et al.* (1972) and Mulder *et al.* (1978). These workers artificially contaminated single carcasses with salmonellae and *E. coli* K12 respectively prior to plucking and showed that the same organisms could be recovered from many carcasses following the contaminated one through the plucking machines. Again, the rubber fingers were implicated in the contamination process. The spread of *Salmonella* contamination during processing has also been noted by Dougherty (1974).

The microbiological hazards of the scalding and plucking processes can be reduced by simultaneous scalding and plucking; a system which utilizes steam or hot water sprays. Veerkamp and Hofmans (1973) and Mulder and Veerkamp (1974) have described and assessed the use of this procedure and noted a large reduction in contamination, especially in the number of *Enterobacteriaceae*.

Unavoidable faecal contamination of the carcass occurs during evisceration procedures (Galton *et al.* 1955; May 1961; Berner and Scholtyssek 1968; van Schothorst *et al.* 1972; Bryan *et al.* 1978; Mackey and Derrick 1979). However, this contamination may be reduced prior to cooling (by up to 0.8 log₁₀ cycle) by judicious use of spray washing (with or without chlorination) after evisceration (Walker and Ayres 1956; Wilkerson *et al.* 1961; Keel and Parmelee 1968; Sanders and Blackshear 1971; Whitehead *et al.*

1972; May 1974; Mulder and Veerkamp 1974; Barnes 1975). Hot spray washing has also been suggested (Thomson *et al.* 1974).

During subsequent processing, contaminants are largely gram negative bacteria derived from wash water, ice and equipment surfaces. Data from three separate studies are compared in Table 1. In each study organisms were recovered by the surface spread technique on non-selective media and identified by similar methods. These contaminants usually include many psychrophilic spoilage types (Barnes 1960). Knoop *et al.* (1971) and Lahellec *et al.* (1975) have demonstrated a build up of numbers of these psychrotrophs during the latter stages of processing, especially during immersion chilling.

Rapid chilling of the carcass following evisceration is therefore essential to avoid the possibility of bacterial multiplication. In commercial practice this is normally achieved by water immersion chilling or air chilling (Thomas 1977). Immersion chilling is the preferred method in Australian poultry processing plants and when properly used can lead to a reduction of bacterial numbers on the carcass (Surkiewicz *et al.* 1969; Mead and Thomas 1973a; Mulder *et al.* 1976; Reddy *et al.* 1978). Continual addition of fresh iced water, possibly with high levels of free chlorine is required to prevent a build up of contamination in the chiller and allow adequate temperature control to minimise the risk of bacterial growth (Peric *et al.* 1971a; Mead and Thomas 1973a; Blood and Jarvis 1974).

The mechanism of carcass contamination during immersion in processing waters was examined by Notermans and Kampelmacher (1974). These authors concluded that motile bacteria attached more readily to broiler carcass skin than non motile bacteria and implicated

TABLE 1

COMPOSITION OF MICROFLORA OF CHICKEN SKIN IMMEDIATELY AFTER
PROCESSING

	Barnes and Thornley (1966) (%)	Barnes ^A (1976) (%)	Daud (1978) (%)
Micrococci	50	-	30
Gram positive rods	14	<10	-
Flavobacteria	14	20	18
Enterics	8	-	8
Pseudomonads	2	<10	26
<i>Acinetobacter/Moraxella</i>	7	50	4
Yeasts and others	5	<10	14

^APsychrophilic flora only.

flagella activity, chemotaxis and extracellular polysaccharide polymers as mechanisms in the contamination process. This inference however does not explain why the bacterial flora of broiler skin immediately after immersion cooling is dominated by non-motile types (Table 2). McMeekin and Thomas (1978) also examined retention of bacteria on broiler skin as a function of time, but were unable to confirm the linear increase with time for motile organisms reported by Notermans and Kampelmacher (1974, 1975b). However, a similar relationship between retention and suspension population density was obtained by both groups of workers. Plots of \log_{10} numbers retained versus \log_{10} suspension population density were in all instances linear with slopes close to unity, irrespective of the motility status of the test organism. Similar results have been obtained for the retention of bacteria on dipslides (McMeekin 1976; Thomas *et al.* 1977) and salivary microorganisms associated with tooth enamel or buccal epithelial cells (Gibbons and van Houte 1975). Therefore the relationship appears to have application for a range of surfaces transiently immersed in bacterial suspensions. On removal from a suspension the test surface retains a film of liquid and the density of bacteria within this film is the same as that in the original suspension (Thomas *et al.* 1977). McMeekin and Thomas (1978) interpreted this relationship as representing a simple dilution effect and concluded that non motile and motile contaminants derived from immersion chiller water were equally well retained within a water film on the skin surface. In both studies, however, any effect of time of immersion is of minor importance compared with the effect of population density on retention. In situations where bacteria accumulate at a surface as a result of chemotaxis or other phenomena, the slope of the

TABLE 2

PROPORTION OF MOTILE AND NON-MOTILE BACTERIA ON CHICKEN SKIN
IMMEDIATELY AFTER PROCESSING

Reference	Motile ^A (%)	Non-Motile ^A (%)
Barnes and Thornley (1966)	26	74
Barnes (1976) ^B	30	70
Daud (1978)	28	72
Thomas and McMeekin ^C	21	79
McMeekin and Thomas (1978)	11	89

^AMotile/non-motile calculated from flora analyses excluding micrococci (autochthonous flora) except for data of McMeekin and Thomas (1978) which was obtained by direct microscopic examination of bacteria recovered from skin.

^BPsychrophilic flora only.

^CUnpublished data.

linear relationship between population density and retention would be expected to deviate from unity (Thomas *et al.* 1977). With undisturbed systems (Adler 1973) which enabled detection of chemotactic responses by motile bacteria toward nutrient sources, slopes of 2 have been reported (Thomas *et al.* 1977). However, on chicken skin, any increase in retention with time is clearly insufficient to significantly affect the population density/retention relationship (McMeekin and Thomas 1978). Thus, from a practical viewpoint, the important consideration is to prevent a build up of bacterial contamination during the immersion chilling and washing procedures, since this increase will proportionately increase carcass bacterial numbers. The relationship between the number of bacteria in the spin chiller and carcass contamination is shown by the work of Mead and Thomas (1973a), Blood and Jarvis (1974) and Peric *et al.* (1971a) which demonstrated that a build up in contamination in the immersion chiller water rapidly leads to an increased level of carcass contamination. In addition, the likelihood of cross contamination is greatly increased when high numbers of bacteria are present in chiller water (Notermans *et al.* 1973). The relationship also suggests the use of counter current spin chillers is appropriate since incoming carcasses meet the cleanest water at the point of removal from the chilling system.

An alternative to immersion chilling is air chilling; a process whereby heat loss from the carcass is effected by a flow of chilled air. Air chilling alone offers no means of reducing carcass contamination derived during evisceration and prior processing procedures, and Mead (1975) and Thomson *et al.* (1975) have reported air chilled carcasses carry higher numbers of microorganisms. However, Knoop *et al.* (1971) has reported lower total viable counts

and psychrophile numbers on carcasses following air chilling as compared to immersion chilling, and Mulder *et al.* (1978) showed air chilling processes reduced cross contamination. The dryness of air chilled carcass skin might be expected to restrict bacterial growth as with New York dressed birds (Barnes 1976), and Knoop *et al.* (1971) showed an increased shelf life of air chilled carcasses compared to immersion chilled carcasses, but Patterson and Gibbs (*pers. comm.* to McMeekin) noted little difference in the flora or the spoilage characteristics.

Chilling by use of water sprays has also been suggested, but has found little commercial application because of high rates of water usage and concomitant effluent disposal problems (Peric *et al.* 1971b; Veerkamp *et al.* 1972; Pietszch and Levetzow 1974; Mead 1975). However, water sprays may be used to advantage to reduce carcass contamination following evisceration and hence reduce the load of organisms entering the spin chiller.

Notermans and Kampelmacher (1975b) showed that a proportion of microorganisms present in the water film on carcass skin surfaces were easily dislodged while the remainder were firmly "attached". Thus mechanical cleaning obtained by spray cooling and/or spin chilling could reduce numbers by tenfold. Similar results were obtained by Mead and Thomas (1973b). To prevent "attachment" from the water film and a build up in the levels of contamination in immersion chillers, Notermans and Kampelmacher (1975b) recommended continuous removal of the water film by spray cleaning especially during plucking and evisceration.

Several approaches have been adopted to develop methods which will reduce, modify or eliminate the contaminant microflora of finished poultry carcasses. A common approach has been to expose

carcasses to different chemicals during the immersion chilling procedures. Chlorine is the most widely used and accepted chemical for this purpose and has been found effective in reducing the bacterial load of chiller water (Thomson *et al.*, 1967; Patterson 1968a; Wahbeck *et al.* 1968b; Sanders and Blackshear 1971; Mead and Thomas 1973a, b; Mead *et al.* 1975; Patterson 1975). However, it is generally accepted that levels of chlorine required to reduce numbers of viable bacteria within chiller water, may not reduce or eliminate all potentially pathogenic organisms contaminating eviscerated carcasses. Mead *et al.* (1975) demonstrated 20 ppm available chlorine in chiller water, reduced counts of faecal (*coli-aerogenes* bacteria) and spoilage bacteria from carcasses by approximately 10-fold after passage through the chilling system, but Patterson (1968c) showed this level of chlorine was ineffective in reducing numbers of faecal streptococci and *Staphylococcus aureus*. Significant reductions in salmonellae contaminating carcasses have only been achieved with high levels of available chlorine (100-200 ppm) (Kotula *et al.* 1967; Thomson *et al.* 1967) while chlorine treatments probably do not eliminate clostridial spores (Dye and Mead 1972). The problems involved in use of chlorine as an antibacterial agent have prompted suggestion of glutaraldehyde as an alternative. Glutaraldehyde has excellent disinfectant properties, is not inactivated by organic matter and is not toxic when used in low concentrations, but its use has not been approved by food authorities. Studies by Thomson *et al.* (1977) and Mast and MacNeil (1978) have shown significant reductions in the bacterial load of experimental chiller systems treated with glutaraldehyde. Glutaraldehyde chilled carcasses were shown to have shelf lives up to 6 days longer than chlorine chilled carcasses.

Many organic and inorganic acids have been evaluated as aids in reducing the bacterial counts of carcasses, thereby prolonging the shelf life of the product (Perry *et al.* 1964; Mounthney and O'Malley 1965; Thomson *et al.* 1967; Patterson 1975). Reduction in numbers by acid treatments is not simply due to effects of exposure to low pH, but is dependent on the acid used and hence Mounthney and O'Malley (1965) showed the greatest prolongation of product shelf life was achieved by use of acetic, adipic or succinic acids in chiller water. A range of other chemical additives have also been evaluated (Islam *et al.* 1978; Islam and Islam 1979) and polyphosphates have been used to control the growth of spoilage bacteria and salmonellae (Spencer and Smith 1962; Elliott *et al.* 1964; Steinhauer and Banwart 1964; Foster and Mead 1976; Thomson *et al.* 1979). Other approaches include:- acid baths followed by heat treatments (Cox *et al.* 1973; Cox, Mercuri, Juven, Thomson and Chew 1974; Juven *et al.* 1974), and carcass pasteurization (Klose *et al.* 1971; Avens and Miller 1972; Cox, Mercuri, Thomson and Gregory 1974; Patterson 1975). However, attempts to destroy contaminant bacteria and potentially pathogenic organisms by heat, and/or chemical treatments are usually ineffective unless the treatment is sufficiently severe to modify the appearance of the exposed carcass surfaces. The use of antibiotics such as oxytetracycline and chlortetracycline (Thornley *et al.* 1960; Thomson *et al.* 1967) in post chill immersion treatments are effective in reducing the level of carcass contamination as well as preventing growth of the normal spoilage microflora, but their use is banned by most food authorities. Radiation sterilization procedures have been found useful in extending shelf life of carcasses (Rhodes 1965; Yndested *et al.* 1972; Mulder *et al.* 1977;

Idziak and Incze 1968). Contaminated chiller water has been used for neck fluming stages following diatomaceous earth filtration and chlorination (Lillard 1978a, b). A novel approach to the problem of shelf life extension has been repression of the spoilage microflora by lactic acid bacteria (Raccach and Baker 1978, 1979; Raccach *et al.* 1979). However, extremely high levels ($>10^8$ cells/g meat) of a mixed inoculum of *Pediococcus cerevisiae* and *Lactobacillus plantarum* were required to inhibit the growth of spoilage bacteria and extend shelf life of deboned poultry meat by 2-3 days (Raccach *et al.* 1979).

(c) Methods Used to Assess Carcass Contamination

The major sites of contamination of dressed eviscerated poultry carcasses are exposed surfaces (skin, visceral linings, cut skin and muscle etc.) and the extent of contamination is normally assessed by a determination of numbers of microorganisms present on the skin. A number of techniques (and their modifications) are available which allow assessment of the level of skin contamination. These include:- contact plating (Silliker *et al.* 1957), swab techniques (Walker and Ayres 1959; Morris and Ayres 1960; Kraft and Ayres 1961; May 1961; Kotula *et al.* 1962; Mercuri and Kotula 1964; Kotula 1966; Notermans *et al.* 1976; and others), whole or part bird rinses (Yacowitz *et al.* 1957; Mallman *et al.* 1958; Goresline and Haugh 1959; Corey and Byrnes 1963; Notermans, Kampelmacher and van Schothorst 1975a, b), a spray rinse (Clark 1965a, b), skin scraping (Williams 1967; Barnes *et al.* 1973), skin tissue removal followed by rinsing (also known as the cut and rinse or dip technique)(Lochhead and Landerkin 1935; May *et al.* 1962; Notermans, Kampelmacher and van Schothorst 1975a, b), skin tissue removal followed by maceration, abrasion or stomaching (Barnes and Shrimpton 1958; Patterson and Stewart 1962; Avens 1968; Avens and Miller 1970a, b; Patterson 1971a, b; Emswiler *et al.* 1977) and whole carcass maceration (Leistner and Szentkuti 1970).

Advantages and disadvantages have been claimed for each method and comparative studies have been made (Barnes and Shrimpton 1958; Mallman *et al.* 1958; Seekins *et al.* 1958; Fromm 1959; Kinsely and Mountney 1966; Avens 1968; Surkiewicz *et al.* 1969; Avens and Miller 1970a; Patterson 1972; Mead and Thomas 1973a, b; Cox and Blankenship 1975; Cox *et al.* 1976; Notermans, Kampelmacher and van Schothorst 1975a, b; Emswiler *et al.* 1977; Thomson and Bailey 1978). The diversity of methods has also been reviewed or surveyed by Patterson and Stewart (1962), Favero *et al.* (1968), Patterson (1971a, b, 1972), Barnes *et al.* (1972), Kitchell *et al.* (1973), Baldock (1974), Barnes (1975), Vanderzant *et al.* (1976) and Gerats and Snijders (1977).

No single technique is preferable to all others in all situations and each may be of particular value in the determination of different parameters of contamination. The limitations of the majority of the methods arise because:-

- (1) there is considerable variation in the numbers and types of microorganisms found on different parts of the carcass;
- (2) different types of bacteria require specialised cultural requirements;
- (3) different methods exert different physical forces to enable release of microorganisms from the skin;
- (4) some microorganisms may be located in specific inaccessible locations; and
- (5) methods differ in their ability to remove bacteria from the skin to a suitable diluent or transport medium.

Although Clark (1968) stated bacteria were uniformly spread over the carcass surfaces and that any sample area was suitable, Ziegler *et al.* (1954) found more bacteria on skin from under the wing and the area around the vent. Kotula (1966) found more

microorganisms on the thighs than on the breast or drumsticks, but the level of contamination of samples from the same parts of opposite sides of the same bird, or several birds from the same lot, were not significantly different. Both Cox, Mercuri, Juven, Thomson and Chew (1974) and Cox *et al.* (1976) confirmed these findings for the total viable count and the *Enterobacteriaceae* count. However, Patterson (1972) found that neck skin and to a lesser extent skin from the vent and the back areas were more contaminated than skin from the leg or under the wing. Similarly Notermans, Kampelmacher and van Schothorst (1975a, b) and van Schothorst *et al.* (1976) found higher total viable counts and *Enterobacteriaceae* counts on neck skin compared to breast and near vent sites. To overcome the variation in distribution of the contaminant flora, Barnes *et al.* (1973) recommended a method developed by Barnes and Shrimpton (1958) in which 2g of underwing skin and 3g of near vent skin (representing approximately 50cm²) are sampled. Other workers have preferred to use neck skin, particularly for the isolation and enumeration of food poisoning bacteria (Mead and Impey 1970; Simonsen 1971; van Schothorst *et al.* 1976). Neck skin has the added advantage that a destructive technique may be used without downgrading the carcass.

Some diluents (such as distilled water, tap water, phosphate water and even physiological saline), used as a vehicle for transfer of microorganisms from the skin to a suitable plating media, may cause rapid destruction of bacteria (Straka and Stokes 1957) and lead to serious errors in a quantitative determination of bacteria in foods. However, Straka and Stokes (1957) showed that the degree of error depended on the number of bacteria present, exposure time to the fluid and the quantity of organic

matter in the fluid. Peptone water (0.1% w/v) did not cause any appreciable destruction of bacteria for one hour. However, studies by Avens and Miller (1970b) have shown peptone water and physiological saline recovered bacteria equally well from poultry skin following blending.

Although there is no direct experimental evidence to demonstrate the location of contaminant bacteria on the skin, Avens and Miller (1970a) have suggested that some of these microorganisms may be located within the skin and Patterson (1971a, b) and Barnes *et al.* (1973) have implicated the feather follicles as location sites. Based on these suggestions, it is not unreasonable to expect that impression techniques, swabbing and rinsing may not be effective in removing those bacteria not superficially located on the skin surface. The ability of swabs, contact methods and skin scraping techniques to remove all those bacteria lying superficially on the skin must be considered unlikely.

Direct contact plating is generally regarded as an inadequate sampling procedure for carcass surfaces. The basic technique suffers three major disadvantages:-

1. clumps of bacteria removed from the skin are counted as a single colony forming unit;
2. even with moderately contaminated carcasses individual colonies are usually not distinguishable because of confluent growth (Seekins *et al.* 1958);
3. skin surface bacteria are incompletely removed for enumeration (Fromm 1959).

However, the first two disadvantages may be overcome by blending the contact surface in an appropriate fluid prior to dilution and plating (Sharf 1966).

Although swab samples recover only a fraction of the total flora present on chicken skin (Seekins *et al.* 1958; Fromm 1959; Patterson and Stewart 1962; Avens 1968; Avens and Miller 1970a; Patterson 1971a and 1972; Barnes 1976; Cox *et al.* 1976), the technique remains attractive in simplicity of operation and in being non destructive. However, Seekins *et al.* (1958), Fromm (1959) and Avens and Miller (1970a) have noted that the method gives incomplete and inconsistent removal of skin surface bacteria for enumeration; the inconsistency may be caused by variable pressure applied to the swab, variable swab moisture content and variable fat content and texture of the skin surface. Thus, where the technique is to be used, duplicate or triplicate swabs are recommended. This point is well illustrated by data of Patterson (1971a, b), who showed that successive swabs removed 38%, 43%, 9%, 8% and 2% of the total recoverable flora. Avens and Miller (1970a) used swab and maceration techniques to evaluate the effectiveness of a scald procedure and notes that swabs removed a greater proportion of the total skin flora from heavily contaminated carcasses than from carcasses with a lower level of contamination. They concluded the use of swab sampling to test the effectiveness of bactericidal treatments would over-estimate the percentage bacterial kill. Mallman *et al.* (1958) compared swab sampling with rinse techniques and found that although the numbers of bacteria recovered by swabbing were subject to wide variation, counts in general paralleled those obtained by rinsing. Hence swab sampling is useful in determining trends of contamination or microbial growth.

Whole bird rinses have been recommended when the aim is to recover microorganisms (such as *Salmonella*) present in small numbers

and unevenly distributed over the carcass surface (Barnes *et al.* 1973). This technique also allows sampling of the body cavity.

Disadvantages of the technique include:-

1. total viable counts cannot be related with any accuracy to a unit area of skin on a carcass (Goresline and Haugh 1959);
2. total carcass counts are of limited value in comparisons unless all carcasses examined are the same size (Avens and Miller 1970a);
3. removal of bacteria from carcass surfaces is incomplete and inconsistent (Avens and Miller 1970a) and Fromm (1959) has suggested the method does not furnish enough friction to effectively remove bacteria from the skin and skin fat prevents water from contacting and dislodging many bacteria;
4. the method is cumbersome when applied to large carcasses such as turkeys.

Simonsen (1971) found shaking carcasses in diluent for 30 sec gave greater recovery than dipping a carcass 15 times in 2 litres of diluent as proposed by Leistner and Szentkuti (1970). This is not surprising in view of the effect of suspension population density on the retention of bacteria on skin (Notermans and Kampelmacher 1974; McMeekin and Thomas 1978). Mead and Thomas (1973b) have noted that without the use of chlorine in spin chiller systems, counts of faecal bacteria showed less reduction after chilling than was obtained by the rinse method. Again this result is not surprising, considering that, in effect, the study compared reduction of counts following immersion of a contaminated carcass into an already contaminated rinse solution with immersion of a similar carcass into a sterile rinse solution. Hence these results do not support their inference that "some microorganisms are more

deeply situated in the skin". A variant of the rinse technique which impinges a spray against a defined area of skin was proposed by Clark (1965a, b). The technique was reported to yield higher total bacteria counts than either the swab or blending techniques when used on freshly contaminated foods, but does not appear to have been extensively adopted.

The cut and rinse methods, particularly when the rinsing technique is aided by an abrasive material (sand, glass fragments or beads) are reported to give superior recovery of microorganisms from chicken skin compared to swab techniques (Fromm 1959; Patterson and Stewart 1962; Patterson 1972; Cox *et al.* 1976). Similarly skin scraping techniques (Patterson and Stewart 1962; Williams 1967) recover more microorganisms than swabbing.

A comparison of the various methods available for enumeration of the skin microflora shows that greatest recovery is achieved by maceration of the sample (Fromm 1959; Avens 1968; Avens and Miller 1970a; Mead and Thomas 1973b). Cox *et al.* (1976), however, equated recoveries obtained by blending with shaking the skin sample in diluent with or without glass beads. Shaking was said to be superior for the examination of crust frozen carcasses for *Enterobacteriaceae*, but gave equivalent results to blending for total viable bacteria. The latter result is difficult to explain if one accepts the "attachment/detachment" process is a function of the chicken skin surface rather than the bacteria concerned, and further illustrates the vagaries of sampling and enumeration techniques. Avens and Miller (1970a) have stated that the skin blending technique enabled enumeration of bacteria on the skin as well as those which "adhere more firmly to intact skin surface cells and bacteria in the follicles and subsurface layers".

Hence, skin blending allows enumeration of bacteria, in an area of skin, with more accuracy and precision than other methods. However, the technique is disadvantaged by significant heat input to the sample homogenate and the need to sterilize blender cups before reuse.

The Colworth Stomacher (A.J. Seward and Co. Ltd., London) is a useful alternative to the laboratory blender and Emswiler *et al.* (1977) have evaluated its use for enumeration of microorganisms on poultry skin. Their results showed counts obtained by blending were not significantly different from those obtained by stomaching. Advantages of the stomacher include minimal sample temperature increase, hence low microbial cell damage. Convenient single use sterile plastic sample bags enable rapid examination of a number of samples.

The work of Notermans, Kampelmacher and van Schothorst (1975a, b) represents a significant advance in the rationale of the use of different sampling procedures. Following the considerations of earlier work (Notermans and Kampelmacher 1974, 1975a, b) on the attachment and detachment of bacteria from chicken skin, they concluded that the dip (cut and rinse) method provided insufficient information about hygiene considerations along the whole processing line. Although this method detected contaminants from immersion chilling, earlier contamination can be enumerated accurately only by skin maceration. Similarly, van Schothorst *et al.* (1976) concluded that drip counts from defrosted frozen broiler carcasses represented only contamination occurring at the end of the slaughter line. These workers also expressed the opinion that bacteria present inside the carcass, or those which are not easily removed from the skin surface, play only a minor role as

far as public health risk is concerned. However, bacteria present in thaw water or those which can be easily rinsed from the carcass are potentially dangerous because they are likely to be involved in cross contamination of utensils or other foods.

Thus it is evident that great variability exists not only in the distribution of microorganisms on carcass surfaces, but also in their recovery by various sampling and enumeration procedures. Maceration procedures yield the greatest recovery of bacteria from skin, but in a practical situation where other considerations, such as carcass damage, may have to be taken into account, swabs or rinses may be considered. Therefore, it is important that the microbiological or quality control personnel can relate the significance of a result obtained by any technique to the concept of good manufacturing practice.

(d) The Development of Microorganisms on Carcass Surfaces
During Storage

Mossel (1971) has drawn a clear distinction between contamination and microbial proliferation in foods. The fate of the initial contaminant microflora depends on various factors listed as extrinsic, intrinsic, processing and implicit (Mossel 1971). For broiler carcasses, temperature of storage is the primary criterion (an extrinsic factor) controlling the rate of spoilage, although this is obviously potentiated by other factors (such as the number of initial contaminants able to grow well at the storage temperature, the gaseous environment, the water activity (a_w) and microbial interactions). If the carcasses are frozen, microbial growth and activity ceases and microbiological problems are usually only encountered during

defrosting and the culinary stages of preparation. However, if ambient temperatures are allowed to rise, carcass spoilage occurs at a faster rate and the advantages of good plant hygiene can be easily lost as a result of inadequate temperature control during transport and retail storage.

The initial contaminant microflora of finished poultry carcasses usually comprises a large number of genera (Barnes and Thornley 1966; Barnes 1976; Daud *et al.* 1979). During chill storage of carcasses in oxygen permeable bags, *Pseudomonas* strains rapidly become the predominant spoilage microorganisms together with a few other gram negative bacteria such as *Acinetobacter-Moraxella* types (Ayres *et al.* 1950; Barnes and Shrimpton 1958; Ayres 1960; Nagel *et al.* 1960; Barnes and Thornley 1966; McMeekin 1975, 1977; Barnes 1976; Daud *et al.* 1979). However, Barnes and Thornley (1966) showed that although the spoilage flora of carcasses stored at 10°C was dominated by *Pseudomonas* strains (33%), this group formed a smaller proportion of the spoilage flora of carcasses stored at 15°C (11%). The microflora of carcasses stored at 15°C was still predominantly gram negative, but the majority of these microorganisms were either *Acinetobacter* or *Enterobacteriaceae*. Barnes *et al.* (1978) noted a large increase in the number and proportion of yeasts present on turkey carcasses stored at -2°C until spoilage and suggested that reduced carcass surface a_w caused by partial freezing of the substrate restricted the growth of gram negative psychrophilic bacteria. Yeast spoilage has been observed previously by Yacowicz *et al.* (1957), Barnes and Shrimpton (1958) and Wells and Stadelman (1958) and others, when growth of many of the spoilage bacteria was delayed or restricted by treatment of

chicken carcasses with chlortetracycline.

At spoilage, pigmented and non pigmented pseudomonads (typically *Pseudomonas* groups I or II according to the scheme of Shewan *et al.* 1960) form more than 70% of the genera isolated from poultry skin or muscle stored at chill temperatures (1° - 4° C) (Barnes and Thornley 1966; McMeekin 1975, 1977; Barnes 1976; Daud *et al.* 1979). At higher storage temperatures both group I and II type pseudomonads represented a smaller fraction of the flora of carcasses at spoilage (Barnes and Thornley 1966). *Alteromonas putrefaciens* (formerly *Pseudomonas putrefaciens*) has been isolated from carcasses stored at chill temperatures (Barnes and Thornley 1966; McMeekin 1975, 1977; Daud *et al.* 1979) and at 10° C and 15° C (Barnes and Thornley 1966). However, this organism forms only a small proportion of the spoilage microflora of carcasses stored at any temperature and it is interesting that Daud *et al.* (1979) did not isolate *Alteromonas putrefaciens* from the skin of carcasses stored at 2° C.

The physico-chemical nature of the poultry substrate has been shown to exert an important selective influence upon the potential spoilage microflora. Barnes and Impey (1968) and McMeekin (1975, 1977) have demonstrated marked differences in the flora of spoiled chicken leg and breast muscle. *Alteromonas putrefaciens* and *Acinetobacter* types were found to be unable to compete with *Pseudomonas* group I and II strains on breast muscle, whereas these microorganisms grew well on leg muscle. This difference has been attributed to the lower pH of breast muscle (approximately 5.8) compared to leg muscle (6.5). There is, however, no evidence as to whether the pH of the skin or the exudate within feather follicles relates at all to the pH of the underlying muscle

(Barnes and Impey 1968) and hence any selective influence of pH on the microflora of intact breast and leg skin is unknown. Fromm and Monroe (1965) have noted only a small difference in rinse water pH of breast and thigh skin areas (6.3 and 6.6 respectively) after processing and a similar increase in skin rinse water pH during storage as observed for muscle tissue.

Packaging of processed foods in gas impermeable films significantly inhibits or delays proliferation of the normal microflora associated with aerobic spoilage. The rate of spoilage is dependent on many factors such as the vacuum level, packaging film gas permeability and the presence of carbon dioxide (CO₂) or other gases (Bailey *et al.* 1979). Vacuum packaging leads to an increase in the partial pressure of CO₂ in non sterile foods due to initial respiration of aerobic microorganisms and fermentative dissimilation processes in anaerobes, although the CO₂ level over packaged foods may be increased artificially (Mosse 1977). Inhibition of food spoilage microorganisms by CO₂ and the consequent increase of shelf life of poultry carcasses packaged in oxygen (O₂) impermeable films with or without vacuum or added CO₂ has been reported by a large number of workers (Valley 1928; Coyne 1933; Haines 1933a, b; Smith 1934; Ogilvy and Ayres 1951; Wells *et al.* 1958; Shrimpton and Barnes 1960; Shank and Lundquist 1963; Taylor *et al.* 1966; Gardner and Carson 1967; King and Nagel 1967; Wahbeck *et al.* 1968b; Clark and Lentz 1969b; Baran *et al.* 1970; Rey and Kraft 1971; Clark and Burki 1972; Roth and Clark 1972; Gardner *et al.* 1977; Bailey *et al.* 1979). This form of packaging has been noted to inhibit or delay the aerobic pseudomonads and *Acinetobacter* types which characterise spoilage of poultry carcasses (Barnes and Shrimpton 1958; Shrimpton and Barnes

1960; Wahbeck *et al.* 1968b; Barnes and Melton 1971; and others), and Rey and Kraft (1971) noted an absence of fluorescent pseudomonads on vacuum packaged carcasses. Further, Barnes and Melton (1971) showed inhibition of pigmented and non pigmented strains of *Pseudomonas* spp. on carcasses packaged in O₂ impermeable film. *Alteromonas putrefaciens* and *Microbacterium thermosphactum* were the predominant microflora when these carcasses were stored at 10°C and 20°C. Although similar trends have been noted for vacuum packaged dark, firm, dry meat stored at 10°C (Gill and Newton 1979), it is interesting that *Alteromonas putrefaciens* grew well under the CO₂ concentration prevailing, especially since Mossel (1977) has noted the inhibitory nature of CO₂ against gram negative bacteria.

Bacteria induced changes leading to off odour of whole birds during refrigerated storage occurs first on the surface of the skin, and early studies emphasised the importance of the skin as a substrate because it was the tissue most readily colonized by psychrotolerant bacteria (Lockhead and Landerkin 1935; Ziegler *et al.* 1954). Further, Ziegler and Stadelman (1955) and Essary *et al.* (1958) showed that hard scalded carcasses spoiled at a faster rate than soft scalded carcasses, and Clark (1968) examined the effect of scalding treatments on the growth of various psychrotolerant bacteria on skin tissue. *Pseudomonas* spp. grew best on hard scalded skin while *Achromobacter* spp. grew best on unscalded skin; soft scalded skin gave intermediate growth rates for both groups of bacteria. More recently, Clark (1970) demonstrated more rapid growth rates of psychrotolerant pseudomonads and achromobacteria on skin compared to other poultry tissues (e.g. visceral lining, leg and breast muscle and muscle fascia). Cell

generation times were nearly identical for all tissues examined and differences in the overall growth rates were attributed to differences in duration of the lag periods. Additionally, tissue pH was found not to be a factor affecting the length of the lag period. Hence the composition, structure and degree of scald damage appear to be significant factors affecting the growth of the spoilage microflora of stored poultry carcasses.

The development of techniques which allow the excision of sterile tissue from carcass meats, has provided a means of characterising that portion of the microflora responsible for organoleptic changes associated with spoilage of flesh foods. Hence, work with fish substrates (Adams *et al.* 1964; Herbert *et al.* 1971) has demonstrated that the development of off odours characteristic of spoiling flesh foods occurs as a result of the growth and metabolism of a restricted group of psychrophiles. The technique has been partially extended to the examination of the organoleptic potential of bacterial isolates associated with spoilage of poultry carcasses. McMeekin (1975, 1977) and Daud *et al.* (1979) showed only a small proportion of the total spoilage microflora of cut muscle sections and the skin surface can produce off odour from a muscle substrate (Table 3). Although the data of McMeekin (1975) showed an apparent selection for spoilers during storage, this result was probably due to the isolation procedures employed (colonies picked from nutrient agar after 7 days at 2°C). The procedure recovered only a few psychrophilic bacteria, most of which were off odour producers as well as possessing the fastest growth rates. However, unlike muscle tissue, sterile intact skin cannot be obtained (Daud *et al.* 1979) and hence studies to date have not been able to assess the organoleptic potential of skin

TABLE 3

OFF ODOUR PRODUCERS AS A PROPORTION OF THE PSYCHROPHILIC FLORA
ON CHICKEN BREAST AND LEG MUSCLE AND SKIN DURING STORAGE AT 2°C.

Days	Breast ^A muscle (%)	Leg ^B Muscle (%)	Skin ^C (%)
0	15	9	11
4	26	17	12
8	46	17	9
12	66	15	13
16	80	21	16

^AMcMeekin (1975)

^BMcMeekin (1977)

^CDaud (1978)

surface psychrotrophic isolates on their original substrate. Thus Daud *et al.* (1979) used breast and leg muscle to test off odour production by their skin isolates. Only Clark and coworkers (Clark 1970; Adamcic and Clark 1970a, b; Adamcic *et al.* 1970) have attempted to prepare skin or skin macerates to examine the growth of various psychrotolerant bacteria on this substrate. Barnes (1960) and Clark (1968) have noted that immediately after scalding procedures, carcass skin is free of viable psychrophilic contaminants and hence it may be possible to obtain psychrophile free skin by careful heat treatment of skin samples. This skin substrate could be easily used for off odour production tests.

The spoilage of meat, poultry and fish stored at refrigeration temperatures is attributed to microbial activity (Ayres *et al.* 1950; Shewan and Jones 1957; Adamcic *et al.* 1970; Herbert *et al.* 1971; Ingram and Dainty 1971; Jay 1972; Herbert and Shewan 1976; Shewan 1976) and not to tissue autolysis (Herbert *et al.* 1971; Ingram and Dainty 1971; Shewan 1976). It is generally agreed that while autolytic chemical changes are considerable, they appear to be relatively unimportant organoleptically in spoiling flesh foods (Partmann 1966). Clark (1968) and Adamcic *et al.* (1970), in an examination of the growth of psychrotolerant pseudomonads and achromobacteria on poultry skin showed that enough free amino acids and other low molecular weight nitrogenous compounds were available in skin to allow excellent growth and off odour production. These compounds were utilized without the need for protein degradation. Similarly, Jay (1967) and Jay and Kontou (1967) reported this result for the spoilage of ground beef flesh. However, a large number of studies have shown *Pseudomonas* spp., associated with

spoiled flesh foods, possess extracellular proteolytic enzymes, but their role in spoilage is not well known (Jay 1967; Jay and Kontou 1967; Rey *et al.* 1969; Adamcic and Clark 1970; Adamcic *et al.* 1970; Barnes and Melton 1971; Rey and Kraft 1971). Evidence suggests these extracellular enzymes may only bring about significant changes in meats when the bacteria are present at near spoilage levels (Adamcic and Clark 1970; Adamcic *et al.* 1970; Borton *et al.* 1970a, b; Hasegawa, Pearson, Price, Rampton and Lechowich 1970; Tarrant *et al.* 1971; Dainty *et al.* 1975; Chung and Kim 1976). This evidence is supported by the negative results of Lea *et al.* (1969a), Lea *et al.* (1969b), Fishwick and Lea (1969), Griffiths and Lea (1969) and Stevens and Lea (1969) which showed no off odours were detected with bacterial counts below 10^7 - 10^8 /g of muscle and autolytic changes were greater than those induced by the microorganisms, although these changes were unimportant organoleptically.

Organoleptic spoilage of flesh foods is due primarily to off odours which are directly related to the volatile chemicals produced by microorganisms (Lee *et al.* 1979). The types of microorganisms present during spoilage are responsible for the chemicals produced, and different conditions favour the growth of different microorganisms and hence affect the nature of spoilage and off odour (Barnes 1976). Generally spoilage odours have only been subjectively described (Ayres *et al.* 1960; Herbert *et al.* 1971; Ingram and Thornley 1959; McMeekin 1975, 1977) and only recently have attempts been made to identify specific volatiles present at spoilage and relate them to the causative organism (Freeman *et al.* 1976).

Most studies of spoilage odours have been confined to fish

substrates, while poultry and other carcass meats have received little attention. Herbert *et al.* (1975) has shown that hydrogen sulphide, methanethiol and dimethyl sulphide were the main compounds responsible for the strong off odour of chilled cod. Dimethyl disulphide, as well as these sulphide compounds, were associated with chill stored commercial fish species from Queensland (Gillespie 1977). A number of other organoleptically less important volatiles contributing to off odour in spoiling chill stored fish have been noted. A musty potato-like odour which commonly developed in chill stored cod, haddock, flounder and halibut during the early stages of spoilage is caused by a pyrazine derivative (2 methoxy-3-isopropylpyrazine) (Miller *et al.* 1973a). Trimethylamine has been associated with "fishy odor" of some spoiled fish (Stansby 1962; Davies and Gill 1936). Lea *et al.* (1969a) has noted the presence of hydrogen sulphide in spoiling poultry meat and Nicol *et al.* (1970) found hydrogen sulphide associated with the spoilage of packaged beef. Freeman *et al.* (1976) evaluated the off odours produced by spoilage microorganisms present on refrigerated chicken breasts. Hydrogen sulphide, methanethiol, dimethyl sulphide, dimethyl disulphide, methyl acetate, ethyl acetate, heptadiene, methanol and ethanol were produced on breasts stored at both 2⁰ and 10⁰C. Xylene, benzaldehyde, 2,3-dithiahexane were detected only on samples stored at 2⁰C while methyl thiolacetate, 2-butanone and ethyl propionate were associated with spoilage at 10⁰C. Castell and Greenough (1957) described a fruity odour associated with spoiled fish, however, these authors did not relate the odour to any particular volatile chemical.

A number of workers have studied the production of spoilage type odours by the action of pure cultures of spoilage bacteria on

sterile muscle sections. *Achromobacter* and *Pseudomonas* species were shown to be primarily responsible for the sulphide like odours of stored cod (Castell and Anderson 1948; Castell *et al.* 1949; Castell and Mapplebeck 1952; Shewan *et al.* 1960b). Miller *et al.* (1973c) found *Pseudomonas putrefaciens* generated propionaldehyde, methanethiol, dimethyl sulphide, dimethyl trisulphide, 3 methyl butanol and triethylamine when grown on fish muscle. Isolates of *Achromobacter* produced the same volatiles except for triethylamine while *Pseudomonas fluorescens* produced methane-thiol and dimethyl disulphide as the major volatile components. However, Herbert and Shewan (1975, 1976) in an examination of off odours produced by pure cultures of *Alteromonas putrefaciens*, *Pseudomonas* spp. (belonging to groups I and II of Shewan's classification) and certain *Vibrio* spp. when inoculated onto sterile cod muscle, concluded that hydrogen sulphide, methanethiol and dimethyl sulphide were the major spoilage volatiles. *Pseudomonas perolens* produced a substituted pyrazine derivative (methyl-3-isopropyl pyrazine), which was implicated as responsible for the musty potato-like odour produced on stored fish (Miller *et al.* 1973a), but also produced a number of other volatiles when grown on fish muscle (e.g. methanethiol, dimethyl disulphide, dimethyl trisulphide, 3-methyl-1-butanol, butanone and 2-methoxy-3-secbutyl pyrazine). Castell and Greenough (1959) and Castell *et al.* (1959) noted *Pseudomonas fragi* was responsible for the fruity odour sometimes detected on spoiling chill stored fish. This fruity odour was caused by ethyl esters of acetate, butyrate and hexanoate (Miller *et al.* 1973b). This organism also produced other volatile compounds including methane thiol, when grown on sterile fish muscle. As with fish substrates, several characteristic spoilage odours have

been noted when pure cultures of bacteria isolated from spoiled poultry tissue are grown on sterile chicken muscle (Table 4).

Alteromonas putrefaciens is normally responsible for production of sulphide-like odours on chicken muscle sections at 2°C, although this property has been noted for some pigmented and non-pigmented pseudomonads (McMeekin 1975, 1977; Freeman *et al.* 1976; Daud *et al.* 1979). Thus, McMeekin (1975, 1977) and McMeekin and Patterson (1975) have shown hydrogen sulphide is produced primarily by *Alteromonas putrefaciens*, while both pigmented and non-pigmented pseudomonads produced methanethiol (Freeman *et al.* 1976; Daud *et al.* 1979) from muscle sections. However, McMeekin (1975, 1977) and Daud *et al.* (1979) also noted that some non-pigmented pseudomonads produced fruity ester-like odours, but these organisms may also produce at least one sulphur compound (Freeman *et al.* 1976). Non-pigmented strains of *Pseudomonas* spp. may also produce an "evaporated milk" odour when grown on muscle at 2°C, while some strains of *Acinetobacter* may cause "fishy" odours (McMeekin 1975, 1977). Flavobacteria and enteric bacteria have also been implicated in production of sulphur volatiles from muscle stored at 5°C (McMeekin *et al.* 1978).

The study of volatiles involved in off odours is extremely complex especially since many of the compounds present can be detected olfactorily at concentrations of ng/g. Generally, the more volatile sulphur compounds (hydrogen sulphide, methanethiol and to a certain extent dimethyl sulphide and dimethyl disulphide) are the major volatiles responsible for the spoilage odours of most flesh foods stored at chill temperatures. More gas chromatography/mass spectrometry studies as described by Freeman *et al.* (1976) and Lee *et al.* (1979), in combination with pure culture studies on sterile substrates, are required to

TABLE 4

SOME OFF ODOURS PRODUCED ON CHICKEN MUSCLE BY SPOILAGE ORGANISMS.

Odour	Substrate	Organism(s) responsible
Sulphide-like	leg muscle	<i>Alteromonas putrefaciens</i> ^A
	leg, breast muscle	Enteric types ^B
	leg, breast muscle	<i>Pseudomonas</i> group I ^{A, B} (e.g. fluorescent types)
	leg, breast muscle	<i>Pseudomonas</i> group II types ^{A, B} (e.g. non-pigmented)
	leg, breast muscle	<i>Pseudomonas</i> group III/IV types ^B
Fruity-ester-like	leg muscle	<i>Acinetobacter/Moraxella</i> -like ^B
	leg, breast muscle	<i>Pseudomonas</i> group II ^{A, B} (e.g. <i>P. fragi</i>)
Evaporated milk	leg, breast muscle	<i>Pseudomonas</i> group II types ^{A, B}
Fishy	leg muscle	<i>Acinetobacter</i> ^A

^AFrom McMeekin (1975, 1977).^BFrom Daud *et al.* (1979).

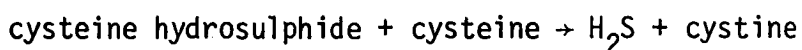
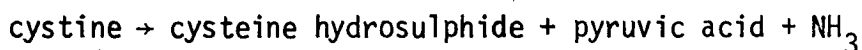
characterise the volatiles, microorganisms and the substrates responsible for off odours produced during chill storage of poultry products.

The mechanism of production of volatile sulphur compounds by microorganisms has received much attention and the subject has been reviewed by Kadota and Ishida (1972) and examined in detail by Jocelyn (1972). Herbert and Shewan (1975) have pointed out that the precursors of volatile sulphides in several food products have already been determined, but much of the data is related to cooked or canned foods. Data related to precursors of volatile sulphur compounds in unprocessed foods is limited mainly to dairy products. Only Herbert and Shewan (1975, 1976) have provided definitive evidence for precursors in flesh foods. Hydrogen sulphide was found to be produced from cysteine by bacteria, growing on cod muscle or by the action of cell free extracts of sulphide producing bacteria on radioactively labelled cysteine *in vitro*. Similarly, methanethiol was produced from methionine while dimethyl sulphide was said to have been formed by condensation of two methanethiol molecules. Other sulphur amino acids were not involved in volatile sulphide production; glutathione disappeared too rapidly from both sterile and spoiling cod muscle for it to be a direct precursor and was probably hydrolysed to glutamic acid, glycine and cysteine. Taurine was totally resistant to degradation by either bacterial or autolytic enzymes. Their data showed that autolytic enzymes in cod muscle were unable to metabolise cyst(e)ine or methionine with the subsequent release of volatile sulphur compounds.

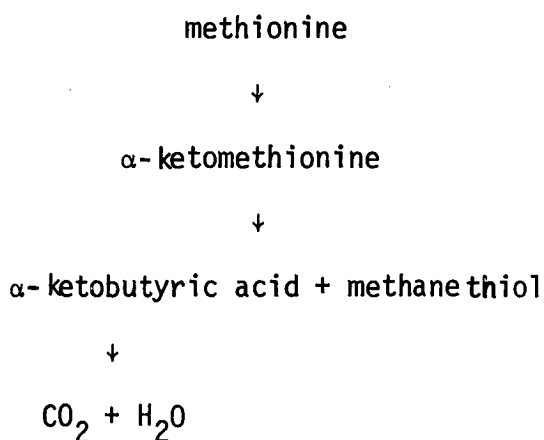
Chatagner and Sauret-Ignazi (1956) have suggested hydrogen sulphide may be produced enzymatically from cyst(e)ine as follows:-



However, Jocelyn (1972) noted that in fact the true reactant is cystine, but this is required only in catalytic amounts because the sulphur of the first formed hydrosulphide intermediate oxidises more cyst(e)ine to cystine during the reaction *viz:-*



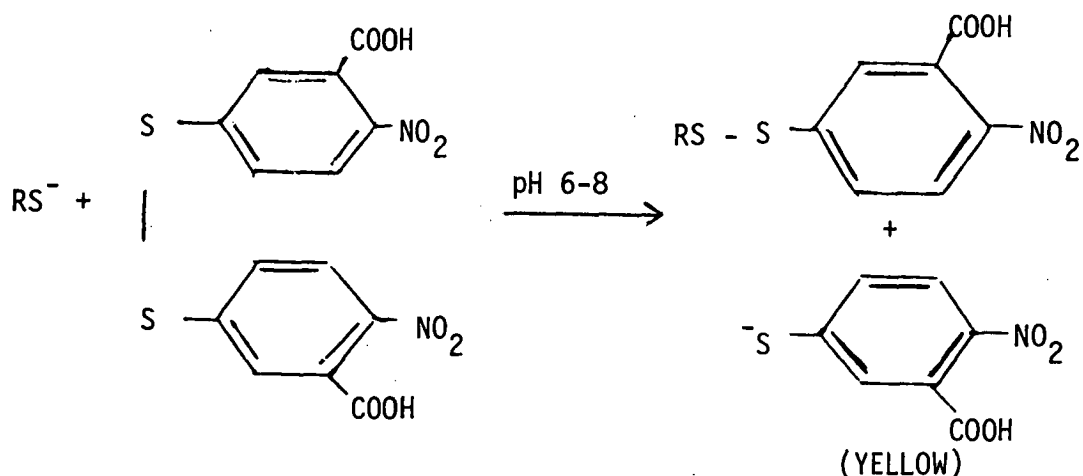
Decomposition of methionine by bacteria to methanethiol has been extensively examined by Segal and Starkey (1969). Methionine is first deaminated and the product demethylated to produce methanethiol as follows:-



Similarly, ethionine may be converted to ethane thiol. There is evidence to suggest a source of energy is required for the demethiolation of α -ketomethionine (Ruiz-Herrera and Starkey 1969) and Segal and Starkey (1969) showed increased production of methanethiol from methionine by some fungi and bacteria (including *Pseudomonas* spp.), when an energy source such as glucose was included in test media. Without this energy source, intermediates such as α -ketomethionine and α -hydroxy- τ -methyl mercaptobutyric acid accumulated. Methanethiol produced by demethiolation of α -ketomethionine may be converted by autoxidation or enzymatic oxidation (Segal and Starkey 1969) to dimethyl disulphide.

The exact mechanism of formation of dimethyl sulphide in spoiling flesh foods is not well established. However, it seems likely that this sulphide is produced from dimethyl- β -propiothetin or by methylation of methane thiol (Kadota and Ishida 1972).

Methods for the isolation and detection of sulphide-producing microorganisms in foods have relied on the use of lead or iron salts. These are of limited value, however, since they only enable detection of those organisms producing large amounts of hydrogen sulphide (Clarke 1953; Levin 1968; Herbert *et al.* 1971; McMeekin and Patterson 1975; Daud *et al.* 1979). Other heavy metal salts are inappropriate because of lethal toxic effects. However, Gillespie (1977) and Sharpe *et al.* (1977) used a modification of Ellman's reagent (Ellman 1959) to detect volatile sulphur-producing bacteria in spoiling fish and cheese. This reagent, 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) reacts specifically with aliphatic thiols such as hydrogen sulphide and methane thiol to yield a highly coloured aromatic thiol as follows:-



McMeekin *et al.* (1978) devised a method, based on DTNB, to allow rapid detection of bacteria capable of producing volatile sulphur compounds. However, the technique will not detect sulphides such as dimethyl sulphide as suggested by these authors, since the reagent can only react with aliphatic thiols. DTNB has also been

used to quantify methane thiol production (Laakso 1976; Law and Sharpe 1978).

(e) Histological/Microbiological Relationships

Although the nature of the bacterium-skin interaction is of obvious importance in contamination and subsequent development of the spoilage flora, the assessment of contamination and growth of the flora is confined almost solely to counts and microflora analyses at various stages of processing and storage. Only Notermans and coworkers have advanced hypotheses to explain the process of contamination although their inferences are a matter of dispute (McMeekin and Thomas 1978). Other authors have made suggestions about the site and nature of skin contamination; these inferences were, however, based on circumstantial evidence. Thus, Avens and Miller (1970a) described bacteria as "in or on the skin" and "in follicles and subsurface layers". Patterson (1971a, b) referred to the rough, somewhat greasy nature of skin with many feather follicles and incorporated rough sand in diluent to remove bacteria from feather follicles (Patterson 1972). Barnes *et al.* (1973) stated that "after storage the spoilage flora will be found growing mainly on the cut muscle surfaces and down in the holes left by removal of the feathers". High numbers of skin bacteria recovered by maceration as compared to swab techniques were attributed to "bacteria growing down in feather follicles rather than at the surface of the skin" or in part to the gelatinous consistency of *Acinetobacter* colonies (Barnes and Shrimpton 1958). Barnes *et al.* (1973) used a spray of triphenyl tetrazolium chloride (TTC) in an attempt to locate the sites of microbial activity on carcass surfaces, and concluded the majority

of the bacteria were located in cut surfaces and feather follicles. Mead and Thomas (1973b), in an examination of faecal bacteria on carcasses, noted that counts after chilling showed less reduction than was obtained by the rinse method and equated these results with the view that some bacteria are "more deeply situated in the skin".

There is no direct evidence to confirm the location of bacteria on the skin of broiler carcasses or to indicate the nature of the bacterium-skin interaction. Therefore, it seems reasonable to suggest that detailed microscopic examination combined with normal cultural studies might elucidate these questions. In only a few food systems has microscopy been used to examine the bacterium-substrate relationship. Paton and Jones (1973) used optical brightness to examine the incidence of bacteria and fungi in foods and Muller and Afifi (1976) used fluorescence microscopy to examine foods for streptococci, tuberculosis bacilli, yeasts and other bacteria. Gill and Penney (1977) used light microscopy to examine penetration of beef muscle by bacteria commonly associated with spoilage. However, the information obtained regarding the microbiology/histology relationships was limited by the low resolution of the light microscopic techniques used.

Scanning and transmission electron microscopy have been used to examine in detail, mouldy bread (Reiss 1977), bacteria in cheese (Kimber *et al.* 1974; Brooker 1976; Kalab 1977; Umemoto *et al.* 1978) and bacterial invasion of hens egg shell (Tung *et al.* 1979). Firstenberg-Eden *et al.* (1979) used scanning electron microscopy (SEM) to examine the attachment of certain groups of bacteria to cows teat tissue, and Abbott *et al.* (1977) used transmission

electron microscopy (TEM) to assess the presence of bacteria in aseptically obtained meat blocks. Dutson *et al.* (1971) used TEM to observe the action of *Pseudomonas fragi* on inoculated pig muscle and implicated "blebs" associated with these bacteria in proteolysis of the myofibrillar muscle fraction. All these studies used microscopy in both a confirmatory role as well as to provide new information about the relationship of the bacteria of interest with their substrate.

This approach could yield useful information about aspects of the microbial ecology of poultry processing and storage. In particular, a knowledge of the location of skin-borne bacteria on carcasses at various stages of processing and storage would enable evaluation and justification of various sampling techniques in current use. Additionally the information may provide a means to effectively decontaminate a carcass during processing, or suggest changes in present processing practice to minimise contamination.

EXPERIMENTAL

SECTION A

Microbial Contamination of Carcasses During Processing

Materials and Methods

(a) Origin and Isolation of Strains

Freshly killed and bled poultry with feathers intact and immersion chilled carcasses which had been drained for 30 secs, were packed in sterile polythene bags and transported under ice to the laboratory. The feathers, on fresh killed poultry, were carefully removed by hand from leg and breast areas.

Pieces of skin (16cm^2) were excised aseptically from the breast and leg areas (Figs. 2a, b) of the hand plucked and the processed carcasses. Each piece was homogenised in 100ml saline (0.8% w/v NaCl) using a model 400 Colworth Stomacher (A.J. Seward and Co. Ltd., London) and 0.1ml aliquots of appropriate serial dilutions of the homogenates surface spread on nutrient agar (Appendix 1) plates. All inoculated plates were incubated aerobically at 22°C for 3 days. Total viable counts (TVC) per 16cm^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.

(b) Characterization of Isolates

Strains isolated from poultry carcass skin were identified using a modified scheme, based on that proposed by Shewan *et al.* (1960a) (Fig. 3). One day nutrient agar slope cultures were used to assess gram reaction, morphology and oxidase reaction (Kovacs 1956). These cultures were also used to determine the mode of utilization of glucose (Hugh and Lefson 1953) and production of

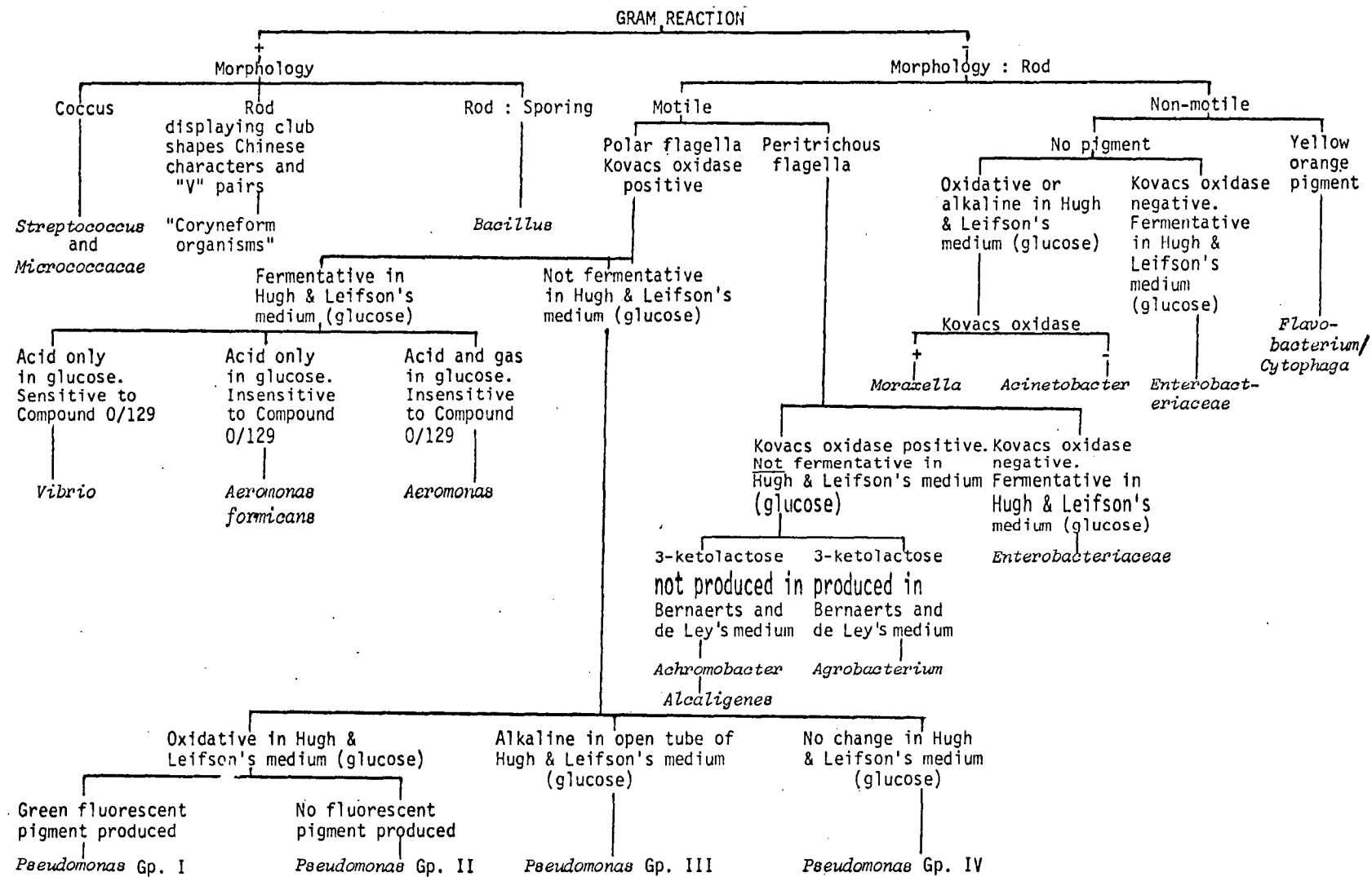
Figure 2. Areas of skin samples from broiler carcasses for use in various microbiological and - electron microscope preparation procedures.

(a) Breast skin

(b) Leg skin



Figure 3. Diagnostic key for the identification of bacteria isolated from poultry (modified scheme based on that proposed by Shewan *et al.* (1960a)).



fluorescent pigment (King *et al.* 1954). Motility was examined by microscopy of overnight broth cultures (Difco nutrient broth, 0.8% w/v). Flagella arrangement was determined by electron microscopy: formvar coated copper grids were placed on drops of nutrient broth cultures for 10-30 sec, removed and placed on a drop of 0.5% aqueous uranyl acetate for 10 sec. Excess stain was removed, the grids were air dried and examined using a Hitachi-H300 transmission electron microscope operated at 72kv.

Gram positive aerobic cocci isolated from poultry carcass skin were classified according to the scheme of Baird-Parker (1966). Nutrient agar plate 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 using media described by Baird-parker (1963). Acetoin production from glucose phosphate peptone water (Appendix 1) was tested using Barritt's modification (Barritt 1936).

(c) Sources of Psychrotrophic Microorganisms Contaminating Carcasses During Processing

Figure 1 outlines a typical poultry processing procedure.

The following samples were taken from a commercial processing plant:-

1. non-processed carcasses
2. scald water
3. fresh water added to the scald tank
4. plucker water
5. plucked carcasses
6. eviscerated carcasses
7. immersion cooler water
8. immersion cooled carcasses

9. ice
10. immersion chiller water
11. immersion chilled carcasses

Carcasses and water samples were placed in sterile polythene bags or bottles, packed under ice and transported to the laboratory. Samples were taken on eight separate occasions.

Pieces of breast skin (16cm^2) aseptically excised from broiler carcasses, were stomached in 100ml of sterile saline (0.8% w/v NaCl) using a model 400 stomacher. Serial decimal dilutions of the homogenates were prepared. Where expected numbers of psychrotrophs were greater than $10^3/16\text{cm}^2$ of skin, 0.1ml aliquots of suitable dilutions of the skin homogenates were spread on the surface of nutrient agar plates. Inoculated plates were incubated aerobically at 4°C for 14 days to enumerate psychrotrophic organisms, or at 22°C for 3 days to determine the TVC. A most probable number technique (MPN) was used to enumerate psychrotrophs where expected numbers were less than $10^3/16\text{cm}^2$ of skin. 10ml, 1ml and 0.1ml aliquots of the undiluted skin homogenate were used to inoculate a five tube glucose tryptone broth (Appendix 1) MPN system. All tubes were incubated aerobically at 4°C for 14 days and examined for growth and/or acid production. All TVC or psychrotroph counts were expressed as numbers/ 16cm^2 of breast skin.

The TVC and numbers of psychrotrophic organisms present in water samples were determined by the surface spread technique (described above). The MPN method was used to enumerate psychrotroph numbers less than 10 per ml of water. All counts were expressed as numbers/ml of water.

(d) Evaluation of Fixation and Dehydration Techniques for Electron Microscopic Examination of Processed Broiler Skin

Pieces of breast skin ($\approx 1\text{cm}^2$) excised from fresh immersion chilled poultry carcasses (Fig. 2a) were pinned to dental wax and prepared for scanning electron microscopy by one of the following methods:-

- (1) Skin pieces were snap frozen in liquid nitrogen and freeze dried at -20°C for two days using a Dynovac FD16 freeze drying unit (Dynovac High Vacuum Pty. Ltd., Victoria, Australia).
- (2) Skin specimens were fixed overnight at 4°C in osmium tetroxide vapour (the specimen was placed over a solution of osmium tetroxide [1% w/v] in sodium phosphate buffer [0.1M, pH 7.2]).
- (3) Skin specimens were fixed overnight at 4°C by immersion in a solution of osmium tetroxide (1% w/v) in sodium phosphate buffer (0.1M, pH 7.2).
- (4) Skin specimens were fixed overnight at 4°C by immersion in 5% glutaraldehyde in sodium phosphate buffer (0.1M, pH 7.2).

Chemically fixed specimens were rinsed with cold sodium phosphate buffer (0.1M, pH 7.2), dehydrated in a graded ethanol series (30 \rightarrow 50 \rightarrow 60 \rightarrow 70 \rightarrow 80 \rightarrow 90 \rightarrow 95 \rightarrow 100% x 3, using distilled water as the diluent) and sliced into small pieces with a razor blade. The pieces were infiltrated with amyl acetate (25 \rightarrow 50 \rightarrow 75 \rightarrow 100% x 2, using absolute ethanol as the diluent) and critical point dried with carbon dioxide in a Polaron E-3000 Critical Point Dryer (Polaron Equipment Pty. Ltd., Watford, England). The skin pieces were examined under a dissecting microscope during chemical fixation, ethanol dehydration and following critical point drying or freeze drying.

Dried specimens were glued to brass SEM stubs with conductive silver paint, coated with about 270\AA of gold in a Dynavac SC150 sputter coater (Dynavac High Vacuum Pty. Ltd., Victoria, Australia) and examined in a JEOL JXA 50-A scanning electron microscope operated at 15kV. All photomicrographs were recorded on Polaroid Type 52 Polaplan film.

At least five separate skin pieces prepared by each of the above schedules were examined.

(e) Microscopic Examination of Broiler Carcass Skin at Various Stages of Processing

Non-processed poultry carcasses, plucked uneviscerated carcasses and immersion chilled broiler carcasses sampled from a commercial processing plant were packed in polythene bags, placed under ice and transported to the laboratory. Pieces of skin ($\approx 1\text{cm}^2$) excised from the breast and outside leg areas of the carcasses (Fig. 2 a, b) were pinned to dental wax.

Skin pieces to be examined by scanning electron microscopy (SEM) were fixed in osmium tetroxide vapour or glutaraldehyde solutions overnight at 4°C , dehydrated in a graded ethanol series, infiltrated with amyl acetate and critical point dried from carbon dioxide as described previously. The dried skin preparations were glued to brass SEM stubs with silver electroconductive paint, coated with about 270\AA of gold by sputtering and examined with a JEOL JXA-50A SEM operated at 15kV. All micrographs were recorded on Polaroid types 52 or 107 Polaplan film.

Skin pieces to be prepared for examination under the transmission electron microscope (TEM) were fixed overnight at 4°C by one of the following methods:-

1. by fixation in osmium tetroxide vapour as previously described;
2. by fixation in a solution of 5% glutaraldehyde in sodium phosphate buffer (0.1M, pH 7.2);
3. by fixation in a solution of 5% glutaraldehyde and alcian blue (1% w/v) in sodium phosphate buffer (0.1M, pH 7.2).

All skin pieces were then rinsed in several changes of cold phosphate buffer or until all unbound alcian blue was removed. All tissue was then postfixed overnight at 4°C in a solution of osmium tetroxide (1% w/v) in sodium phosphate buffer (0.1M, pH 7.2), rinsed in phosphate buffer, and dehydrated in a graded ethanol series (30 → 50 → 60 → 70 → 80 → 90 → 95 → 100% x 3, using distilled water as the diluent). The skin was then sliced into suitable small pieces, rinsed in absolute ethanol followed by two 30 minute rinses in 1,2 epoxy propane and embedded in Araldite M according to Glauert and Glauert (1958). Silver sections of the embedded tissue were cut using a LKB type 4801A ultramicrotome and placed on formvar coated copper electron microscope grids. All sections were stained with 1% (w/v) aqueous uranyl acetate followed by lead citrate (Reynolds 1963) and examined with either a Phillips EM201 or a Hitachi H-300 transmission electron microscope operated at 60kV or 72kV respectively. All micrographs were recorded on Ilford electron microscope film.

(f) Recovery of Microorganisms from Broiler Carcass Skin by the Stomacher

Pieces of skin (16cm² in area) were excised from the breast area (Fig. 2a) of fresh immersion chilled broiler carcasses. Each piece was homogenised in 100ml saline (0.8% w/v) for 1, 2, 3, 5 or

10 minutes, using a model 400 stomacher, to examine the effect of time of stomaching on the recovery of bacteria from the skin. Four replicate skin samples were examined for each duration of stomaching. Serial decimal dilutions of each homogenate were prepared and duplicate 0.1ml aliquots of suitable dilutions spread on the surface of nutrient agar plates. All inoculated plates were incubated aerobically at 22⁰C for 3 days. Colony counts were recorded as the total viable count (TVC) per 16cm² of skin. Skin homogenates were examined visually and by positive phase contrast microscopy following each duration of stomaching.

The stomacher and the Atomix Blender (MSE, London) were compared by stomaching or blending skin pieces in 100ml saline (0.8% w/v) for 5 minutes or 2 minutes (after Avens and Miller 1970a) respectively. Skin samples to be homogenised by the stomacher were taken from one side of the breast area of fresh immersion chilled broiler carcasses, and samples from the other side were examined using the Atomix Blender. The total viable count per 16cm² of breast skin was determined as above.

Breast skin excised from fresh immersion chilled broiler carcasses or immersion chilled carcasses stored at 10⁰C for 5 days (these were stored in sealed and inflated sterile oxygen permeable polythene bags), were homogenised in 100ml of saline for 5 mins using the Colworth stomacher. Pieces of the stomached skin were rinsed in 5 x 100ml volumes of saline. Rinsed stomached skin and intact control skin samples were fixed overnight at 4⁰C in glutaraldehyde, dehydrated in a graded ethanol series, infiltrated with amyl acetate and critical point dried from carbon dioxide as described previously. The dried specimens were glued to brass SEM stubs with silver conductive paint,

coated with about 270\AA of gold by sputtering and examined in a JEOL JXA-50A scanning electron microscope operated at 15kV. All micrographs were recorded on Polaroid type 52 Polaplan film.

(g) Characterisation of Some Water Soluble Components Present in Immersion Chiller Water and on the Skin of Fresh and Stored Immersion Chilled Broiler Carcasses

Fresh immersion chilled broiler carcasses and carcasses stored at 2°C for 4 days in polythene bags were swabbed separately on leg and breast skin areas with cotton swabs. These swabs were rinsed in deionized water. Swab rinse fluid and immersion chiller water were centrifuged at $25,000 \times g$ at 4°C for 30 minutes to remove particulate matter, frozen at -20°C and freeze dried with a Dynavac model FD16 freeze dryer. All freeze dried solids were kept in a dessicator over silica gel until required.

Blood from fresh killed poultry was centrifuged, using a bench top centrifuge and the serum removed and stored at -20°C until required.

Freeze dried solids from immersion chiller water and skin swabs were examined for the presence of amino acids by thin layer chromatography (TLC). All solids were dissolved in distilled water ($\approx 20\text{mg/ml}$) and $20\mu\text{l}$ aliquots applied to Kieselgel 60 F_{254} aluminium foils (Merck, Darmstadt, Germany). $2\mu\text{l}$ samples of L-cyst(e)ine (0.1% w/v), L-methionine (0.1% w/v) and taurine (0.1% w/v) were applied as standards. The TLC foils were run in a butanol:acetic acid:water = 60:15:25 solvent system until the solvent front had moved 10cm from the origin, removed and dried. Amino acids were located using a ninhydrin spray (0.2% w/v in acetone) with heating at 100°C for 3 min.

Electrophoresis of soluble protein present in freeze dried solids from skin swabs and immersion chiller water was performed using acrylamide slab gel electrophoresis. A 6% gel (Appendix 2) was used in conjunction with a discontinuous buffer system (Appendix 2). All freeze dried solids were dissolved in gel buffer ($\approx 20\text{mg/ml}$) and used to fill the sample wells. Bovine serum albumin (Calbiochem, San Diego, California, U.S.A.) in gel buffer (10mg/ml) and chicken blood serum (1% v/v in gel buffer) were used as standards. The tracker dye used was Cromophenol blue (0.05% w/v in gel buffer). All gels were run at a constant 15 ma per gel at room temperature. Following electrophoresis, the buffer front was marked and the gels stained for protein with amido black, protein bound carbohydrate by the periodic acid Schiff method, and lipoprotein by the oil red O method (see Appendix 2).

RESULTS

SECTION A

(a) Numbers and Incidence of Different Bacteria Present on Non-Processed and Immersion Chilled Broiler Carcass Skin

The numbers and types of bacteria found on breast and leg skin of non-processed and immersion chilled broiler carcass skin is presented in Table 5. Greater numbers of bacteria were found on both leg and breast skin of non-processed carcasses compared to the skin of immersion chilled carcasses.

Micrococcus spp. were almost the only bacteria isolated from breast and leg skin of non-processed poultry carcasses. All strains of *Micrococcus* isolated were assigned to either subgroup 5 or subgroup 6 of Baird-Parker's classification scheme (Baird-Parker, 1966). Subgroup 6 predominated on both leg and breast skin. Only one other type of bacterium, an actinomycete, was isolated from non-processed poultry skin.

The flora of immersion chilled carcass skin was composed of a variety of types of bacteria (Table 5). 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.

(b) Sources of Psychrotrophic Microorganisms Contaminating Poultry Carcasses During Processing

Tables 6a and 6b show the incidence of psychrotrophs and related total viable counts present in processing waters and on the skin of broiler carcasses samples from various stages of processing. In general, while poultry processing procedures brought about a reduction

TABLE 5. Aerobic microbial flora of breast and leg skin
from non-processed poultry and immersion
chilled carcasses.

Table 5.

GROUP	PERCENTAGE OF POPULATION			
	NON-PROCESSED POULTRY SKIN		IMMERSION CHILLED CARCASS SKIN	
	BREAST	LEG	BREAST	LEG
<i>Micrococcus</i> Subgroup 5	29	9	25	3
Subgroup 6	71	87		
<i>Coryneforms</i>	-	-	-	3
<i>Moraxella/Acinetobacter</i>	-	-	-	74
<i>Flavobacterium/Cytophaga</i>	-	-	11	8
Group I	-	-	50	-
<i>Pseudomonas</i> Group II	-	-	-	-
Group III/IV	-	-	7	5
ENTEROBACTERIACEAE	-	-	4	8
Yeasts/Other	-	4	-	-
TOTAL NUMBER OF ISOLATES	24	23	28	38
TOTAL VIABLE COUNT /16 cm ² SKIN	3.55x10 ⁶	6.25x10 ⁶	2.5x10 ⁴	4.95x10 ⁴

TABLE 6a. Incidence of psychrotrophic organisms on
carcasses and in processing waters sampled
at various stages of processing.

Table 6a.

	1	2	3	4	5	6	7	8	MEAN 147	
SAMPLE NO.	0	240	0	-	-	-	-	350		
NON-PROCESSED POULTRY SKIN NO./16cm ²										
SCALD WATER NO./ml	1.8	0	0	-	-	-	-	-	<0.45	
FRESH SCALD INPUT NO./ml	0.46	16	6	-	-	-	-	9.2	<8	
FRESH PLUCKER INPUT NO./ml	5.4	8	6	-	-	9.2	20	9.2	9.6	
PLUCKED CARCASS SKIN NO./16cm ²	330	240	33	-	-	-	-	350	238	
EVISCERATED CARCASS SKIN NO./16cm ²	-	-	-	-	350	900	540	240	508	
ICE NO./ml	23	30	5600	85	120	267	30	10	770	
IMMERSION WASHER WATER NO./ml	1270	1540	84400	4500	1150	425	990	830	2393	
WASHED CARCASS SKIN NO./16cm ²	350	1000	48000	920	280	540	240	540	968	
IMMERSION CHILLER WATER NO./ml	1180	5800	11700	9920	3600	13000	340	1930	6596	
IMMERSION CHILLED CARCASS SKIN NO./16cm ²	540	19000	1500	17500	920	2000	2650	350	5558	

- Sample not tested.

TABLE 6b. Total viable counts of carcass skin
and processing waters sampled at
various stages of processing.

Table 6b.

SAMPLE NO.	NON-PROCESSED POULTRY SKIN NO./16cm ² SKIN	SCALD WATER NO./ml WATER	FRESH SCALD INPUT NO./ml WATER	FRESH PLUCKER INPUT NO./ml WATER	PLUCKED CARCASS SKIN NO./16cm ² SKIN	EVISцерATED CARCASS SKIN NO./16cm ² SKIN	ICE NO./ml MELT	IMMERSION WASHER WATER NO./ml WATER	WASHED CARCASS SKIN NO./16cm ² SKIN	IMMERSION CHILLER WATER NO./ml WATER	IMMERSION CHILLED CARCASS SKIN NO./16cm ² SKIN
1	2.50x10 ⁵	2.4x10 ⁴	75x10 ³	75x10 ³	2.4x10 ⁵	-	9x10 ²	2.9x10 ⁵	6.45x10 ⁵	9.5x10 ⁴	6.76x10 ⁵
2	-	-	-	-	-	-	2.80x10 ²	3.00x10 ⁵	5.10x10 ⁵	1.73x10 ⁵	5.7x10 ⁵
3	-	-	-	-	-	-	2.51x10 ⁴	8.30x10 ⁵	6.45x10 ⁴	2.35x10 ⁵	1.75x10 ⁵
4	-	-	-	-	-	-	2.82x10 ³	8.00x10 ⁵	3.11x10 ⁵	3.40x10 ⁵	6.7x10 ⁴
5	-	-	-	-	-	2.11x10 ⁵	1.53x10 ³	2.57x10 ⁵	1.22x10 ⁵	1.73x10 ⁵	1.5x10 ⁴
6	-	-	3.60x10 ²	3.60x10 ²	-	1.74x10 ⁵	1.92x10 ⁴	3.75x10 ⁵	9.2x10 ⁴	3.16x10 ⁵	4x10 ⁴
7	-	-	1.33x10 ²	1.33x10 ²	-	4.40x10 ⁴	3.70x10 ³	3.00x10 ⁵	9.8x10 ⁴	1.78x10 ⁵	2.81x10 ⁴
8	4x10 ⁵	-	5.95x10 ³	2.57x10 ³	3.75x10 ⁵	1.45x10 ⁵	8.90x10 ²	1.01x10 ⁵	-	6.5x10 ⁴	4.85x10 ⁴
MEAN	3.25x10 ⁵	2.4x10 ⁴	2.15x10 ³	1.02x10 ³	3.08x10 ⁵	1.44x10 ⁵	6.80x10 ³	4.07x10 ⁵	2.63x10 ⁵	1.97x10 ⁵	7.58x10 ⁴

- Sample not tested.

in the total viable flora present on broiler carcass breast skin, a large increase in the number of psychrotrophic contaminants occurred.

Prior to scalding, small numbers of psychrotrophs were found on breast skin of poultry carcasses ($\approx 150/16\text{cm}^2$ skin). However, during plucking and subsequent stages of processing large increases in psychrotroph contamination of the 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 organisms (i.e. $<10/\text{ml}$). 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.

(c) Evaluation of Fixation and Dehydration Techniques for Electron Microscopic Examination of Processed Broiler Skin

Fresh untreated excised skin pieces from immersion chilled carcasses were observed to be covered by a liquid film, presumably derived from immersion chiller water. This film remained intact throughout osmium tetroxide vapour fixation. During the 30% and 50% stages of ethanol dehydration of vapour fixed tissue, a white gelatinous material formed in the channels and folds on the skin surface and remained intact in all subsequent preparative procedures. This material appeared as a bluish-white filling in the channels and folds of critical point dried tissue, but was not present in tissue which had been fixed by immersion in solutions of osmium tetroxide or glutaraldehyde. However, a white material was present in the channels

and folds of freeze dried skin preparations.

Examination of freeze dried skin preparations by SEM confirmed that the channels and folds were filled with a smooth surfaced material (Figs. 4a, b, c) which obstructed examination of any skin cell surface detail. Large droplets on the surface (Figs, 4b, c) were usually associated with skin channels and seemed to have come from beneath the skin surface.

SEM examination of skin preparations fixed in osmium tetroxide vapour also showed the presence of a substance filling the channels on the skin (Fig. 5a). Smaller channels on individual skin cells were also filled (Fig. 5b) and cell surface detail was obscured (Fig. 5b). The large droplets on the surface of freeze dried tissue were not present on vapour fixed critical point dried preparations.

Channels on the surface of skin fixed by immersion in osmium tetroxide or glutaraldehyde solutions were not filled with any of the material observed on freeze dried or vapour fixed tissue (Figs. 6a, 7a) and cell surface detail was visible. Individual skin cells were always partially covered by small rounded particles about $1\mu\text{m}$ to $2\mu\text{m}$ in diameter (Figs. 6b, 7b).

(d) Microscopic Examination of Broiler Carcass Skin at Various Stages of Processing

SEM was used to examine the skin surface of non-processed poultry carcasses. The rough and folded surface (Fig. 8a) was covered with thin, flattened skin cells in various stages of exfoliation (Fig. 8b). Parts of the surface of these cells were often covered by dense clumps of particulate matter (Fig. 8c). The size range of individual particulates was $0.5\mu\text{m}$ to $3.0\mu\text{m}$.

Figure 4 SEM of freeze dried breast skin from an immersion chilled broiler carcass.

(a) Low magnification SEM showing a material filling the large channels in the skin surface (solid arrows) and droplets on the skin surface (open arrows). Bar = 200 μ m.

(b) Note the large droplets always seen on freeze dried skin. The skin cell surface is covered by the material observed in 4a. Bar = 100 μ m.

(c) Higher magnification SEM of the droplets shown in 4b. Bar = 30 μ m.

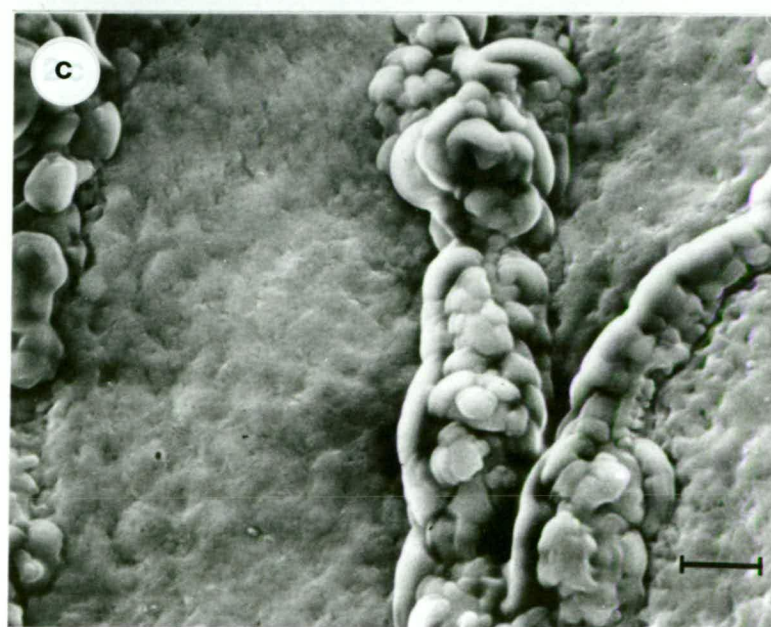
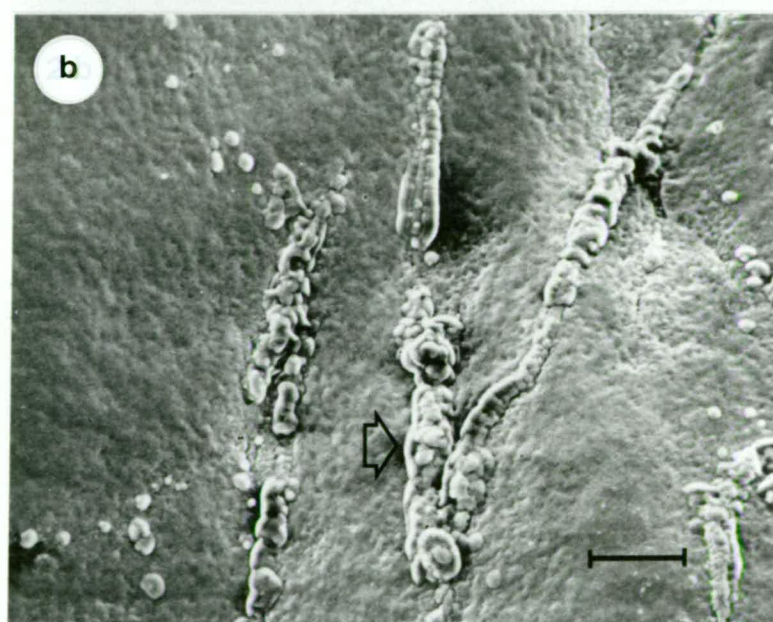
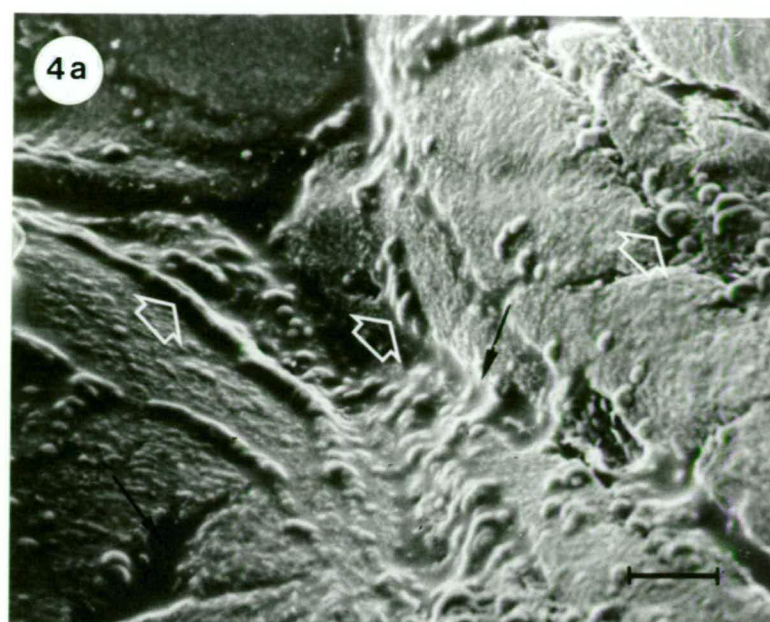


Figure 5. SEM of breast skin from fresh immersion chilled broiler carcasses fixed in osmium tetroxide vapour followed by critical point drying.

- (a) Low magnification SEM of the skin surface. The large channels on the surface are partly filled with a material (arrows) and skin cell surface detail is partially obstructed from view.

Bar = 30 μ m.

- (b) High magnification SEM of the surface of a skin cell. The small channels (arrowed) are filled with material and no cell surface detail is visible. Bar = 3 μ m.

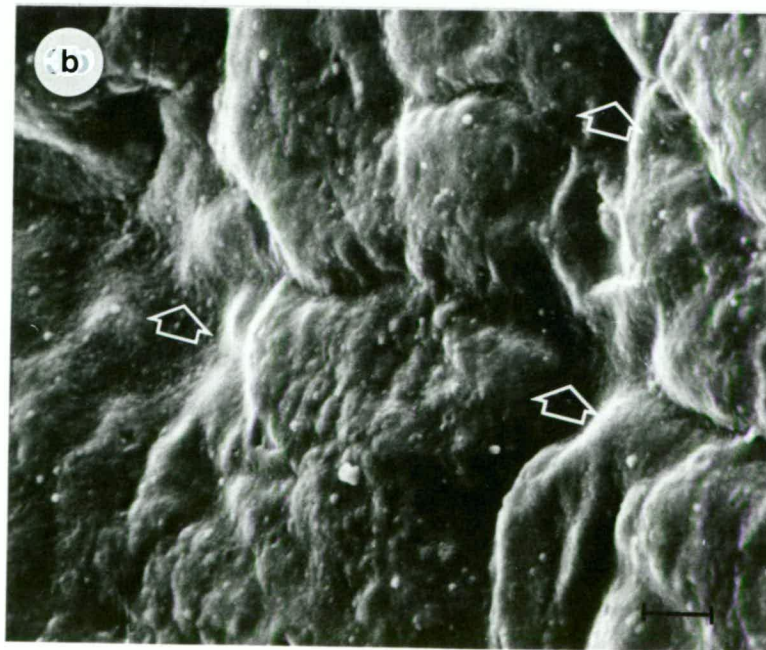
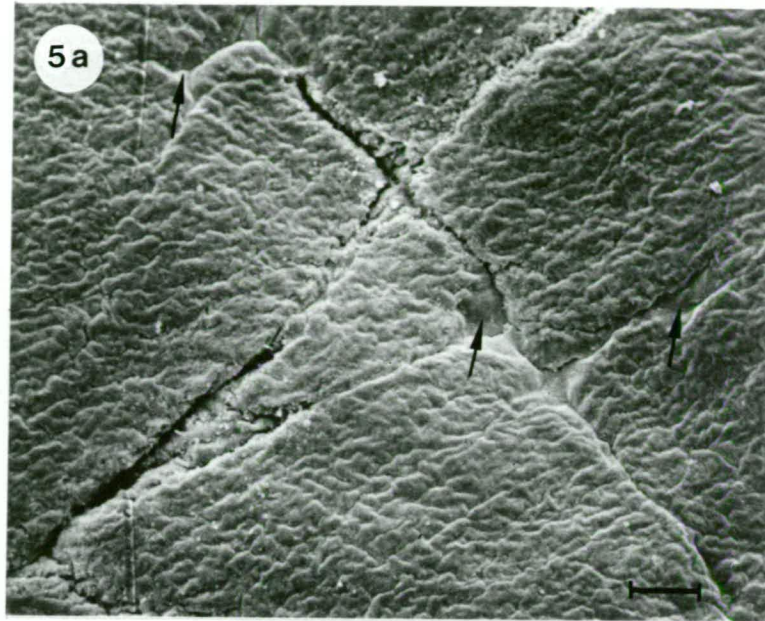


Figure 6. SEM of breast skin from fresh immersion chilled broiler carcasses, fixed by immersion in a glutaraldehyde solution followed by critical point drying.

(a) Low magnification SEM of the skin surface. The large channels and the small crevices on individual skin cell surfaces are free of material observed in Figures 4 and 5. Bar = 30 μ m.

(b) High magnification SEM of the surface of an individual skin cell. Note the absence of any layer material as seen in Figure 5b. The surface is partially covered by rounded particles. Bar = 3 μ m.

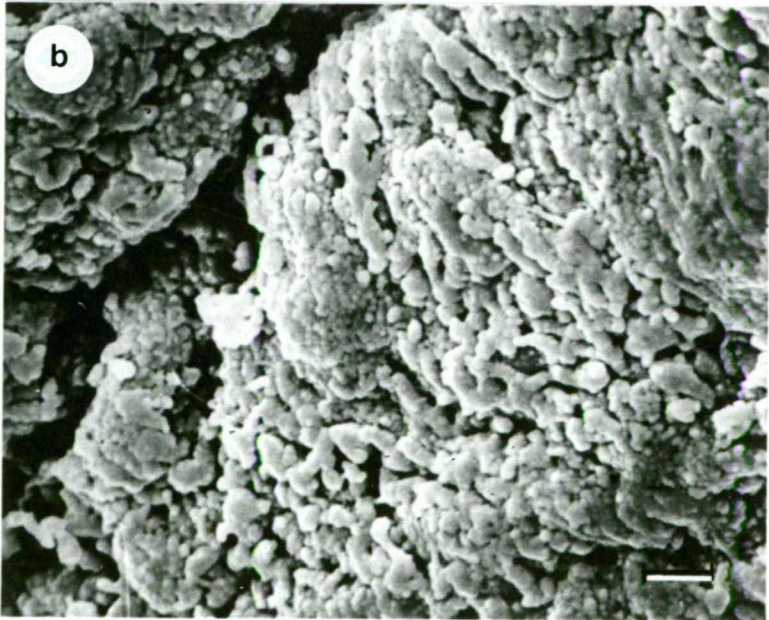
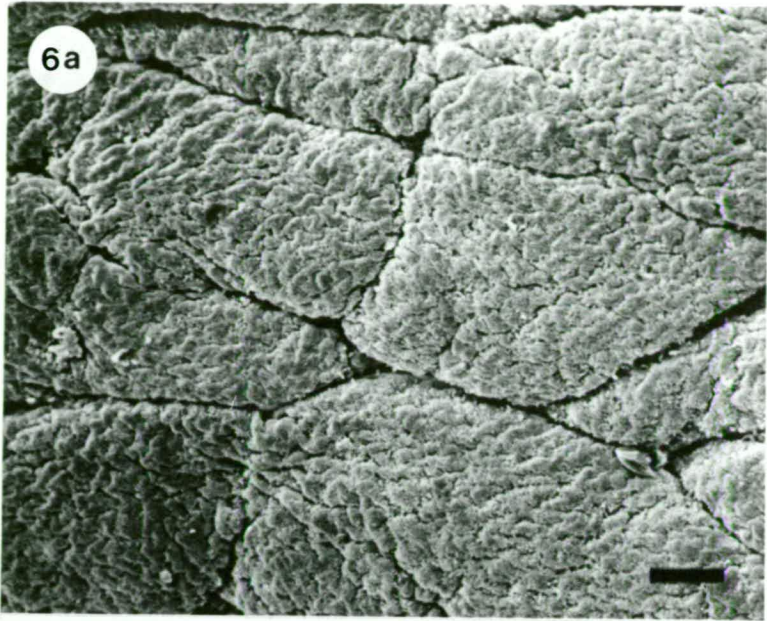


Figure 7. SEM of breast skin, from fresh immersion chilled broiler carcasses, fixed by immersion in an osmium tetroxide solution followed by critical point drying.

(a) Low magnification SEM of the skin surface. Note the similarity in appearance to Figure 6a. The large channels in the skin are free of occluded material and surface detail is visible. Bar = $30\mu\text{m}$.

(b) Higher magnification SEM of the surface of an individual skin cell. Note the similarity in appearance to Figure 6b, and the rounded particles on the surface. Bar = $3\mu\text{m}$.

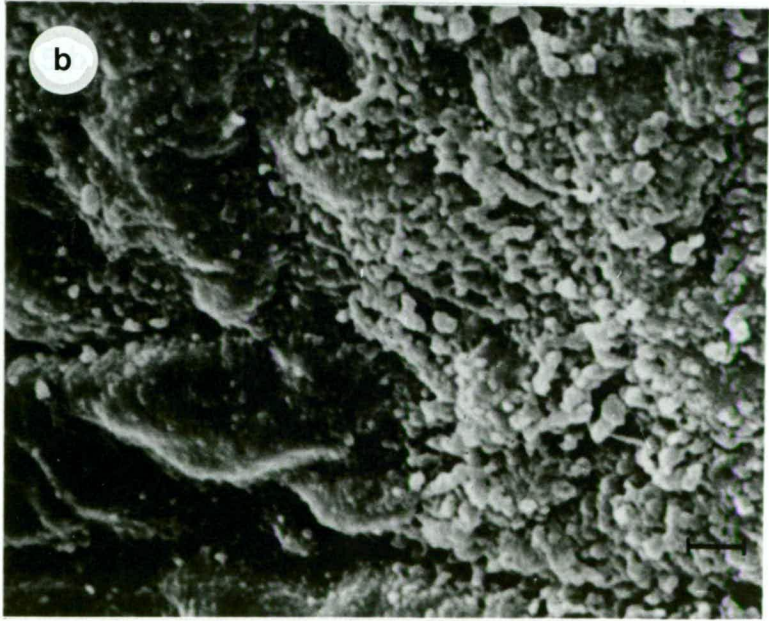
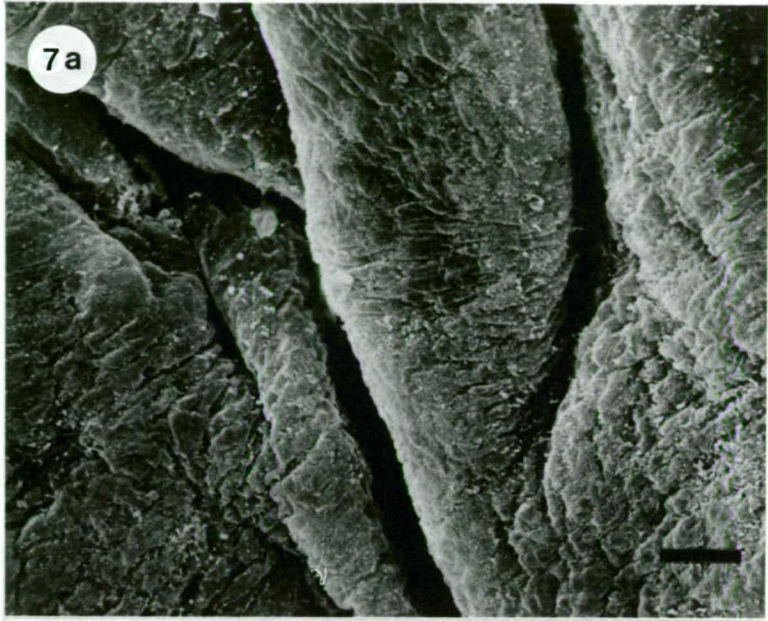
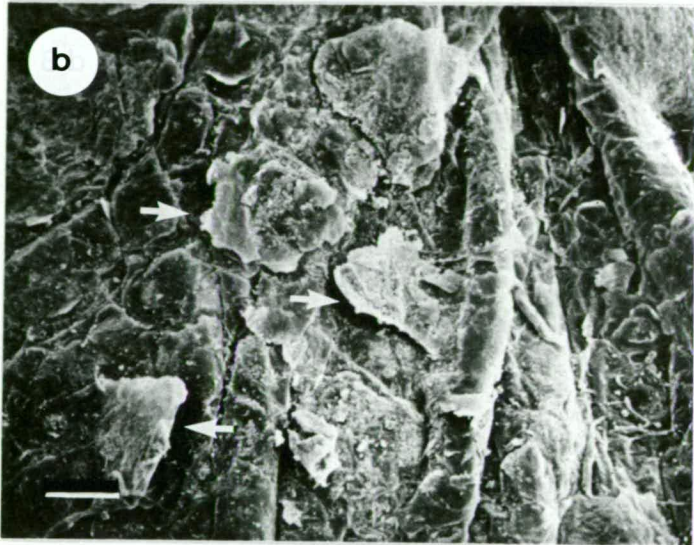
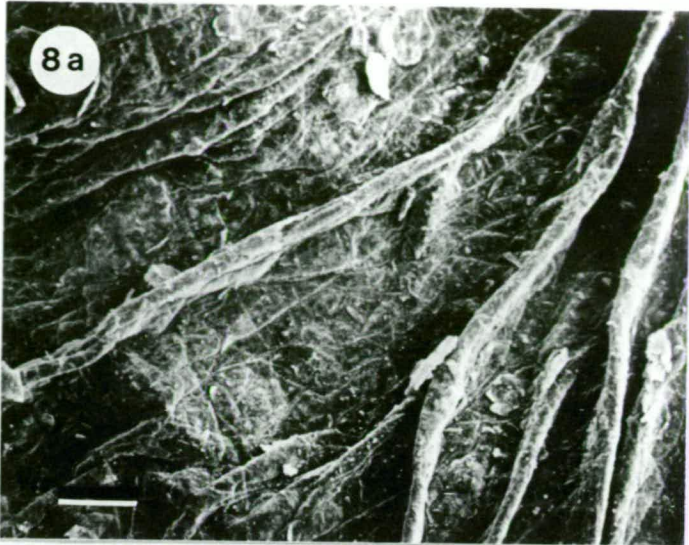


Figure 8. SEM of surface features on skin from fresh killed poultry sampled prior to scalding. Skin samples were fixed in osmium tetroxide vapour.

(a) Low magnification SEM of the surface of breast skin. Note the rough, folded nature of the surface. A similar topography was observed for leg skin. Bar = 250 μ m.

(b) Surface skin cells are thin and flattened and commonly in various stages of exfoliation (arrowed). Bar = 80 μ m.

(c) The skin surface is partially covered by dense patches of particulate matter (arrows). Coccoid shaped bacteria (c) were often found associated with these clumps. Bar = 3 μ m.



The general histology of the skin of non-processed carcasses examined by TEM of thin sections of Araldite embedded tissue was similar in detail to that already described by Matoltsy (1969) and Lucas and Stettenheim (1972). 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 (Figs. 9a, 9b). 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 characterised by several plies of orthogonally oriented collagen fibrils.

Three morphologically different microbial cell types were located on the surface of the corneous layer of skin from non-processed carcasses. Low numbers of yeasts and rod-shaped bacteria were found lying on the skin amongst dust and other debris (Figs. 10a, b). Gram positive cocci were regularly found within the clumps of particulate material (Figs. 8c; 11a, 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 as the microflora of non-processed 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

Figure 9. TEM of a transverse section through the skin of a fresh killed carcass sampled prior to scalding. Skin was treated with a glutaraldehyde solution containing alcian blue and post fixed with osmium tetroxide. Sections stained with uranyl acetate/lead citrate.

(a) Low magnification TEM of a transverse section through the epidermal and dermal skin tissue. The superficial *stratum corneum* (sc), composed of thin, flattened, highly cornified cells joined at their edges, overlays the *stratum germinativum* (sg). These two layers constitute the epidermis. The epidermis is separated from the dermis by the basal lamina (bl). Dermal tissue (d) is characterised by plies of orthogenally oriented collagen fibrils and is predominantly a layer of connective tissue. Bar = 3 μ m.

(b) Higher magnification TEM of the basal lamina (bl) which separates epidermal tissue (e) from dermal tissue (d). Note the bundles of collagen fibrils within the dermal tissue. Bar = 1 μ m.

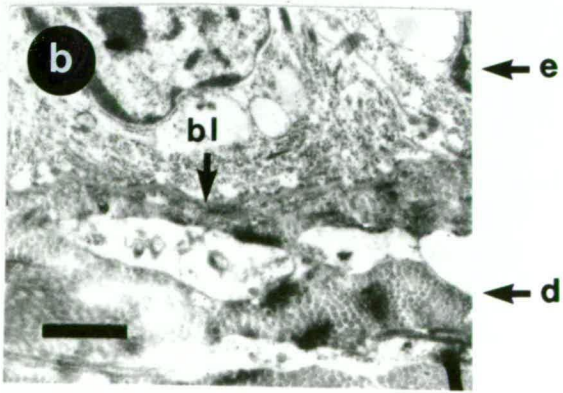
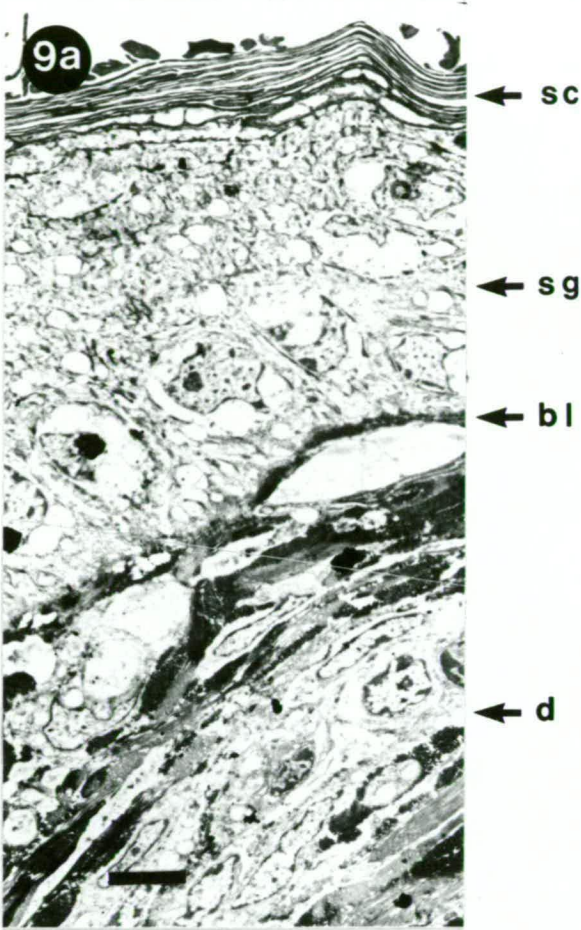
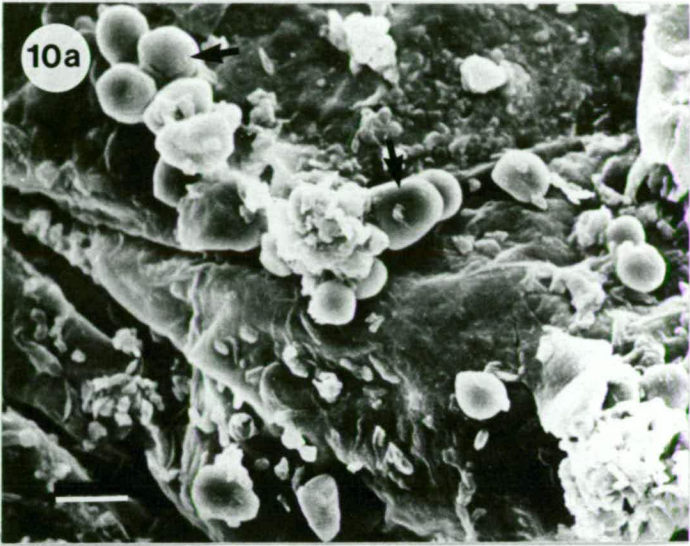


Figure 10. SEM of microorganisms on the surface of skin from fresh killed poultry sampled prior to scalding. Skin tissue fixed in osmium tetroxide vapour.

(a) Yeast cells (arrowed) on the edge of a corneous skin cell. Bar = 10 μ m.

(b) A rod shaped bacterium (open arrow) among debris on the skin surface. Bar = 3 μ m.



the skin surface (Figs. 11a, b) indicate that this material is composed of substances stained by osmium tetroxide, uranyl acetate and/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 Figures 11a, b.

Comparison of TEM's of thin sections of skin from non-processed 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. 9a and Fig. 12a). Small epidermal cell fragments remained attached to the basal lamina (Fig. 12a) where this membrane remained intact. These fragments correspond in size and shape to particulates observed on processed skin examined in the SEM (Figs. 6a; 7a). The newly exposed dermal surface was relatively smooth compared to the surface of skin from non-processed poultry, but was deeply channelled where the basal lamina conformed to channels and folds in the epidermal surface or dermal intercellular spaces (Figs. 12a, c, d).

The skin surface of plucked carcasses, sampled prior to 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 vapour. 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 (Figs. 5a, b; 12b), or thin sections of skin examined by TEM (Figs. 12d, e; 13a, b). Most of the layer material was present in folds and channels in the

Figure 11. TEM of sections of skin from fresh killed poultry samples prior to scalding. All skin was treated with a solution of glutaraldehyde containing alcian blue and post fixed in osmium tetroxide. Sections were stained with uranyl acetate/lead citrate.

- (a) Section through the *stratum corneum* showing a gram +ve coccoid bacterium (arrow) associated with a clump of particulate matter. Bar = 1 μ m.

- (b) Section through a clump of particulate matter present on the *stratum corneum* (sc) showing a number of associated gram +ve bacteria (arrowed). Areas within the clump cleared of any material probably represent lipid material dissolved by 1,2 epoxy propane used during embedding. Bar = 1 μ m.

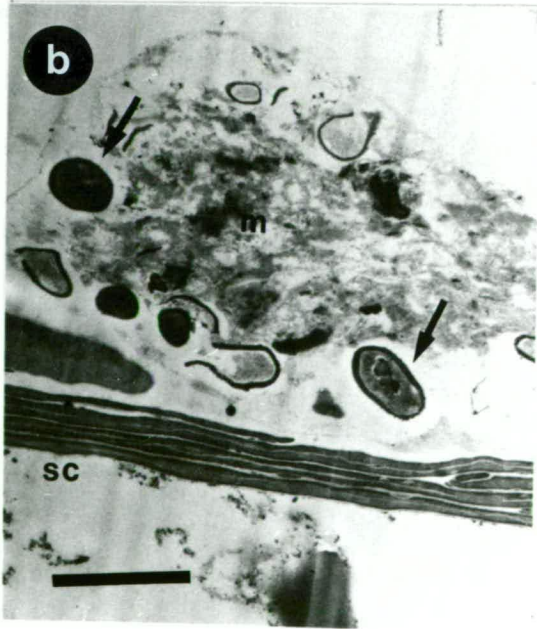


Figure 12. Microscopic examination of skin of scalded and plucked poultry carcasses sampled prior to evisceration.

- (a) TEM of a transverse section through the skin. Note that the scalding and plucking processes have removed the epidermal tissue to expose dermal (d) tissue. The dark stained particles (ef) are fragments of the epidermal tissue not removed by plucking. The basal lamina (not resolved in this micrograph) may or may not be left intact. Similar observations were made for both breast and leg skin. Skin tissue was fixed in a solution of glutaraldehyde containing alcian blue and post fixed in osmium tetroxide. Sections were stained with uranyl acetate/lead citrate. Bar = 1 μ m.
- (b) and (c). SEM of breast skin fixed in osmium tetroxide vapour (b) or glutaraldehyde solutions (c). Note that the skin surface detail on vapour fixed tissue is obscured by a material not present on glutaraldehyde fixed skin. Similar observations were made for leg skin. Bar = 100 μ m.
- (d) and (e). TEM's of transverse sections through skin showing a layer of material (lm) filling channels in the surface. Lipid droplets (l) and bacteria (arrowed in 12e) were often found in the layer material. Tissue fixed as for 12a. Bar = 1 μ m.

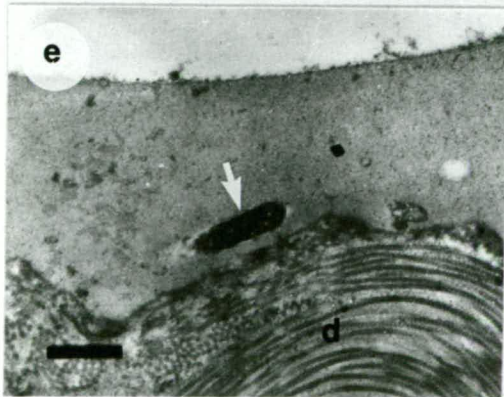
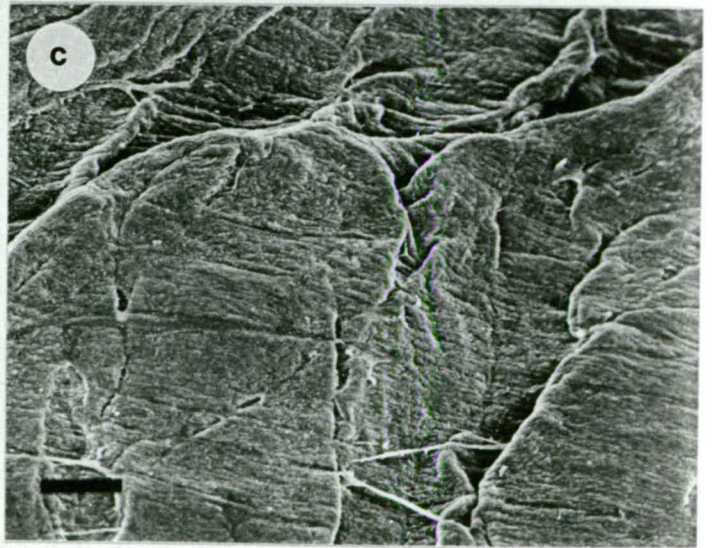
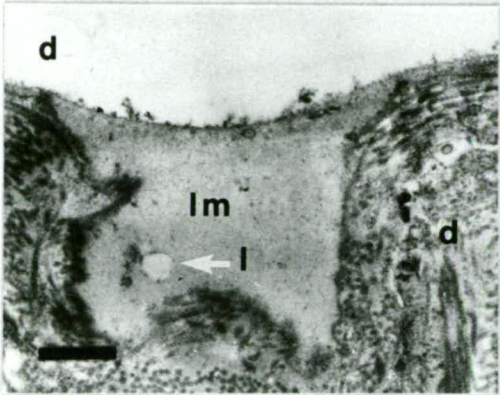
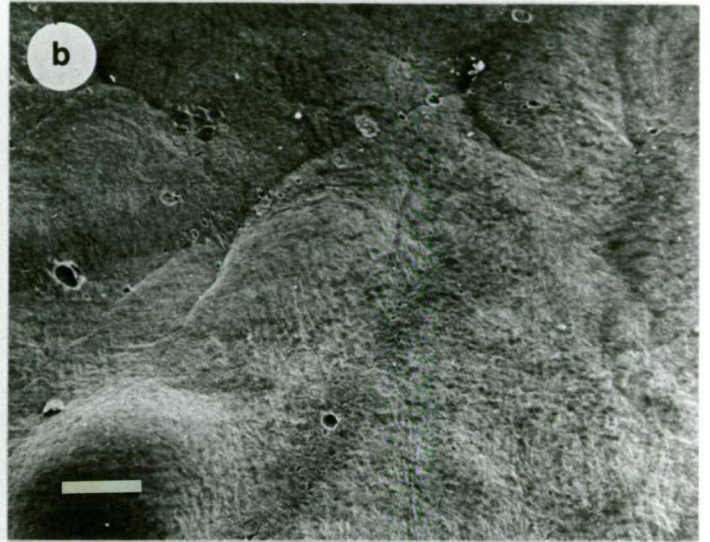
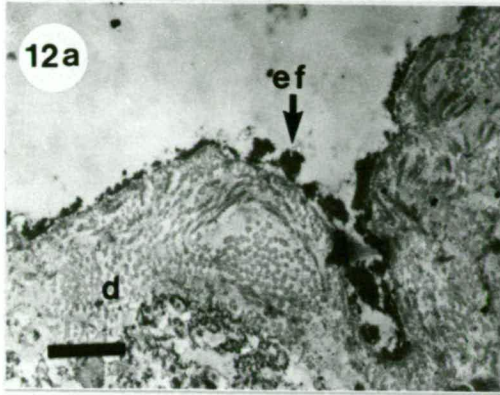
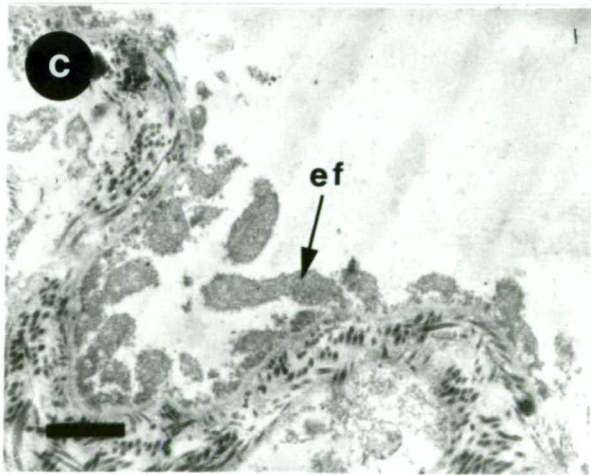
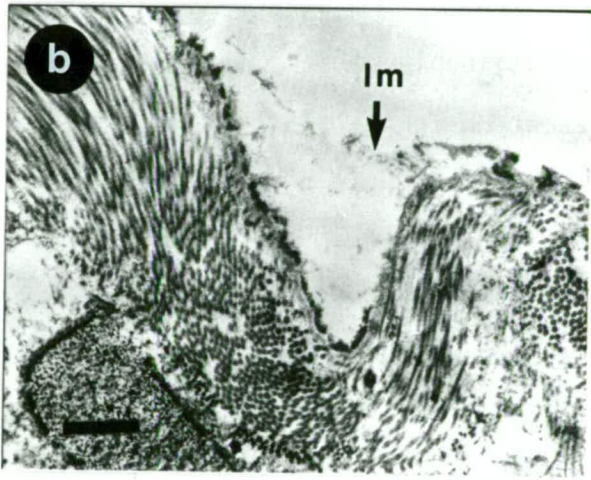
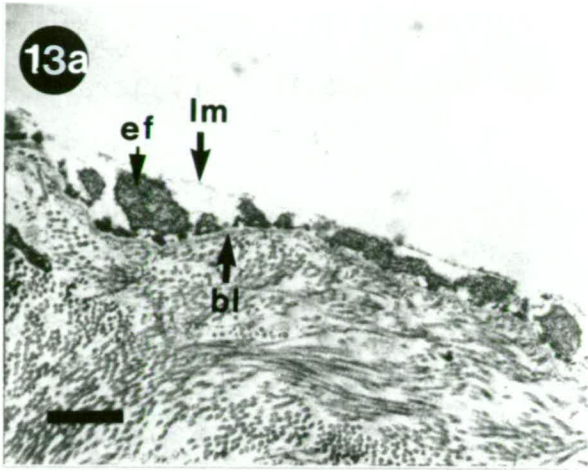


Figure 13. Microscopic examination of skin from fresh immersion chilled broiler carcasses. All sections stained with uranyl acetate/lead citrate.

(a) and (b). Sections of skin fixed with osmium tetroxide vapour. Note the layer material (lm) filling channels in the surface and covering epidermal fragments (ef) still attached to the basal lamina (bl). The layer material in this tissue contains less stainable organic material than that present on freshly scalded and plucked skin (*cf.* Figure 12d, e). Bar = 1 μ m.

(c) Section of skin fixed by immersion in a solution of glutaraldehyde and post fixed in osmium tetroxide. Note that no layer material is present on the surface of the skin. The epidermal fragments (ef) are exposed on the skin surface. Bar = 1 μ m.



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 glutaraldehyde solutions (Figs. 6a, b; 13c). 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 (Fig. 12a). 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.* Figs. 12c, d; 13a, b).

In some thin sections of skin from carcasses sampled immediately after plucking, lipid droplets (Fig. 12d) and bacteria (Fig. 12e) were found within the layer of preserved fluid film components. However, 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 (Figs. 14a, b). Generally, bacteria within preserved film materials could not be examined by SEM and, 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. The fact that

Figure 14. Microorganisms present on the skin surface of fresh immersion chilled broiler carcasses. Tissue fixed in osmium tetroxide vapour.

(a) SEM of yeasts found in a cavity on breast skin. Bar = 25µm.

(b) SEM of a yeast cell on the surface of leg skin. Bar = 10µm.



bacteria were not found on skin from immersion chilled carcasses, does not imply higher numbers of bacteria on skin from fresh plucked carcasses, or an absence of microorganisms on this skin, since TVC confirm otherwise (Table 6b).

Significant changes in the appearance of surface features of skin occurred during immersion chilling. Figure 6a shows the swollen appearance of skin from immersion chilled carcasses as compared to skin from fresh plucked carcasses shown in Figure 12b.

(e) Recovery of Microorganisms from Broiler Carcass Skin by the Stomacher

Higher numbers of microorganisms were recovered from skin homogenised by the stomacher when longer times of stomacher action were employed (Table 7), but analysis of data variance (Appendix 3) showed this increase was not significant (at the 95% confidence level). Macroscopic and microscopic examination of homogenates showed that maximised emulsion of lipid released from subcutaneous adipose tissue occurred only after about 5 minutes of stomacher action.

Comparable numbers of bacteria were recovered from paired skin samples macerated by either the stomacher or the blender techniques

TABLE 7. Effect of time of stomaching of breast
skin samples on the number of
microorganisms recovered.
(Data from Appendix 3a).

Table 7.

DURATION OF STOMACHING (MINS)	1	2	3	5	10
MEAN TOTAL VIABLE COUNTS FROM 16cm ² BREAST SKIN	1.22×10^5	2.04×10^5	2.56×10^5	1.99×10^5	1.75×10^5
STANDARD DEVIATION*	0.72×10^5	1.44×10^5	1.50×10^5	0.38×10^5	1.07×10^5
NUMBER OF SKIN SAMPLES EXAMINED	4	4	4	4	4

*Transformed and calculated from data presented in the Table of Analysis of Variance (Appendix 3a).

(Table 8). Statistically, numbers recovered by other techniques were not significantly different at the 95% confidence level.

SEM of intact and stomached skin from fresh immersion chilled broiler carcasses showed that although the surface integrity of the skin was maintained (*cf.* Figs. 6a; 15a), large amounts of particulate matter were removed from the surface by stomacher action (*cf.* Figs. 6b; 15b). Stomaching of skin from carcasses stored 5 days at 10°C removed most microorganisms present on the skin (Figs. 16a, b, c, d) and only a few firmly attached bacteria remained present on the skin (Figs. 16c, d and inset).

(f) Water Soluble Compounds Present in Immersion Chiller Water and on the Skin of Fresh and Stored Broiler Carcasses

TLC of freeze dried solids, detected only one component amino acid in immersion chiller water and four components in skin swab fluids from fresh and stored poultry carcasses (Fig. 17). The amino acid present in chiller water was also present in all swab fluids and had the same R_F value as the taurine standard (R_F 0.24). This component, based on colour intensity of the ninhydrin-amino acid complex, was the major amino acid present. All other components were present in relatively small quantities. No cysteine or methionine were detected.

Electrophoresis was used to separate any proteins present on the freeze dried solids of chiller water and skin swab fluids. The only protein separated from all samples had similar electrophoretic mobility to bovine serum albumin and chicken blood serum albumin (Fig. 18). Based on the stain intensity of the protein bands, swab fluids from carcasses stored 4 days at 2°C had greater amounts of protein present than skin swab fluids from fresh chilled poultry

and chiller water. No protein bound carbohydrate or lipoprotein was detected in either skin swab fluids or immersion chiller water.

TABLE 8. Comparison of recovery of microorganisms
from broiler carcass breast skin by the
stomacher and Atomix blender techniques.
(Summary of data presented in Appendix 3b).

Table 8.

	SAMPLING METHOD	
	STOMACHER	ATOMIX BLENDER
MEAN TOTAL VIABLE COUNT PER 16cm ² BREAST SKIN	2.22 x 10 ⁵	1.40 x 10 ⁵
STANDARD DEVIATION*	3.87 x 10 ⁵	2.04 x 10 ⁵
NUMBER OF SKIN SAMPLES EXAMINED	18	18

*Transformed and calculated from data presented in the Table of Analysis of Variance (Appendix 3b).

Figure 15. SEM examination of the effect of stomacher action on skin from fresh immersion chilled broiler carcasses. All tissue fixed by immersion in a solution of glutaraldehyde.

(a) Low magnification SEM of stomached skin.

Note that the surface integrity has been maintained (*cf.* Figure 6a). Bar = 30 μ m.

(b) High magnification SEM of the surface of an individual skin cell. Much of the particulate material observed on unstomached control samples (Figure 6b) has been removed. Bar = 3 μ m.

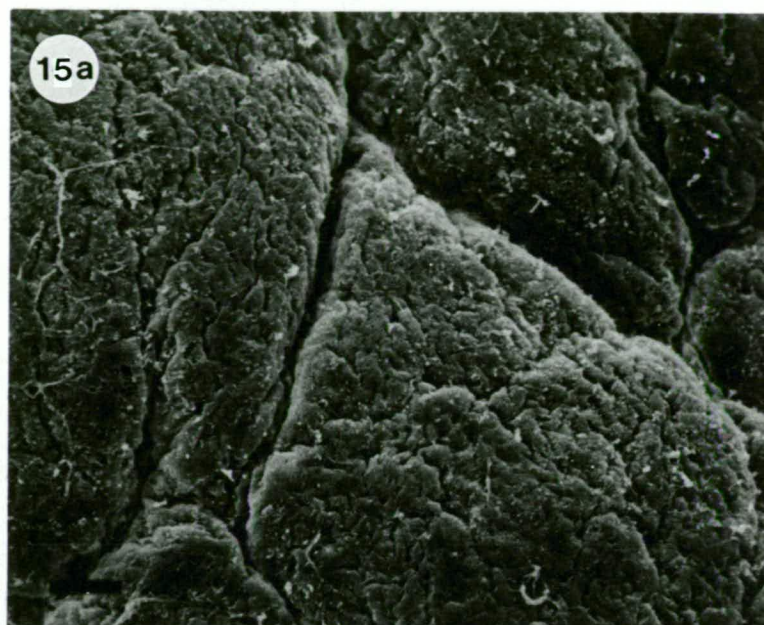


Figure 16. SEM examination of the effect of stomacher action on skin from a broiler carcass stored 5 days at 10°C. All tissue fixed by immersion in glutaraldehyde solutions.

(a) and (b). Skin prior to stomaching. Note the extent of microbial proliferation over the skin surface. No skin channels are visible. Bars = 30µm and 10µm respectively.

(c) Skin after stomaching in saline diluent. Most microorganisms have been removed from the skin channels and exposed surfaces. Bar = 30µm.

(d) Higher magnification SEM of spoiled skin after stomaching showing a number of surface microorganisms not removed. Bar = 10µm. Inset shows these microorganisms are covered by a mass of extracellular material, and appear to be interconnected by thin strands. Bar = 3µm.

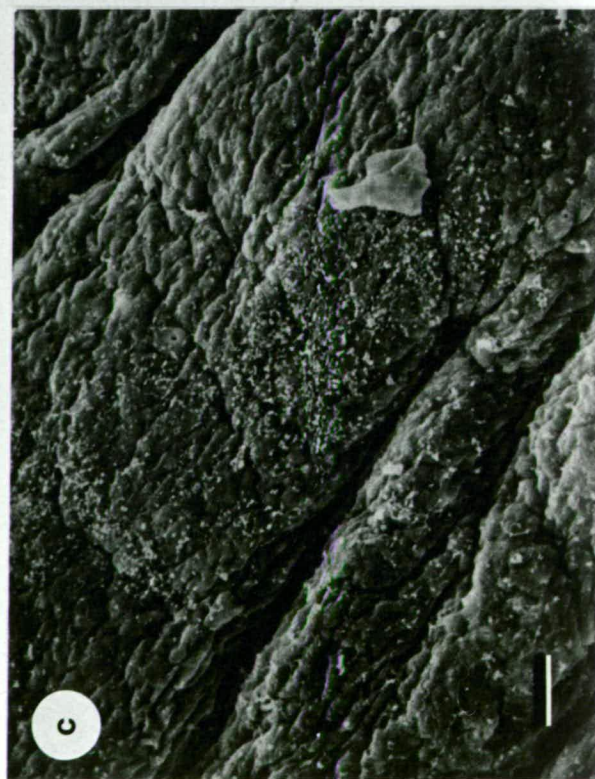
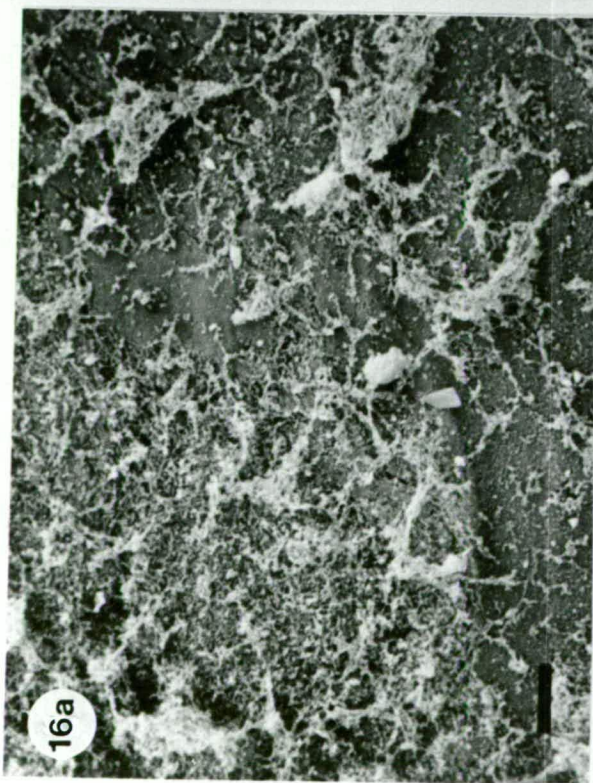
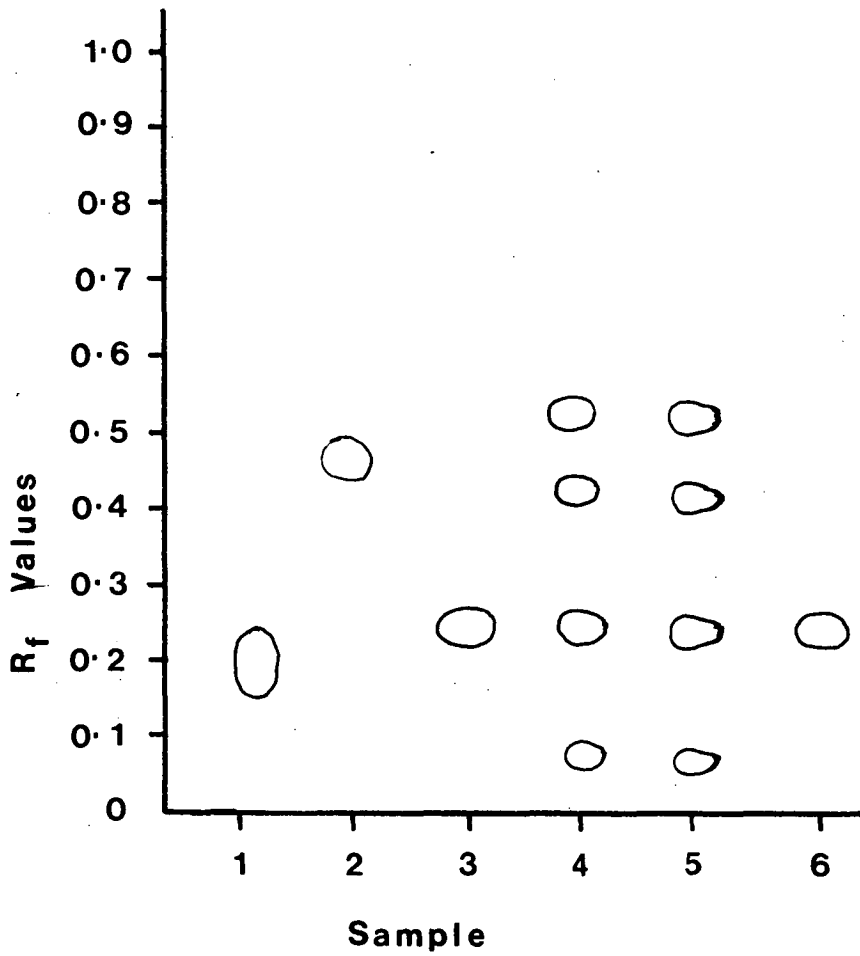


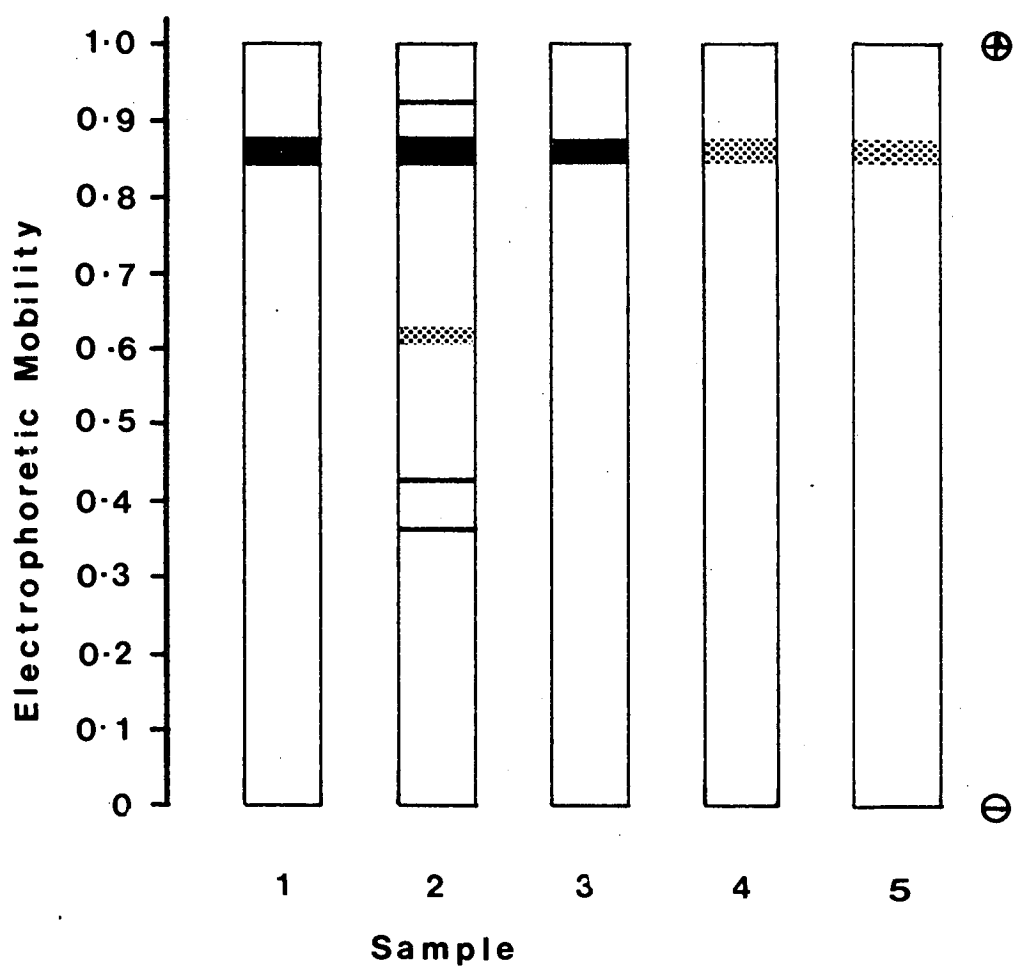
FIGURE 17. Diagram of TLC of ninhydrin +ve amino compounds present in freeze dried solids of immersion chiller water and skin swab fluids from fresh and stored immersion chilled broiler carcasses.

Figure 17



- SAMPLE 1. L-CYSTEINE CONTROL
2. L-METHIONINE CONTROL
3. TAURINE CONTROL
4. SKIN SWAB FLUID FROM CARCASSES STORED 4 DAYS AT 2°C
5. SKIN SWAB FLUID FROM FRESH CARCASSES
6. IMMERSION CHILLER WATER

FIGURE 18. Diagram of acrylamide slab gel electrophorogram of proteins separated from freeze dried solids of chiller water and skin swab fluids.

Figure 18

- SAMPLE 1. BOVINE SERUM ALBUMIN CONTROL
2. 10% (w/v) CHICKEN BLOOD SERUM
3. SKIN SWAB FLUIDS FROM CARCASSES STORED 4 DAYS AT 2°C
4. SKIN SWAB FLUIDS FROM FRESH IMMERSION CHILLED CARCASSES
5. IMMERSION CHILLER WATER

SECTION B

Development and Metabolism of Microorganisms on Broiler

Carcass Skin During Storage at Chill Temperatures

Materials and Methods

(a) Origin and Isolation of Strains

Broiler carcasses removed from an immersion chiller were drained for 30 secs and placed breast up in sterile oxygen permeable polythene. The bags were inflated with air and sealed to maintain conditions of high humidity around the carcass as well as prevent contact of the leg and breast areas with the bag walls. All carcasses were incubated at 2°C for 0, 4, 8, 12 or 16 days.

Following storage, pieces of skin (16cm²) were excised from the leg and breast areas (Fig. 2a, b). Each piece of skin was homogenised in 100ml saline (0.8% w/v NaCl) using a model 400 stomacher. Serial decimal dilutions of the homogenate were prepared and 0.1ml aliquots of suitable dilutions were spread on the surface of nutrient agar plates. Inoculated plates were incubated aerobically for 14 days at 4°C or 3 days at 22°C to enumerate psychrotrophic organisms and the TVC.

In separate storage experiments, numbers of coliforms and yeasts present on skin were monitored in addition to psychrotrophs and the TVC. Coliforms were enumerated by standard pour plate techniques using 1ml aliquots of suitable dilutions of the skin homogenate and MacConkey agar (Oxoid, Code CM7); inoculated plates were incubated for 24 hours at 37°C. Yeasts were enumerated by the surface spread technique using 0.1ml aliquots of the skin homogenate and Sabouraud agar plates with added chloramphenicol (Appendix 1) to inhibit bacterial growth; inoculated plates were incubated for 5

days at 15°C. Counts per 16cm² of skin were recorded for each sampling time and site.

Colonies growing on TVC plates from separate storage experiments were isolated and maintained on nutrient agar slopes at 2°C.

Colonies from nutrient agar plates incubated at 4°C were isolated and maintained as above.

(b) Characterisation of Isolates

The modified scheme of Shewan *et al.* (1960a) presented in Figure 3 was used to identify the isolates.

(c) Production of Off Odours by Isolates Grown on Sterile Excised Leg Muscle

Pieces of leg muscle were aseptically excised from freshly processed broiler carcasses using a technique described by McMeekin (1977) and placed in sterile 30ml McCartney bottles. All muscle sections were used immediately.

Psychrotrophic organisms isolated from leg and breast skin of carcasses stored for periods up to 16 days at 2°C, were grown overnight at 22°C on nutrient agar slopes. The slopes were washed with 4ml sterile quarter strength Ringer's solution (Oxoid Code BR52) and 0.1ml aliquots of these suspensions used to inoculate the sterile muscle sections. The inoculated muscle pieces, along with uninoculated control muscle pieces, were incubated at 2°C for 14 days. Sensory examinations of headspace vapours were carried out after 7 and 14 days and the presence of sulphide volatiles tested using DTNB papers (McMeekin *et al.* 1978) and lead acetate paper.

(d) Production of Off Odours by Isolates Grown on Broiler Skin

Preparations

Pieces of skin ($\approx 1\text{cm}^2$), excised from the outside leg area of fresh immersion chilled broiler carcasses, were scalded in water at 62°C for 3 minutes, removed and drained. Using sterile forceps, the skin pieces were transferred to sterile filter pads soaked in quarter strength Ringer's solution which were then placed in 20ml plastic capped glass vials. These preparations were inoculated with 0.05ml aliquots of suspensions of psychrotrophic organisms (prepared as above) which were previously shown to produce off odours when grown on leg muscle sections. Inoculated skin preparations were incubated at 2°C and sensory examinations carried out after 14 days. The presence of sulphide volatiles in headspace vapours were tested using DTNB and lead acetate papers as above.

(e) Production of Sulphur Volatiles by Isolates Grown in

Methionine or Cystine Supplemented Peptone Broth

Peptone broth (0.8% w/v, Oxoid Code L37) supplemented with L-methionine (0.1% w/v), L-methionine (0.1% w/v) plus glucose (0.1% w/v) or L-cystine (0.1% w/v) was aseptically dispensed in 10ml volumes in 30ml sterile McCartney bottles. Bottles of each media were inoculated with a loopful of overnight nutrient agar slope cultures of psychrotrophic organisms isolated from leg and breast skin of broiler carcasses stored for periods up to 16 days at 2°C . Inoculated media were incubated at 2°C for 14 days. Headspace vapours were examined as previously described. Clinistix glucose test strips (Ames Co., Melbourne, Australia) were used to test presence or absence of glucose in incubated bottles of glucose methionine peptone broth.

(f) Analysis of Sulphide Odours Produced by Isolates Grown on Muscle, Skin or Artificial Substrates

An objective description of the sulphide odours produced by inoculation of sterile muscle sections, skin preparations and sulphur amino acid supplemented peptone broths was provided by gas chromatography and organic mass spectrometry. The gas chromatograph used was a Pye Unicam GCD fitted with a flame ionization detector. The column used was a 1.5 metre, 4mm ID 80/100 mesh Poropak Q operated isothermally at 150°C, with a nitrogen carrier gas flow rate of 40ml/min. The injector port and detector temperatures were 150°C and 210°C respectively. Mass spectra of odour volatiles were obtained with a VG 7070F mass spectrometer (V.G. Micromass Ltd., Winsford, England) interfaced to a Pye Unicam 204 gas chromatograph. The column used was a 25 metre Carbowax 20M capillary column run isothermally at 80°C with an argon flow rate of 2ml/min. The injector port temperature was 100°C. Authentic samples of hydrogen sulphide, methanethiol (Merck, Darmstadt, Germany), dimethyl sulphide (Sigma Chemical Co., St. Louis, U.S.A.) and dimethyl disulphide (Sigma) were used as controls.

(g) Detection Thresholds of DTNB and Lead Acetate Papers

The minimum concentrations of methanethiol and hydrogen sulphide detected by lead acetate and DTNB papers (McMeekin *et al.* 1978) were determined by two techniques. Mercuric mercaptide was prepared and purified as described by Sharpe *et al.* (1976) and examined in a Carey Laser Raman Spectrometer to check authenticity and purity (Canty *et al.* 1978). Methanethiol was released from accurately known weights of this mercuric salt by the addition of 0.2ml of 5N hydrochloric acid (Manning 1974) in glass vessels of known volume and tested for a positive DTNB reaction.

Alternatively, 10ml volumes of serial dilutions of methanethiol and hydrogen sulphide in Tris-HCl buffer (0.1M, pH 8.0) were equilibrated in sealed test tubes of 25ml total volume. The headspace of each dilution was tested for positive DTNB and lead acetate reactions. The concentration of methanethiol or hydrogen sulphide in solution was determined quantitatively using DTNB reagent [0.01M in Tris-HCl buffer (0.1M, pH 8.0)] and the spectrophotometric technique described by Ellman (1959). The spectrophotometer used was a Hitachi Perkin-Elmer model 139.

(h) Microscopic Examination of Microorganisms on Skin from Broiler Carcasses Stored at Various Temperatures

Fresh immersion chilled carcasses were placed breast up in sterile oxygen permeable polythene bags. The bags were inflated with air, sealed and the carcasses stored for 4, 8, 12 or 16 days at 2°C or at 4°C, 10°C, 15°C or 22°C until off odour production was detected olfactorily. Other carcasses were stored uncovered at 2°C for 16 days. After storage, pieces of skin ($\approx 1\text{cm}^2$ in area) excised from breast and outside leg skin areas of the carcasses, were pinned to dental wax and prepared for scanning and transmission electron microscopic examination.

Skin pieces from carcasses stored under all the conditions described were prepared for SEM following overnight fixation at 4°C in osmium tetroxide vapour to glutaraldehyde solutions (5% w/v). Other control skin samples were excised from freshly processed broiler carcasses which had been immersed for 2 minutes in a bucket of immersion chiller water containing an inoculum ($\approx 10^8$ cells/ml of chiller water) of a washed cell suspension of a *Pseudomonas* group I strain originally isolated from spoiled poultry. Following immersion, the carcasses were drained for

30 seconds and the skin samples excised and fixed in osmium tetroxide vapour overnight at 4°C. Fixed skin specimens were then rinsed in cold sodium phosphate buffer, dehydrated in a graded ethanol series, infiltrated with amyl acetate and critical point dried from carbon dioxide as described previously. Dried skin pieces were glued to brass SEM stubs with silver electroconductive paint, coated with about 270Å of gold by sputtering and examined in a JEOL JXA 50-A scanning electron microscope unit operated at 15kV. All micrographs were recorded on Polaroid type 52 or type 107 Polaplan film.

Other skin pieces from carcasses stored 4, 8, 12 or 16 days at 2°C under high humidity conditions and 16 days at 2°C under drying conditions were prepared for TEM following overnight fixation at 4°C in sodium phosphate buffer (0.1M, pH 7.2) containing glutaraldehyde (5% v/v) and alcian blue (1% w/v). All pieces were rinsed in cold phosphate buffer to remove unbound dye and postfixed overnight at 4°C in a solution of osmium tetroxide (1% w/v) in sodium phosphate buffer (0.1M, pH 7.2). Pieces of skin from carcasses stored 16 days at 2°C under high humidity conditions were treated with ruthenium red during fixation using methodology described by Luft (1971); control skin pieces were fixed without the presence of ruthenium red. Other skin pieces from carcasses stored for 4 days at 2°C were fixed overnight at 4°C in osmium tetroxide vapour. Fixed specimens were rinsed in the appropriate buffer, dehydrated in a graded ethanol series, soaked in two 30 minute changes of 1,2 epoxypropane and embedded in Araldite M according to Glauert and Glauert (1958). Silver sections of the embedded tissue were cut on an LKB 4801A ultramicrotome and mounted on formvar coated copper electron microscope grids. Sections of skin treated with ruthenium red

during fixation were stained for 5 minutes at room temperature with Reynolds lead citrate. Sections of all other tissue were stained in aqueous uranyl acetate (1% w/v) followed by Reynolds lead citrate; or potassium permanganate (Glauert 1965) followed by Reynolds lead citrate. Stained sections were examined with either a Phillips EM201 or a Hitachi H-300 operated at 60kV or 72kV respectively. Micrographs were recorded on Ilford electron microscope film.

One micron survey sections of Araldite embedded skin tissue were stained using a polychrome stain described by Sato and Shamoto (1973), or a crystal violet solution. These sections were examined by bright field light microscopy. Light micrographs were recorded on Kodak Kodachrome 64 slide film or Kodak Plus X Pan ASA 125 film.

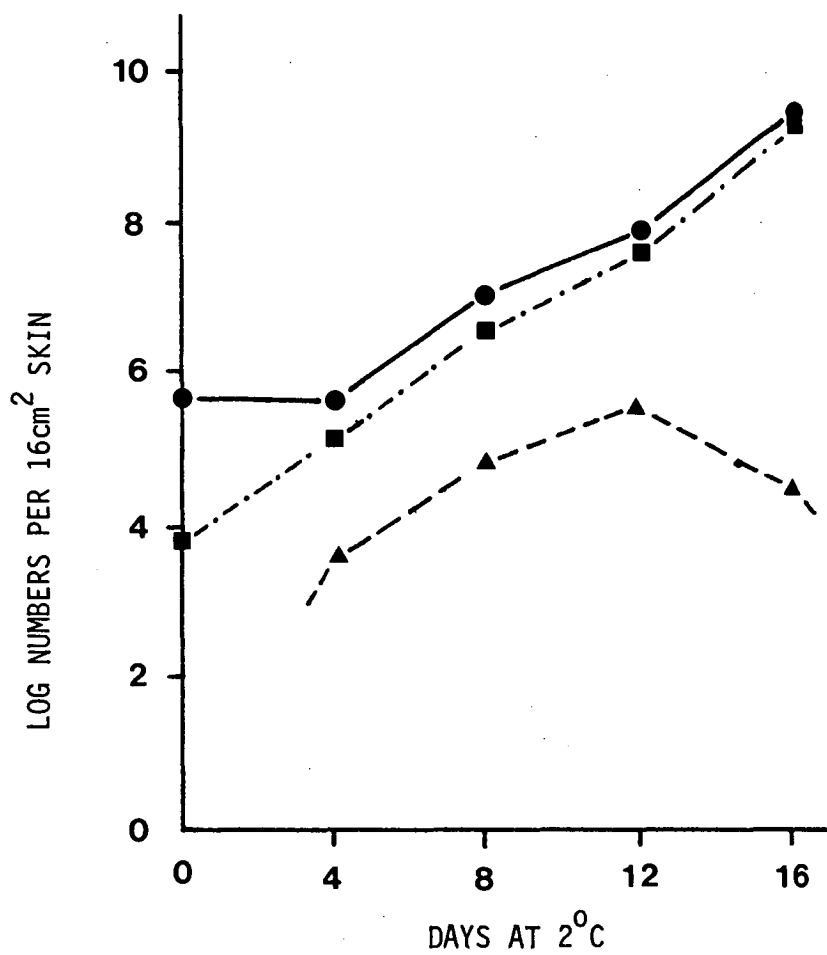
RESULTSSECTION B

(a) Numbers and Incidence of Different Bacteria During Spoilage of Naturally Contaminated Skin

Changes in viable numbers of various groups of microorganisms present on the skin of naturally contaminated broiler carcasses during storage at 2°C were monitored over a period of 16 days. The results are presented in Figures 19 and 20. Similar trends were noted for both breast and leg skin. After a 4 day lag phase, the TVC rapidly increased to about 10^9 organisms/16cm² of skin after 16 days of storage, while numbers of psychrotrophs increased rapidly from the beginning of storage to about the same level at 16 days. Psychrotrophic yeasts were not recovered until the 4 day stage of storage. Their numbers reached a maximum of about 10^5 /16cm² of skin after 8 to 12 days and remained stable or decreased slightly during subsequent storage. Coliform numbers usually decreased to below detectable numbers after 8 days of storage.

A total of 261 strains were isolated from TVC plates of leg and breast skin from carcasses stored up to 16 days at 2°C. Tables 9a and 9b illustrate the distribution of the various genera of bacteria on breast and leg skin during storage. Freshly processed carcasses were contaminated by a wide variety of generic types, all of which could be recovered up to 4 days of storage. However, pseudomonads rapidly predominated with a few other gram negative types such as *Acinetobacter*, *Moraxella* and flavobacteria. *Pseudomonas* groups I and II represented more than 80% of the total

Figure 19. Changes in numbers of microorganisms present on breast skin of broiler carcasses stored at 2°C. (Data from Appendix 4a).



SYMBOLS

TOTAL VIABLE COUNT

● — ●

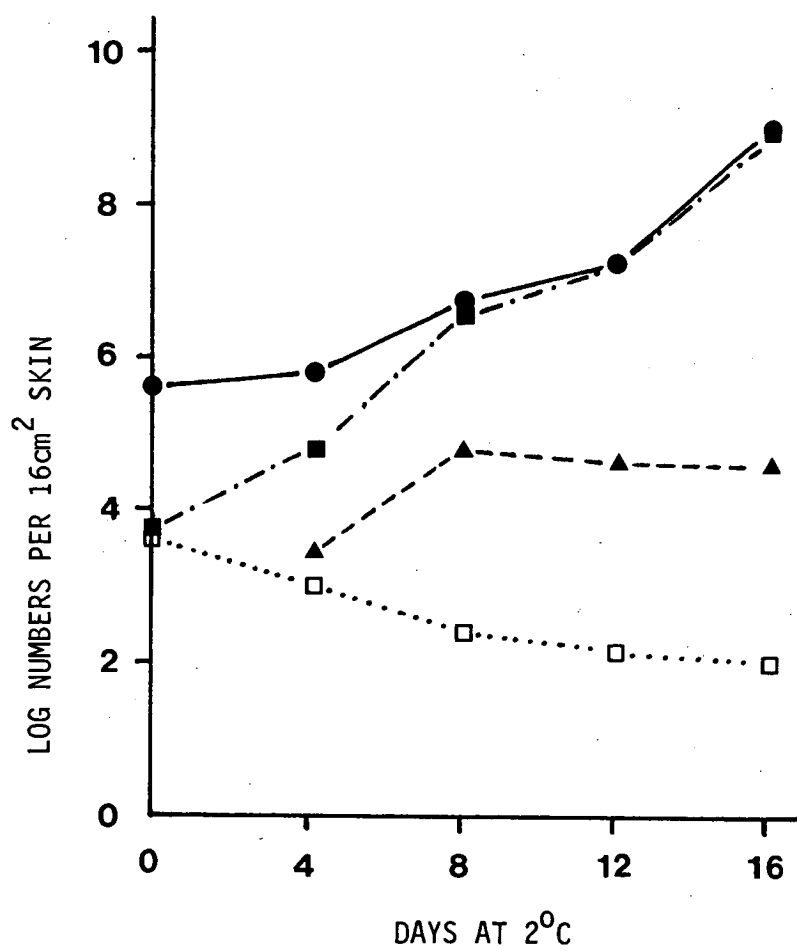
PSYCHROTROPH COUNT

■ - · - · ■

YEASTS

▲ - - - ▲

Figure 20. Changes in numbers of microorganisms
present on leg skin of broiler
carcasses stored at 2⁰C. (Data from
Appendix 4b).



SYMBOLS

TOTAL VIABLE COUNT	●——●
PSYCHROTROPH COUNT	■- - -■
YEASTS	▲- - -▲
COLIFORM COUNT	□.....□

**TABLE 9a. Aerobic microbial flora of breast
skin from broiler carcasses stored
at 2°C.**

Table 9a.

GROUP	PERCENTAGE DISTRIBUTION OF GROUPS ON SKIN FROM CARCASSES STORED AT 2°C FOR:				
	0 DAYS	4 DAYS	8 DAYS	12 DAYS	16 DAYS
<i>Micrococcus</i>	25	3	-	-	-
<i>Coryneforms</i>	-	-	-	-	-
<i>Acinetobacter</i>	4	16	7	-	-
<i>Moraxella</i>	-	-	-	-	-
<i>Flavobacterium/Cytophaga</i>	11	25	-	-	-
<i>ENTEROBACTERIACEAE</i>	4	13	-	-	-
Group I	50	6	41	64	43
<i>Pseudomonas</i> Group II	-	22	52	36	57
Group III/IV	7	13	-	-	-
Yeasts/Others	-	3	-	-	-
TOTAL VIABLE COUNT/16cm ² SKIN	2.5x10 ⁴	3.1x10 ⁴	2.11x10 ⁷	2.35x10 ⁹	3.68x10 ¹⁰
TOTAL NUMBER OF ISOLATES	28	32	26	21	20

TABLE 9b. Aerobic microbial flora of leg skin
from broiler carcasses stored at
2°C.

Table 9b.

GROUP	PERCENTAGE DISTRIBUTION OF GROUPS ON SKIN FROM CARCASSES STORED AT 2°C FOR:-				
	0 DAYS	4 DAYS	8 DAYS	12 DAYS	16 DAYS
<i>Micrococcus</i>	3	4	-	-	-
<i>Coryneforms</i>	3	4	-	-	-
<i>Acinetobacter</i>	66	27	21	-	-
<i>Moraxella</i>	8	-	-	-	-
<i>Flavobacterium/Cytophaga</i>	8	27	-	4	5
<i>ENTEROBACTERIACEAE</i>	8	8	-	-	-
Group I	6	4	48	56	60
<i>Pseudomonas</i> Group II	3	8	31	40	35
Group III/IV	3	15	-	-	-
Yeasts/Others	-	4	-	-	-
TOTAL NUMBER OF ISOLATES	38	25	28	24	19
TOTAL VIABLE COUNT/16cm ² SKIN	4.95x10 ⁴	6.15x10 ⁴	6.91x10 ⁷	2.65x10 ⁹	1.66x10 ¹⁰

viable flora after 8 days at 2°C and more than 90% after 16 days storage. The remainder of the types isolated (e.g. *Micrococcus* spp., coryneforms, *Enterobacteriaceae* and yeasts) were unable to grow and multiply on the skin of carcasses stored at 2°C or could not successfully compete with the pseudomonads. *Alteromonas putrefaciens* was not isolated from either breast or leg skin sampled from fresh or stored carcasses.

Tables 10a and 10b show the distribution of psychrotrophic genera isolated from breast and leg skin from broiler carcasses stored up to 16 days at 2°C. Few psychrotrophic types were isolated from freshly processed broiler carcass skin by the plate count method. Only after 4 days of storage at 2°C were sufficient numbers present to enable a useful analysis of generic distribution. As with the TVC flora analysis, pseudomonads were the predominant bacteria growing on leg and breast skin (more than 80% of the psychrotrophic microflora after 16 days storage). The remainder, were *Acinetobacter*, *Moraxella*, flavobacteria and some coryneforms. *Pseudomonas* Groups I and II were the major types present on skin at all stages at all sampling times, but *Pseudomonas* Group I progressively predominated after 4 days at 2°C. *Pseudomonas* group III/IV organisms were recovered as a small fraction of the spoilage microflora present on carcasses after 12 days and 16 days of storage.

(b) Production of Off Odours by Isolates Grown on Sterile Leg Muscle and Skin Preparations

The proportion of psychrotrophic strains of bacteria, isolated from the skin of stored broiler carcasses, which produced off odours and DTNB positive odours when grown on

TABLE 10a. Psychrotrophic bacteria present on
breast skin from broiler carcasses
stored at 2°C.

Table 10a.

GROUP	PERCENTAGE OF FLORA PRESENT ON SKIN OF CARCASSES STORED AT 2°C FOR:				
	0 DAYS	4 DAYS	8 DAYS	12 DAYS	16 DAYS
<i>Coryneforms</i>	-	14	-	-	-
<i>Acinetobacter</i>	-	-	5	-	-
<i>Moraxella</i>	-	-	9	5	-
<i>Flavobacterium</i>	-	-	-	-	-
Group I	-	43	86	52	75
<i>Pseudomonas</i> Group II	-	43	-	37	6
Group III/IV	-	-	-	5	19
Yeasts/Others	(a)	-	-	-	-
TOTAL NUMBER OF ISOLATES	1	7	21	17	16
TOTAL VIABLE COUNT/16cm ² SKIN	8.5x10 ³	7.9x10 ⁴	2.9x10 ⁵	2.03x10 ⁶	1.55x10 ⁹
PSYCHROTROPHIC COUNT/16cm ² SKIN	≈500	1.75x10 ⁴	2.90x10 ⁵	7.5x10 ⁵	8.20x10 ⁸

(a) PERCENTAGES NOT EXPRESSED FOR LOW NUMBERS OF ISOLATES.

TABLE 10b. Psychrotrophic bacteria present on
leg skin of broiler carcasses
stored at 2°C.

Table 10b.

GROUP	PERCENTAGE OF FLORA PRESENT ON SKIN OF CARCASSES STORED AT 2°C FOR:				
	0 DAYS	4 DAYS	8 DAYS	12 DAYS	16 DAYS
<i>Coryneforms</i>	(a)	9	22	-	-
<i>Acinetobacter</i>	(a)	-	6	-	-
<i>Moraxella</i>	-	-	-	-	-
<i>Flavobacterium</i>	-	18	-	5	5
Group I	-	36	44	24	57
<i>Pseudomonas</i> Group II	-	36	28	67	33
Group III/IV	-	-	-	5	5
Yeasts/Others	-	-	-	-	-
TOTAL NUMBER OF ISOLATES	3	11	19	21	20
TOTAL VIABLE COUNT/16cm ² SKIN	1.1x10 ⁴	3.65x10 ⁴	2.7x10 ⁶	1.10x10 ⁸	7.3x10 ⁹
PSYCHROTROPH COUNT/16cm ² SKIN	≈1000	1.5x10 ⁴	2.0x10 ⁶	5.6x10 ⁷	5.7x10 ⁹

(a) PERCENTAGES NOT EXPRESSED FOR SMALL NUMBERS OF ISOLATES.

leg muscle is presented in Tables 11a and 11b. Similar trends were noted for isolates from breast and leg skin. A higher proportion of strains isolated from carcasses stored for 16 days at 2°C were able to produce off odours when grown on leg muscle than was the case for strains isolated from carcasses stored for shorter periods of time. The proportion of all isolates able to produce off odour compared to those which produced only DTNB positive odours was about 2:1. Only sulphide-like headspace vapours were DTNB positive. Of the 45 isolates which produced off odour when grown on leg muscle, only 3 gave lead acetate positive headspace reactions. Isolates producing fruity odours when grown on leg muscle were a small proportion of the off odour producers present on skin from carcasses at any stage of storage. 11% of strains isolated from carcasses stored 8 days at 2°C were able to produce fruity type odours whereas less than 5% of all isolates from carcasses stored 16 days produced these odours.

Psychrotrophic strains of bacteria isolated from breast and leg skin which were able to produce off odour when grown on leg muscle, were also able to produce odour when grown on skin preparations (Tables 11a, b). However, not all isolates which produced DTNB positive odours from muscle, produced DTNB positive odours when grown on skin, but isolates which produced fruity odours from leg muscle did so when grown on skin.

TABLE 11a. Off odour production from various
substrates by psychrotrophic bacteria
isolated from breast skin of broiler
carcasses stored at 2°C.

Table 11a.

	DAYS CARCASS STORED AT 2°C				
	0	4	8	12	16
TOTAL NUMBER OF ISOLATES	1	7	21	17	16
ISOLATES PRODUCING OFF ODOUR FROM EXCISED LEG MUSCLE	0(a)*	3(a)	5(23)	6(35)	7(44)
ISOLATES PRODUCING DTNB +VE OFF ODOURS FROM EXCISED LEG MUSCLE	0(a)	3(a)	3(14)	3(18)	3(19)
ISOLATES PRODUCING OFF ODOUR ON LEG MUSCLE WHICH ALSO PRODUCE OFF ODOUR ON SKIN	0(a)	3(a)	4(19)	4(24)	7(44)
ISOLATES PRODUCING DTNB +VE ODOUR IN METHIONINE PEPTONE BROTH	0(a)	3(a)	11(52)	7(41)	8(50)
ISOLATES PRODUCING DTNB +VE ODOURS IN GLUCOSE METHIONINE PEPTONE BROTH	0(a)	1(a)	5(23)	3(18)	1(16)
ISOLATES PRODUCING DTNB +VE ODOURS IN PEPTONE BROTH	0(a)	0(a)	4(19)	3(18)	3(19)
ISOLATES PRODUCING LEAD ACETATE +VE ODOUR IN CYSTINE PEPTONE BROTH	0(a)	0(a)	2(10)	0(a)	0(a)

*FIGURES IN PARENTHESES ARE PERCENTAGES OF THE TOTAL NUMBER OF ISOLATES.

(a) PERCENTAGES NOT EXPRESSED FOR SMALL NUMBERS OF ISOLATES.

TABLE 11b. Off odour production from various
substrates by psychrotrophic
bacteria isolated from leg skin
of broiler carcasses stored at 2°C.

Table 11b.

	DAYS CARCASS STORED AT 2 ⁰ C				
	0	4	8	12	16
TOTAL NUMBER OF ISOLATES	3	11	19	21	20
ISOLATES PRODUCING OFF ODOUR FROM EXCISED LEG MUSCLE	0(a)*	5(a)	5(26)	6(29)	8(40)
ISOLATES PRODUCING DTNB +VE OFF ODOURS FROM EXCISED LEG MUSCLE	0(a)	4(a)	3(15)	4(19)	5(25)
ISOLATES PRODUCING OFF ODOUR ON LEG MUSCLE WHICH ALSO PRODUCE OFF ODOUR ON SKIN	0(a)	3(27)	5(26)	6(29)	8(40)
ISOLATES PRODUCING DTNB +VE ODOUR IN METHIONINE PEPTONE BROTH	0(a)	6(a)	8(44)	7(33)	10(48)
ISOLATES PRODUCING DTNB +VE ODOURS IN GLUCOSE METHIONINE PEPTONE BROTH	0(a)	3(a)	6(33)	4(19)	3(14)
ISOLATES PRODUCING DTNB +VE ODOURS IN PEPTONE BROTH	0(a)	1(a)	3(16)	5(24)	4(19)
ISOLATES PRODUCING LEAD ACETATE +VE ODOUR IN CYSTINE PEPTONE BROTH	0(a)	1(a)	0(a)	2(10)	3(14)

*FIGURES IN PARENTHESES ARE PERCENTAGES OF THE TOTAL NUMBER OF ISOLATES

(a) PERCENTAGES NOT EXPRESSED FOR SMALL NUMBERS OF ISOLATES.

Within each of the *Pseudomonas* groups were found strains which caused no detectable odour and strains which produced either sulphide-like or fruity odours. The distribution of types of bacteria producing off odour from leg muscle is presented in Table 12. A greater proportion of *Pseudomonas* group I types caused off odour when incubated with leg muscle than did *Pseudomonas* group II types. *Pseudomonas* group III/IV types were also found to produce off odour.

The predominant odour produced by all strains was the sulphide-like odour. The majority of *Pseudomonas* group I strains producing this odour produced DTNB positive odours when incubated with either leg muscle or skin. Only two group I strains produced fruity odours although these also produced a sulphide component. *Pseudomonas* group II strains were responsible for the fruity odours produced from leg muscle and skin. However, members of this group also produced weak DTNB negative sulphide odours and DTNB positive sulphide odours. Three strains produced DTNB positive, lead acetate positive sulphide-like odours when grown on leg muscle, but did not produce this odour when grown on skin although a sulphide-like odour was detected olfactorily.

Table 12. Incidence and distribution of
off odour producers isolated from
the skin of broiler carcasses
stored at 2⁰C.

Table 12.

DAYS CARCASS STORED	NUMBER OF OFF ODOUR PRODUCERS		
	ALL STRAINS	<i>PSEUDOMONAS</i> GROUP I	<i>PSEUDOMONAS</i> GROUP II
0	0	0	0
4	8	3(37)*	5(63)
8	10	7(70)	3(30)
12	12	7(58)	5(42)
16	15	10(67)	4(27)
TOTALS	45	27(60)	17(38)

*Numbers in brackets represent percentages of all strains producing off odours.

(c) Production of Sulphide Volatiles from Methionine and Cyst(e)ine
Supplemented Peptone Broth

The proportion of psychrotrophic isolates which produced DTNB positive, lead acetate negative odours when grown in methionine broth is presented in Tables 11a and 11b. Almost half the breast or leg skin isolates from all stages of carcass storage produced odour. Isolates which produced sulphide like odours from leg muscle and skin preparations also produced DTNB positive odours when grown in methionine peptone broth. No isolate produced lead acetate positive headspace vapours from this medium.

The presence of glucose in methionine peptone broth caused a reduction in the number of isolates able to produce odours (Tables 11a and 11b). Isolates which produced DTNB positive odours in glucose methionine peptone broth also produced DTNB positive odours in methionine peptone broth. No glucose was detected in incubated tubes of glucose methionine peptone broth which showed presence of DTNB positive odours. However, where sulphide volatiles were absent or present in concentrations not detectable by DTNB, glucose was found present, but in reduced amounts relative to uninoculated control tubes of glucose methionine peptone broth.

Tables 11a and 11b present numbers of psychrotrophic isolates able to produce DTNB positive odours from peptone broth. Fewer isolates produced DTNB positive odours from peptone than from any other substrate tested, however, isolates which produced odour from peptone also produced off odour from methionine peptone broth and glucose methionine peptone broth. All odours produced were lead acetate negative.

A higher proportion of isolates produced lead acetate positive odours when grown in cystine peptone broth compared to muscle sections (Tables 11a and 11b). Positive lead acetate headspace vapours were also DTNB positive.

The incidence and distribution of strains producing DTNB positive odours from methionine supplemented peptone broth is presented in Table 13. A higher proportion of *Pseudomonas* group I types produced odour from this medium than did *Pseudomonas* Group II. Only *Pseudomonas* group II strains produced DTNB positive, lead acetate positive sulphide odours from cystine supplemented peptone broth.

(d) Types and Identity of Off Odours Produced by Psychrotrophic Isolates Grown on Various Substrates

Two distinct types of odour were produced by strains of psychrotrophic bacteria, isolated from spoiled poultry, when these were incubated with leg muscle sections, skin preparations or amino acid supplemented peptone broth. These odours were characterised as sulphide-like or fruity. Some isolates produced both fruity and sulphide-like odours from the same substrate. Bacteria producing sulphide-like odours were the predominant off odour producers present on the skin from carcasses stored at 2°C.

Because of the difficulty in interpretation of the subjective description of sulphide odours objective analyses were provided by chemical tests, gas chromatography and mass spectroscopy. Hydrogen sulphide in DTNB positive headspace vapours was tested with lead acetate paper. DTNB positive, lead acetate negative vapours contained aliphatic thiols other than hydrogen sulphide. DTNB does not detect the presence of dimethyl sulphide or dimethyl disulphide.

Only 3 psychrotrophic strains of bacteria isolated from the skin of stored carcasses produced hydrogen sulphide and none produced this volatile when incubated with skin preparations.

Table 13. Incidence and distribution of psychrotrophic strains isolated from the skin of broiler carcasses stored at 2°C, which produced DTNB positive odours when grown in methionine supplemented peptone broth.

Table 13.

DAYS CARCASS STORED	NUMBER OF ISOLATES PRODUCING ODOUR		
	ALL STRAINS	<i>PSEUDOMONAS</i> GROUP I	<i>PSEUDOMONAS</i> GROUP II
0	0	0	0
4	9	3(33)	5(55)
8	19	15(79)	4(21)
12	14	10(71)	3(21)
16	18	12(67)	5(27)
TOTALS	60	40(66)	17(28)

*Numbers in brackets represent percentages of all strains producing off odours.

However, 8 isolates produced hydrogen sulphide when grown in cystine supplemented peptone broth. Detection of hydrogen sulphide by gas chromatography was not possible because the flame ionization detectors fitted were insensitive to this gas.

Representative headspace vapours produced by isolates incubated with muscle sections, skin or methionine supplemented media were examined by gas chromatography and organic mass spectrometry. All DTNB positive headspace vapours contained large quantities of methanethiol (retention time 3 mins on Poropak Q; Figs. 21a, b, c) while some contained dimethyl sulphide (retention time 7 mins on Poropak Q; Fig. 21d). The presence of methanethiol and dimethyl sulphide was confirmed by authentic control samples and organic mass spectrometry. Dimethyl disulphide, although not separated on the Poropak Q column used, was detected by organic mass spectrometry in all DTNB positive samples tested. Sulphide-like DTNB negative headspace odours contained only small quantities of methanethiol, relative to DTNB positive samples, with or without the presence of dimethyl sulphide (Figs. 21e, f). Isolates which produced hydrogen sulphide or fruity odours with a sulphide component, also produced methanethiol (Figs. 21a, j). Hydrogen sulphide, methanethiol, dimethyl sulphide and dimethyl disulphide were not detected in the headspace vapours of uninoculated control substrates and substrates inoculated with isolates which did not produce odours (Figs. 21g, h, i).

(e) Detection Thresholds of DTNB and Lead Acetate Papers

The minimum concentration of gaseous methanethiol detected by DTNB test strips was approximately 0.034 ppm (v/v). This gas concentration was produced by evolution of 7.838×10^{-8} g of

Figure 21. Gas chromatography profiles of headspace vapours produced by psychrotrophic bacteria when these were grown at 2⁰C on leg muscle, skin preparations or in amino acid supplemented peptone broth. The column used was a Poropak Q operated isothermally at 150⁰C with a nitrogen carrier flow rate of 40ml/min. Signal attenuation as indicated. Peak numbers 1. air peak; 2. methanethiol; 3. dimethyl sulphide.

(a) Methanethiol produced by a *Pseudomonas* Group II strain grown on excised leg muscle. Headspace vapour was DTNB positive, lead acetate positive, and possessed a strong sulphide-like odour. The organism was isolated from leg skin of a carcass stored 4 days at 2⁰C.

(b) Methanethiol produced by a *Pseudomonas* Group I strain grown on skin. Headspace vapour was DTNB positive, lead acetate negative and possessed a strong sulphide-like odour. The organism was isolated from breast skin of a carcass stored 16 days at 2⁰C.

(c) Methanethiol produced by a *Pseudomonas* Group II strain grown in methionine supplemented peptone broth. Headspace vapour was DTNB positive, lead acetate negative, and possessed a strong sulphide-like odour. The organism was isolated from leg skin of a carcass stored 4 days at 2⁰C.

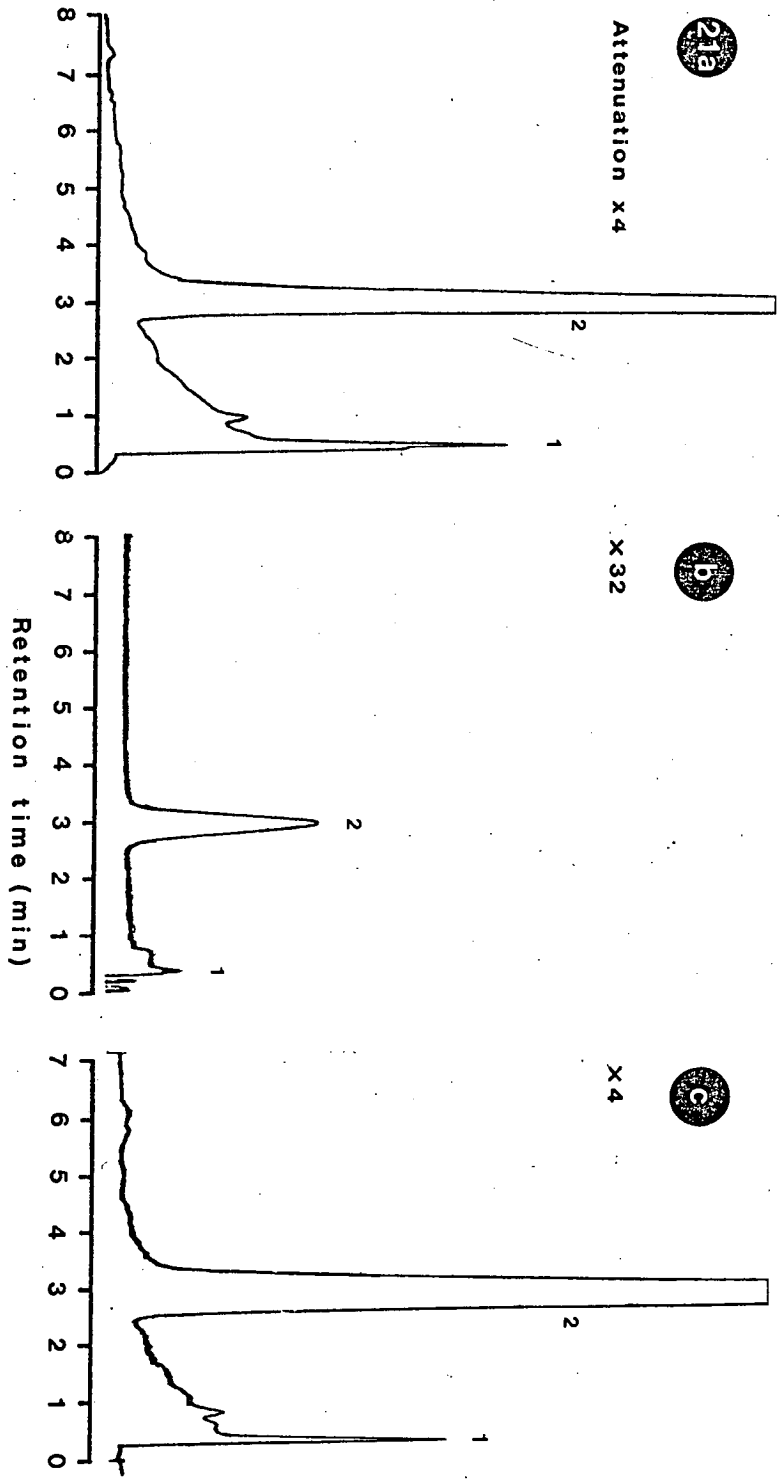


Figure 21. (Continued)

(d) Methanethiol and dimethyl sulphide produced by a *Pseudomonas* Group I strain grown on leg muscle.

Headspace vapour was DTNB positive, lead acetate negative and possessed a strong sulphide-like odour.

The organism was isolated from leg skin of a carcass stored 16 days at 2⁰C.

(e) Methanethiol and dimethyl sulphide produced by a *Pseudomonas* Group II strain grown in glucose and

methionine supplemented peptone broth. Headspace

vapour was DTNB and lead acetate negative, but

possessed a weak sulphide-like odour. The organism

used was the same strain as used for (c) above.

Note that the presence of glucose has inhibited production of methanethiol.

(f) Methanethiol and dimethyl sulphide produced

by a *Pseudomonas* Group II strain grown on leg

muscle. Headspace vapour was DTNB and lead acetate

negative, but possessed a weak sulphide-like odour.

The organism was isolated from leg skin of a

carcass stored 12 days at 2⁰C.

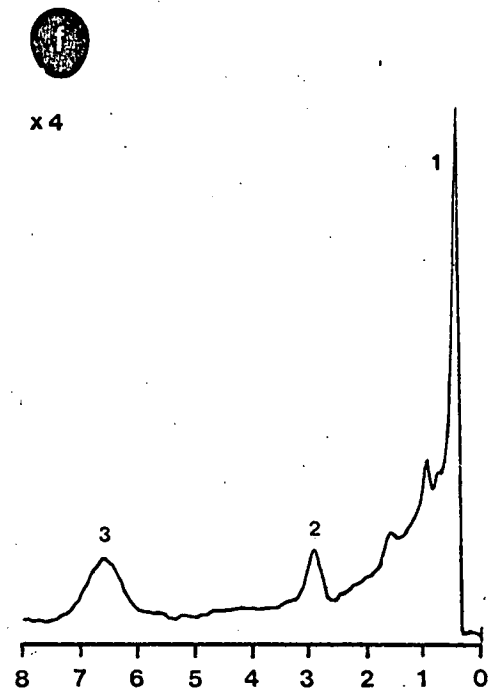
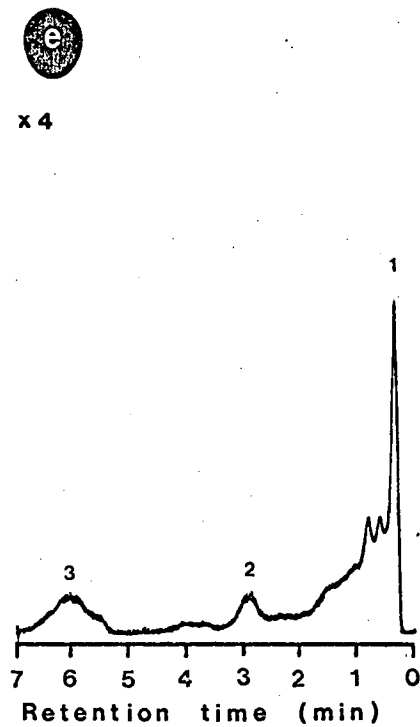
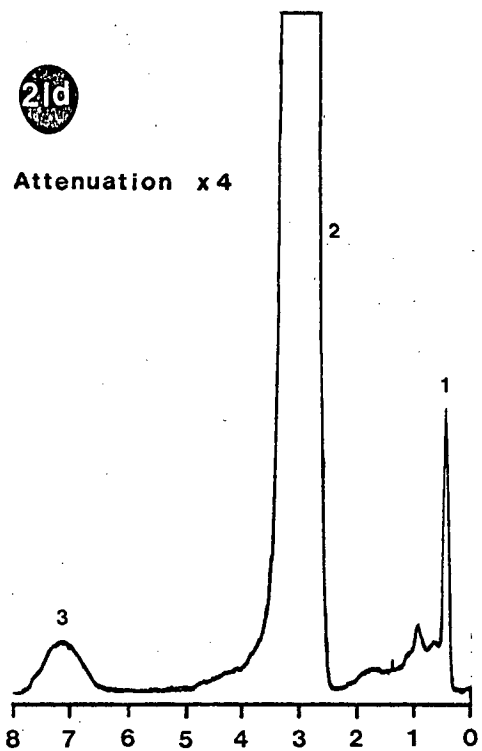


Figure 21. (Continued)

(g) Headspace profile of a *Pseudomonas* Group I strain which produced no off odours when grown on meat. Headspace vapour was DTNB and lead acetate negative. The organism was isolated from breast skin of a carcass stored 16 days at 2°C.

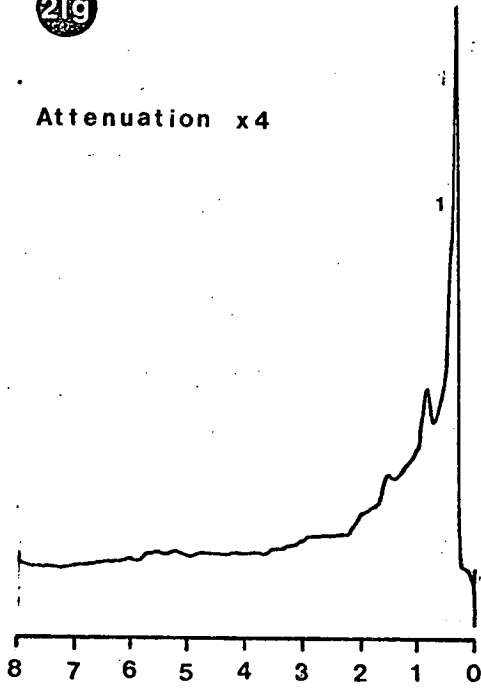
(h) Headspace profile of sterile, leg muscle stored 1 month at 2°C.

(i) Headspace profile of uninoculated skin stored 1 month at 2°C.

(j) Methanethiol produced by a *Pseudomonas* Group I strain grown on leg muscle. Headspace vapour was DTNB and lead acetate negative, but possessed a fruity type odour with a sulphide component. The organism was isolated from skin of a carcass stored 8 days at 2°C.

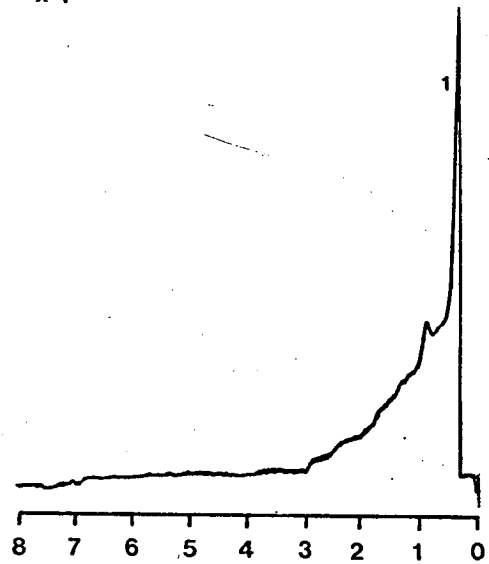
21g

Attenuation x4



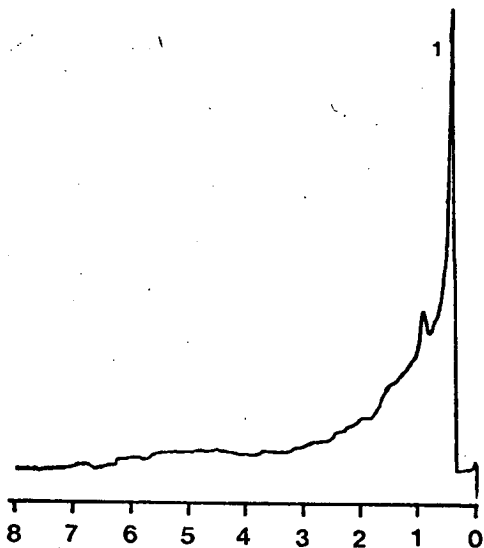
h

x4



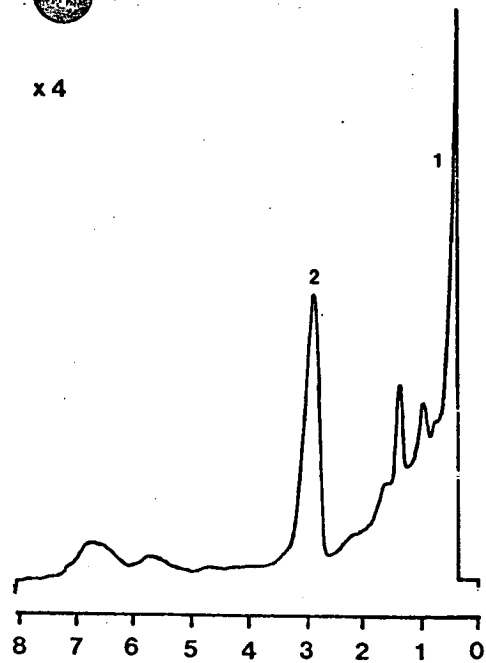
11

x4



1

x4



Retention time (min)

methanethiol from 2.40×10^{-7} g of mercuric mercaptide into a 106ml volume (Appendix 5). An aqueous methanethiol solution concentration of 19.70 ppm (v/v) produced a threshold gas concentration within the headspace of equilibrated closed systems (Appendix 5). Lead acetate test strips only detected very high concentrations of methanethiol. Positive lead acetate reactions to this gas produced a yellow coloured lead salt.

DTNB and lead acetate test strips were equally sensitive to gas mixtures of hydrogen sulphide. The minimum gas concentration of hydrogen sulphide detectable by DTNB or lead acetate was produced by an aqueous hydrogen sulphide solution concentration of 19.73 ppm (v/v) (Appendix 5). The hydrogen sulphide gas threshold concentration was not determined.

(f) Microscopic Examination of Microorganisms on Skin from Broiler Carcasses Stored at Various Temperatures

(1) Broiler carcasses stored at 2°C under high humidity conditions

As with skin from fresh immersion chilled carcasses, skin from stored carcasses could be examined with or without a layer of material present on the skin surface by choosing the fixation regime. However, the amount of fluid material present in cracks and channels on the skin of stored carcasses increased compared to fresh immersion chilled carcass skin (Figs. 5a; 22a; 22b). Storage also induced an increase in the density of electron opaque micro-granules present in the layer material on the skin surface as compared to freshly processed skin (Figs. 13a; 13b; 22c). Similar trends were noted for both breast and leg skin.

Figure 22. Microscopic examination of storage induced changes in skin surface features of carcasses stored at 2°C. All specimens fixed in osmium tetroxide vapour.

(a) SEM of skin from a carcass stored for 4 days.

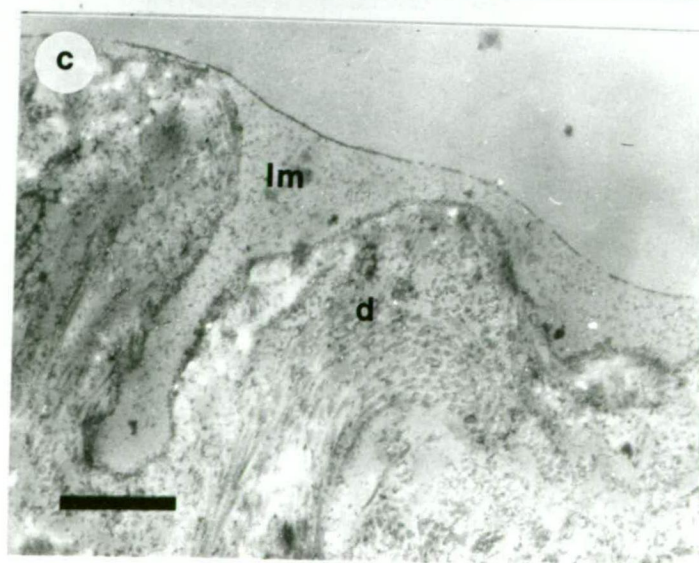
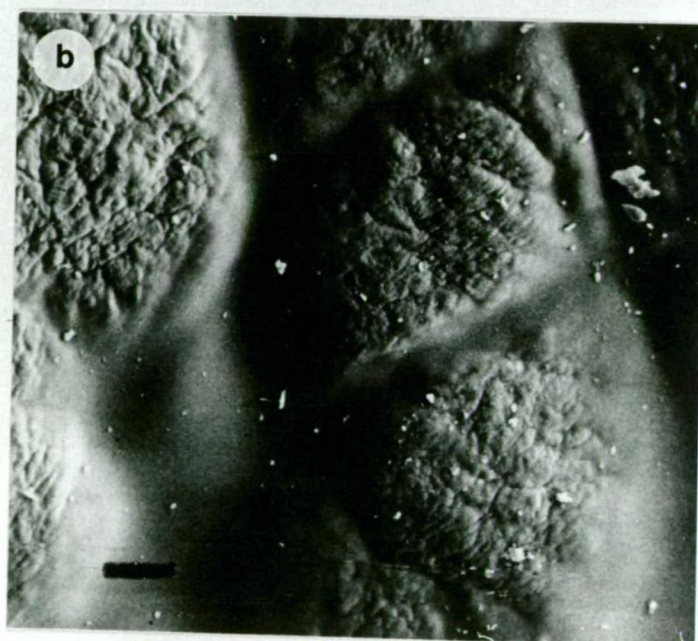
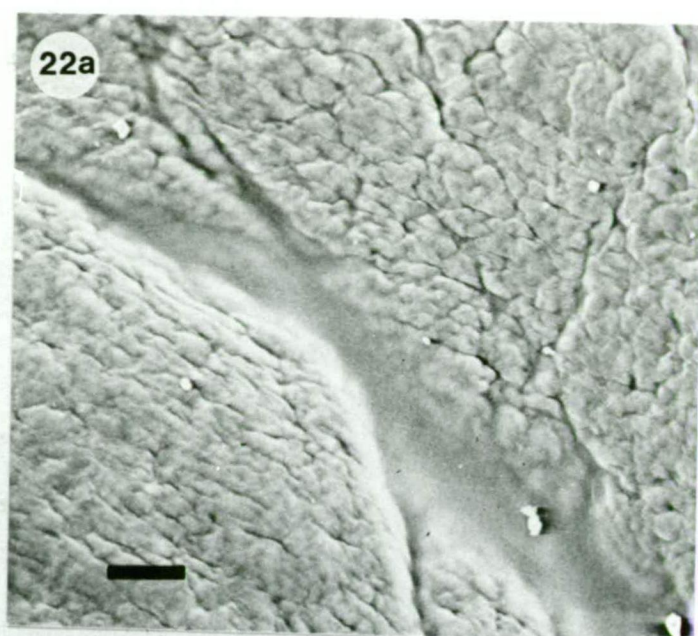
The amount of layer material present in the channels on the skin surface has increased as compared to fresh immersion chilled carcass skin (*cf.* Figure 5a). Bar = 30µm.

(b) SEM of skin from a carcass stored for 8 days.

Note the increased amount of material present in channels on the skin surface as compared to (a) above. Bar = 30µm.

(c) Section through a channel on the skin of a

carcass stored for 4 days. Note that the amount of densely stained material constituting the layer material has increased as compared to skin from a fresh immersion chilled carcass (*cf.* Figure 13a, b). Bar = 1µm.



The inclusion of alcian blue in glutaraldehyde fixative solutions used to prepare skin tissue for examination by TEM, allowed good preservation of the layer material present on the skin of stored carcasses. However, this treatment introduced large electron opaque granules into the layer material (Figs. 23a, 23b), which could be distinguished on the basis of size from similar micro-granules observed in osmium vapour fixed skin (Fig. 23c). These granules were not uniformly distributed within the depth of the layer material but were more concentrated toward the surface (Fig. 23a). Considering the size and distribution of these granules, it seems probable that they are precipitates of alcian blue rather than reaction products between layer material components and alcian blue.

Although a few rod shaped bacteria were located by SEM on the skin surface of carcasses stored 4 days at 2°C (Fig. 24), microorganisms were only observed in sections of Araldite embedded skin after carcasses were stored 8 days or longer. In general, electron microscopic techniques did not usually locate large numbers of bacteria on either leg or breast skin until total viable counts were 10^7 - 10^8 bacteria/16cm² of skin and off odour production was detectable. These conditions occurred after about 10-12 days of carcass storage at 2°C.

Microscopic examination of skin from carcasses stored 12 days or more, showed the spoilage microflora grew within the fluid layer on the skin surface. SEM allowed examination of bacteria on the surface of the layer (Figs. 25a, b) as well as adjacent to the skin surface (Figs. 26a, b, c). All bacteria observed were long rod shaped organisms. TEM of thin sections of skin allowed a more comprehensive examination of the extent of growth

Figure 23. Comparison of preservation of fluid film organic components by various fixation techniques.

- (a) Section through the layer material on the surface of skin from carcasses stored 4 days at 2⁰C. Specimen fixed in a solution of glutaraldehyde containing alcian blue and post fixed in osmium tetroxide. Note the granular nature of the layer material components. The largest granules are concentrated more towards the surface of the layer. d = dermis. Bar = 2 μ m.
- (b) Higher magnification of area outlined by square in (a). Note the size of the granules. Bar = 0.25 μ m.
- (c) High magnification micrograph of a section through the layer material present on osmium tetroxide vapour fixed skin from a carcass stored 4 days at 2⁰C. Note the size of the granules compared to those present in (b) above. Bar = 0.25 μ m.

Figure 24. SEM of rod shaped bacteria on the skin surface of a broiler carcass stored at 2⁰C for 4 days. Specimen fixed in osmium tetroxide vapour. Note that each bacterium is lying within pits probably caused by highly localised enzyme degradation of layer material components. Bar = 3 μ m.

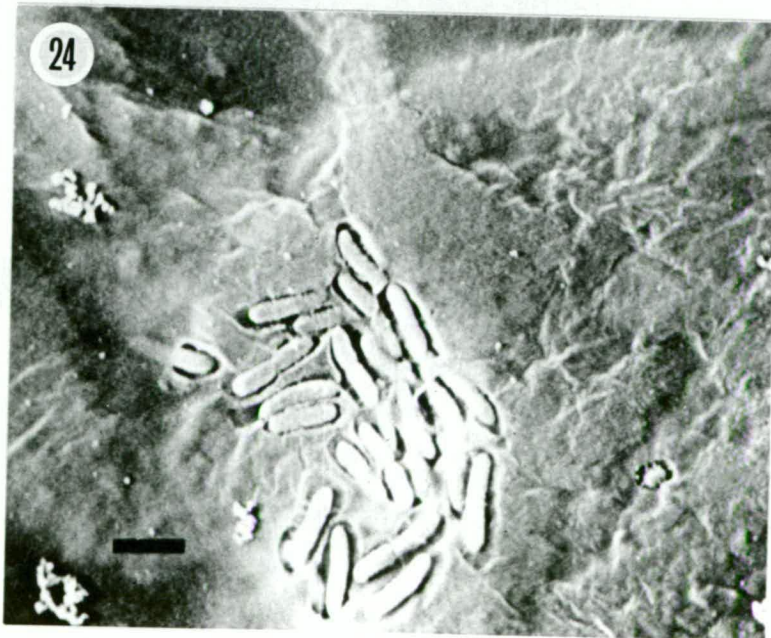
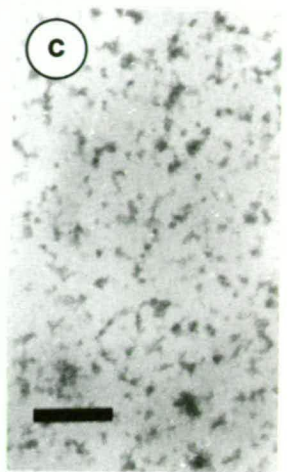
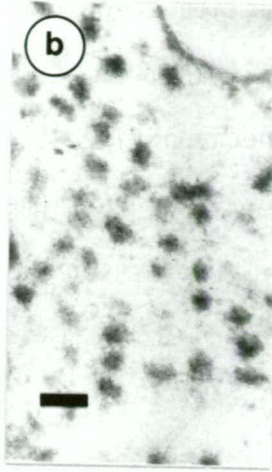
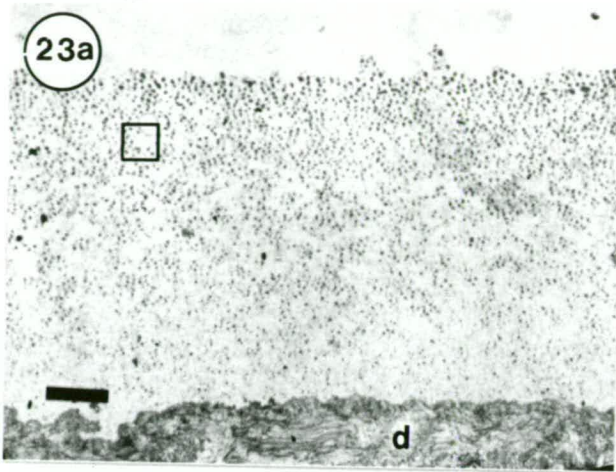


Figure 25. SEM of bacteria in the surface of layer material on skin from carcasses stored at 2°C.

Note that the rod shaped bacteria are lying in pits in the layer material surface. Bacteria are usually connected to the edges of the pits by thin strands. Skin samples fixed in osmium tetroxide.

(a) Skin from a carcass stored for 12 days.

Bar = 3µm.

(b) Skin from a carcass stored for 16 days.

Bar = 3µm.



Figure 26. SEM of microorganisms on the skin surface of carcasses stored at 2⁰C. Note that bacteria are located on the skin surface and deep within the channels in the skin. Skin specimens were fixed by immersion in a glutaraldehyde solution.

(a) Breast skin from a carcass stored 12 days.

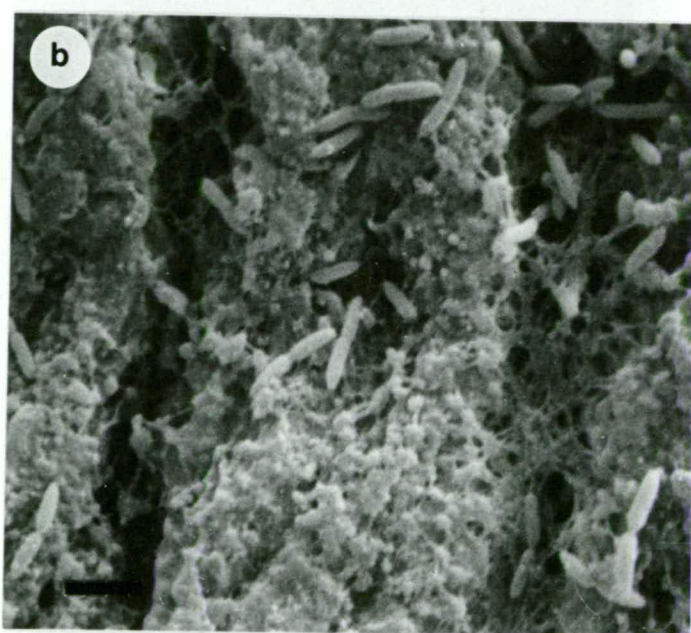
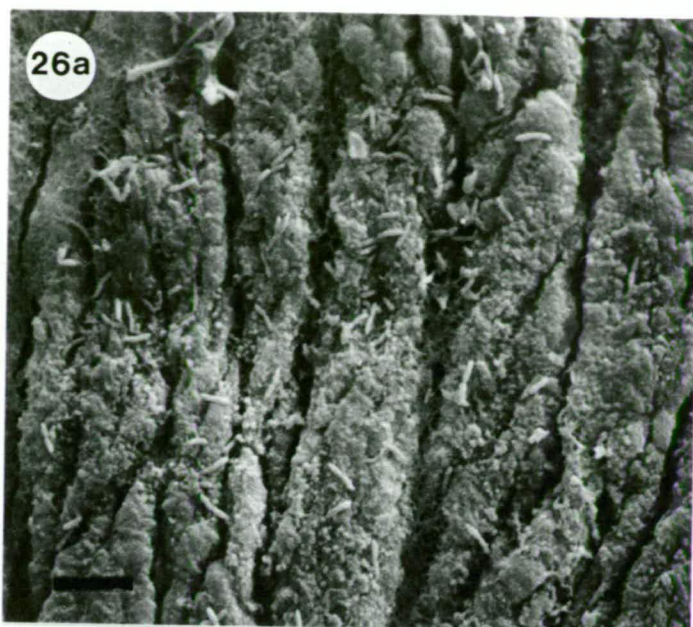
Bar = 10 μ m.

(b) Higher magnification SEM of (a) above.

Bar = 3 μ m.

(c) Skin from a carcass stored 16 days.

Bar = 3 μ m.



of the spoilage microflora within the fluid film. Most bacteria were found throughout this layer (Figs. 27; 28), but were also found deep inside cracks and channels in the skin (Figs. 29a, b, e) as well as empty feather follicle shafts (Figs. 29c, d). No bacteria were found within dermal tissue at any stage of storage.

Generally, the number of bacteria growing within the fluid film on the skin surface, was dependent upon the depth of the fluid over the skin surface. Areas of skin covered only by a thin fluid layer, supported fewer microorganisms (Fig. 30), whereas large numbers of bacteria were always found where the fluid film was deep (Figs. 27; 28).

No discrete colonies of bacteria were observed on leg or breast skin from carcasses stored at 2°C. However, where growth of bacteria was sufficiently prolific, the mass of bacteria grew through the fluid layer surface and produced colony-like formations (Figs. 31a, b). Most bacterial growth was confined to the fluid layer over the skin surface.

Gram negative rod shaped bacteria were the major morphological types found in the thin sections of the liquid film, deep skin channels and feather follicle shafts examined (Figs. 32a, b, c). However, yeasts and short rod shaped gram positive bacteria were sometimes located within the liquid film on the surface of carcasses stored for 16 days at 2°C (Figs. 32d, e, f; 33). Yeasts found on the skin within this layer were always associated with other bacteria, but the microscopic evidence did not suggest any specific interaction or association.

Figure 27. TEM of a section through the fluid layer on the skin surface of a carcass stored for 16 days at 2°C. Note the bacteria within the preserved layer material (lm) on the dermal skin tissue (d). Most bacteria in the preserved film are surrounded by a zone cleared of layer material. Skin treated in a solution of glutaraldehyde containing alcian blue and post fixed in osmium tetroxide. Sections stained with uranyl acetate/lead citrate. Bar = 1µm.



Figure 28. TEM of a section through the fluid layer close to the skin surface of a carcass stored for 16 days at 2⁰C. Note that bacteria close to the skin surface have not damaged the integrity of the skin dermal tissue (d). Skin treated with a solution of glutaraldehyde containing alcian blue and post fixed in osmium tetroxide. Section stained with uranyl acetate/lead citrate. Bar = 1μm



Figure 29. Microorganisms growing in skin channels and feather follicles of carcasses stored 16 days at 2°C.

- (a) Section through a skin channel showing bacteria located in the channel within layer material (lm). d = dermis. Skin specimen fixed as for Figure 28 and sections stained with uranyl acetate/lead citrate. Bar = 2µm.
- (b) As for (a) except deep inside the skin. Bar = 2µm.
- (c) SEM of a razor blade transverse section through a feather follicle deep inside dermal tissue (d). Note the mass of bacteria within the follicle. ep = epidermal tissue. Skin fixed in osmium tetroxide vapour. Bar = 150µm.
- (d) High magnification SEM of the area defined by the square in (c) showing bacteria within the follicle. Bar = 3µm.

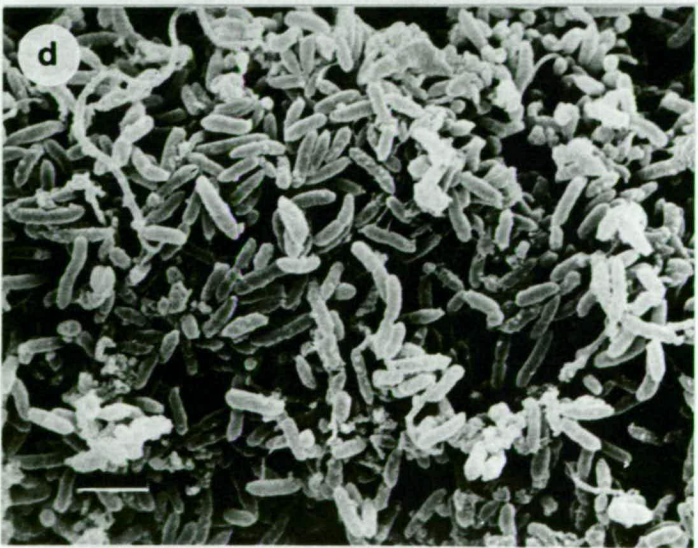
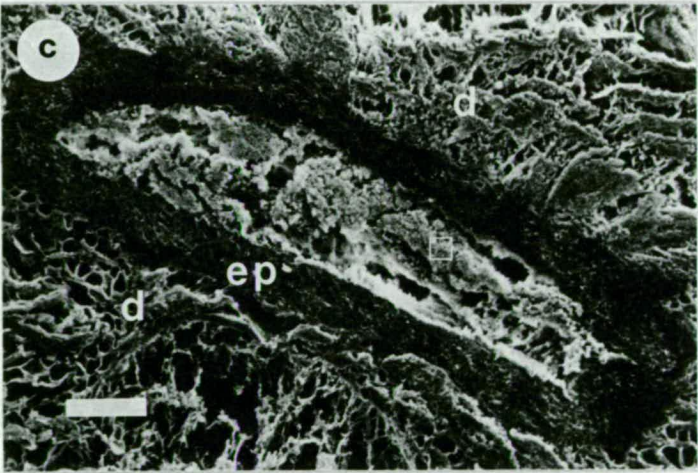
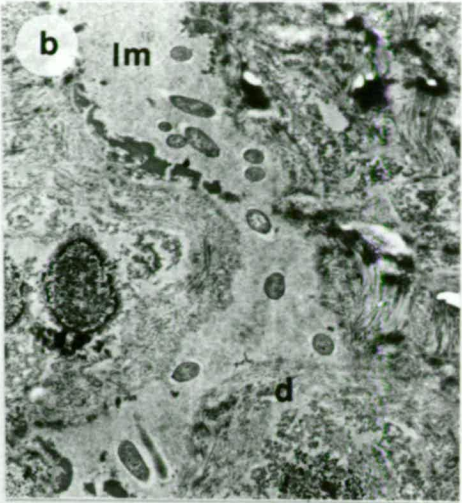
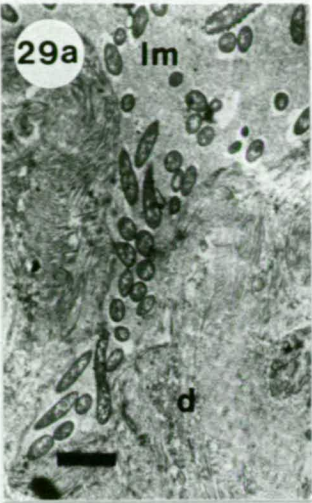


Figure 29e. Light micrograph of a thin section through skin from a carcass stored 16 days at 2⁰C. Note the bacteria deep within the channel in the skin surface. Skin treated with glutaraldehyde containing alcian blue; postfixed in osmium tetroxide. Section stained with polychrome stain (Sato and Shamoto, 1973). Magnification x1200.

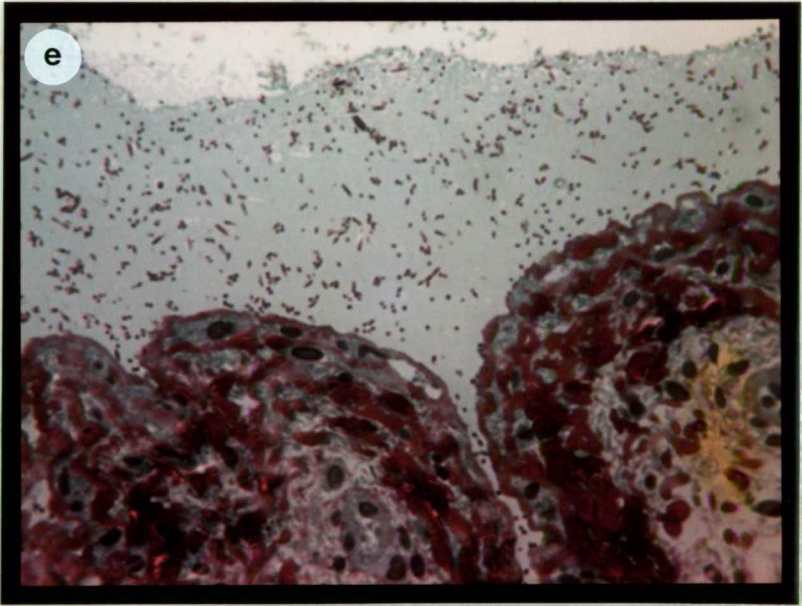


Figure 30. TEM showing bacteria lying on an area of skin not covered by layer material from a carcass stored 16 days at 2°C. Skin samples were treated with glutaraldehyde and alcian blue. Bar = 0.25µm.

Figure 31. Colony-like formations of bacteria on skin from a carcass stored 16 days at 2°C.

(a) SEM of breast skin. Bar = 10µm.

(b) SEM of leg skin. Bar = 10µm.

All specimens fixed in osmium tetroxide vapour.

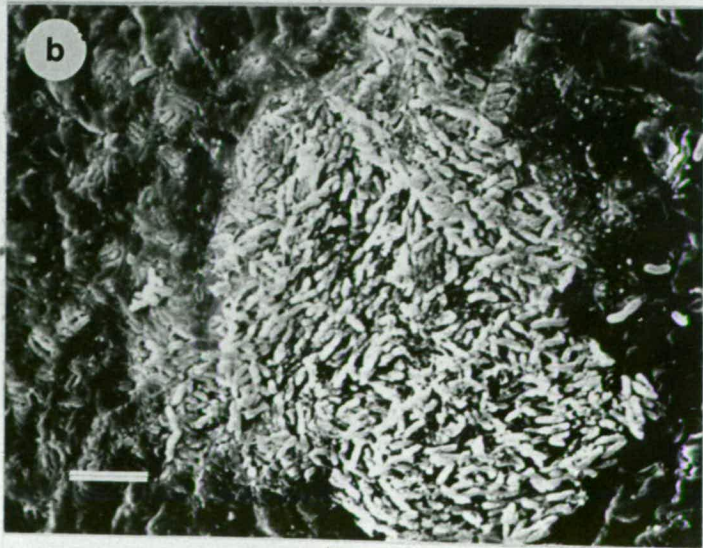
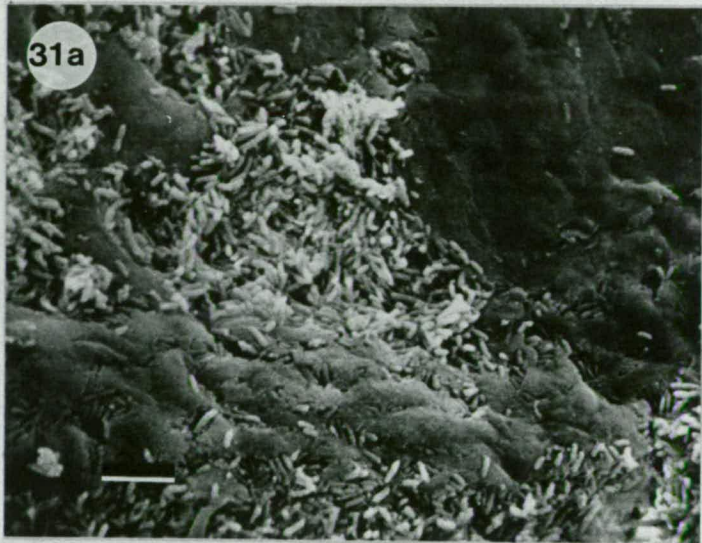
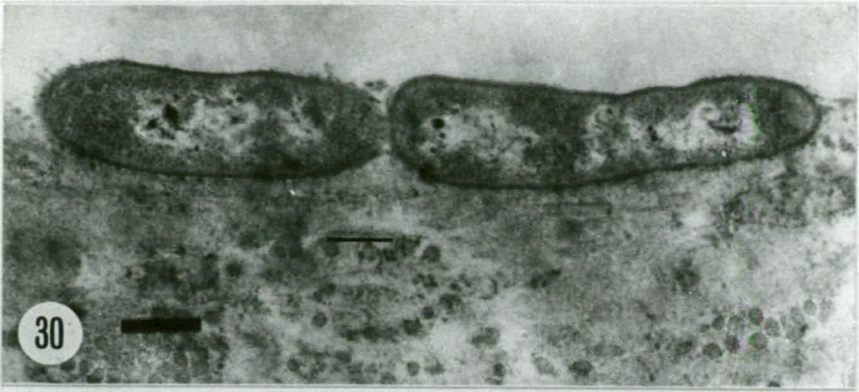


Figure 32. Types of microorganisms present in the layer material and within feather follicles of skin from carcasses stored 16 days at 2⁰C. Specimens were treated with glutaraldehyde and alcian blue and post fixed with osmium tetroxide. Sections stained with uranyl acetate/lead citrate.

- (a) Section through typical gram -ve rod shaped bacteria within the liquid layer on the skin surface. f = flagella. Bar = 0.2 μ m.
- (b) Section through fimbriate gram -ve bacteria within the layer material and close to the dermal skin surface (d). Bar = 0.2 μ m.
- (c) Section through gram -ve rod shaped bacteria within a feather follicle. Bar = 0.2 μ m.
- (d) and (e). Sections through intact layer material showing presence of gram +ve rod shaped bacteria (arrowed) and gram -ve rod shaped organisms. d = dermis. Bar = 1 μ m.
- (f) High magnification TEM of the area defined in (e) showing the gram +ve cell wall structure of the small rod shaped bacteria. Bar = 0.2 μ m.

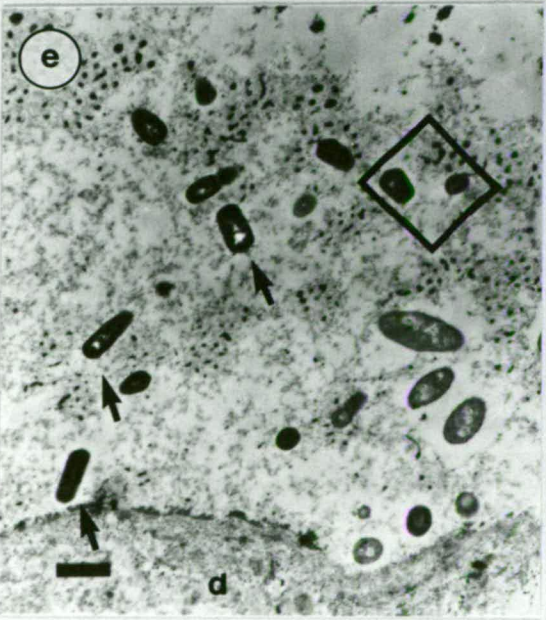
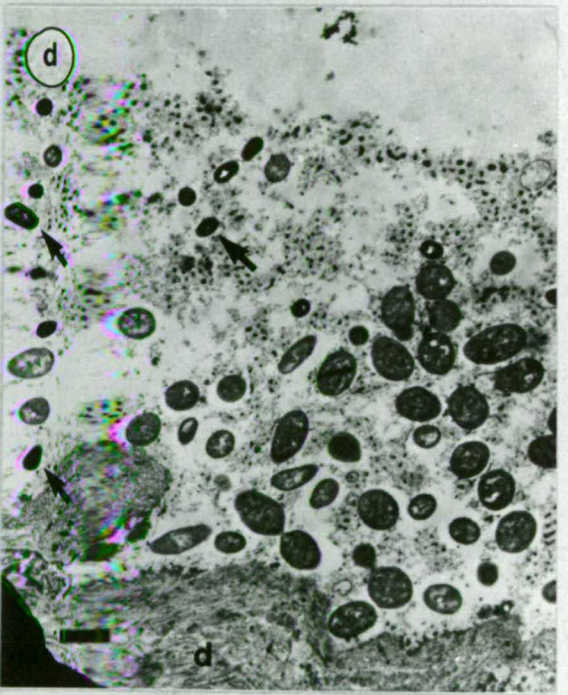
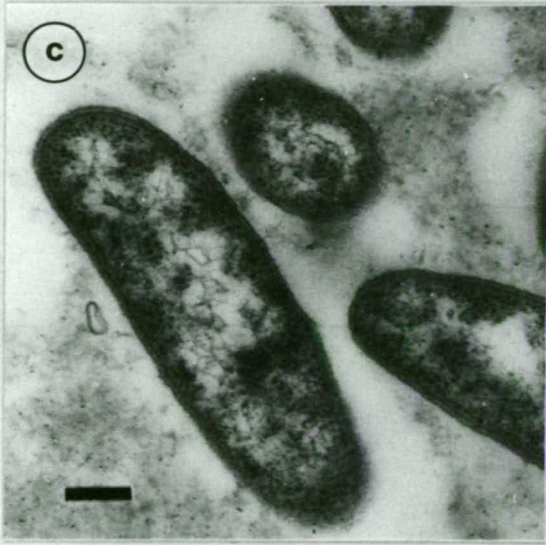
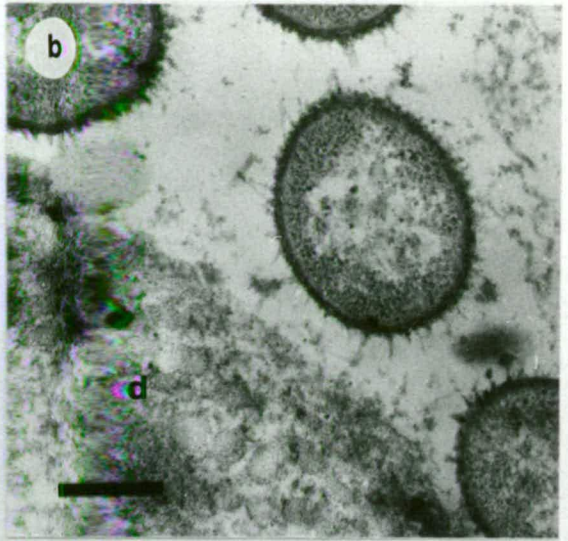
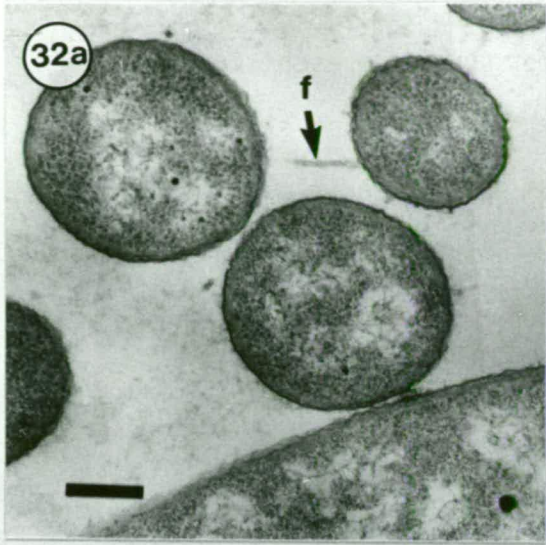
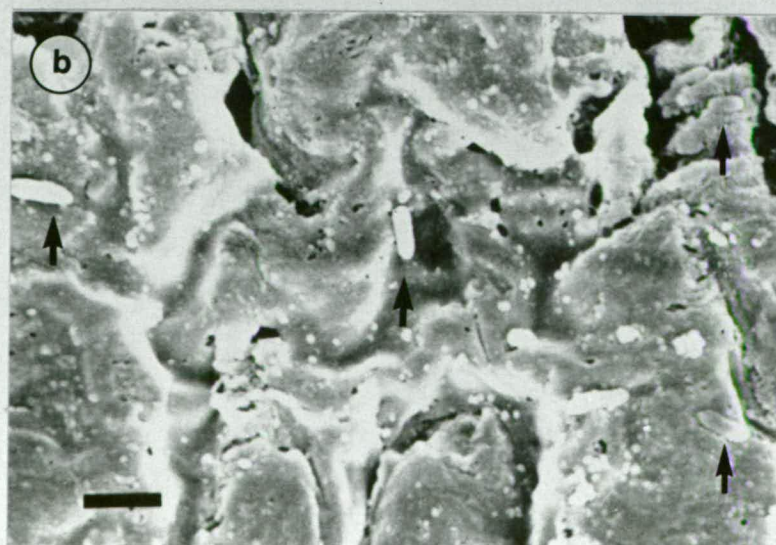
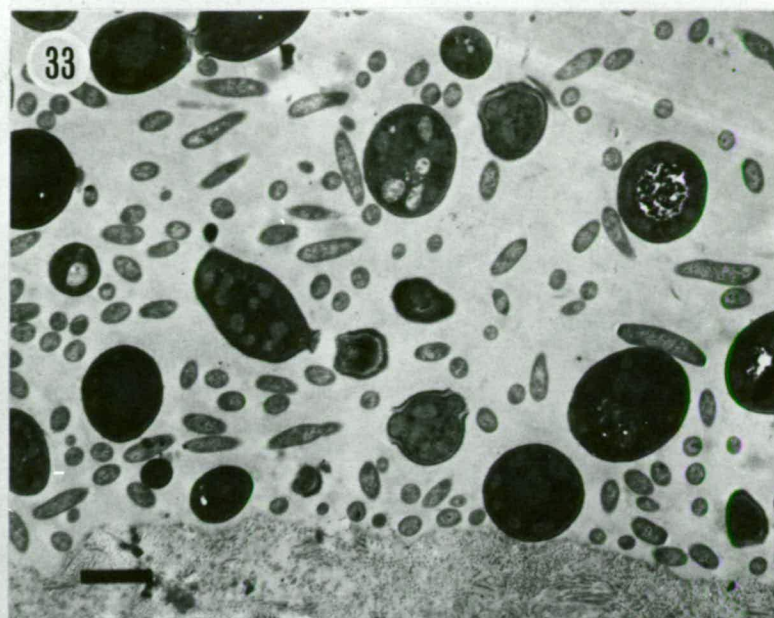


Figure 33. Yeasts associated with bacteria in the layer material on the skin of carcasses stored 16 days at 2°C. Specimen fixed as for Figure 27. Section stained with uranyl acetate/lead citrate. Bar = 3µm.

Figure 34. SEM of fresh immersion chilled broiler skin artificially contaminated by addition of washed cell bacterial suspensions to the spin chiller water. Specimens fixed in osmium tetroxide vapour.

(a) Bacteria within the layer material (arrows) on the skin surface. Bar = 15µm.

(c) Bacteria (arrowed) partially embedded within the surface of the layer material on the skin. Note that the bacteria are not lying in pits within this material. Bar = 3µm.



Bacteria at the surface of the preserved fluid film components were always surrounded by a cleared zone or pit when examined by SEM (Figs. 24; 25a, b). Several strands or fibres normally connected these bacteria to the edges of the pits. Cleared zones were always widest adjacent to the lateral edges of rod shaped bacteria and narrowest at their polar edges. Control specimens of inoculated skin, from immersion chilled carcasses, showed the typical layer of preserved material, but no cleared zones were observed around bacteria on or close to the surface of this material (Figs. 34a, b). This evidence suggests the cleared zones are not an artefact of preparation or the result of extracellular enzyme action during fixation.

Cleared zones around bacteria within the preserved fluid layer components were also observed in all survey sections of spoiled skin stained with the polychrome stain or crystal violet. Thin sections of this layer material examined by TEM, showed most bacteria were surrounded by electron transparent zones similar to those observed in the survey sections (Figs. 27; 28; 35a, b, c, d, e,). The fact that these zones could not be stained with alcian blue, ruthenium red or potassium permanganate suggests these zones are not capsular glycocalyx.

Although examination of spoiled skin specimens by SEM and TEM did not provide any evidence of gross dermal tissue degradation, bacteria growing on skin where only a thin layer of fluid covered the surface did show evidence of localised degradation of the dermal tissue (Figs. 36a, b). Degradation was defined by hemispherical zones of lysis beneath the bacteria involved. Yeasts growing close to the skin surface also showed evidence

of localised dermal erosion, but no disruption of collagen fibril arrangement was obvious (Figs. 36c, d).

Alcian blue and ruthenium red staining schedules as well as potassium permanganate staining confirmed that bacteria were not attached to other bacteria or the skin surface by acidic micro-polysaccharide strands or other bridging substances (Figs. 27; 28; 35a, b, c, d, e). Although peritrichously fimbriate bacteria were regularly observed within the fluid film of spoiled skin, fimbriae could not be shown to be involved in attachment of these bacteria to the skin surface. However, fimbriae may be involved in attachment of bacteria to other bacteria (Figs. 37a, b, c). The majority of bacteria observed were free within the fluid film on the skin of spoiled carcasses.

Some bacteria present within the preserved layer material on alcian blue treated tissue were surrounded by heavily stained zones close to the cell wall. Figure 38a shows a bacterium with fimbriate-like appendages agglutinated by the alcian blue and Figure 38b shows specific staining of cell wall substances.

(2) Broiler carcasses stored under high humidity conditions at 4, 10, 15 or 22°C until onset of spoilage

The skin microflora of spoiled carcasses stored at 4, 10, 15 or 22°C were located largely within the fluid layer on the skin surface. SEM allowed examination of the bacteria on the surface of preserved layer material (Figs. 39a, b, e, f) as well as those within the layer and associated with feather follicles, cracks and channels on the skin surface (Figs. 40a, b; 41a, b, c; 42a, b, c, d). Although the spoilage microflora was confined largely within

Figure 35. Microscopic examination of thin sections of bacteria in the preserved layer material on skin of carcasses stored 16 days at 2⁰C. Note the absence of any bridging polymers between bacteria or between bacteria and the skin surface. No capsular material is present. All bacteria shown are surrounded by a cleared zone as for Figure 27.

(a) Ruthenium red treated. d = dermis.
Section stained with lead citrate.

(b) As for (a) above. Bar = 0.5 μ m.

(c) As for (a) above. Bar = 0.5 μ m.

(d) Skin fixed in glutaraldehyde and
osmium tetroxide and sections
stained with potassium permanganate.
Bar = 0.5 μ m.

(e) As for (d) above. Bar = 0.5 μ m.

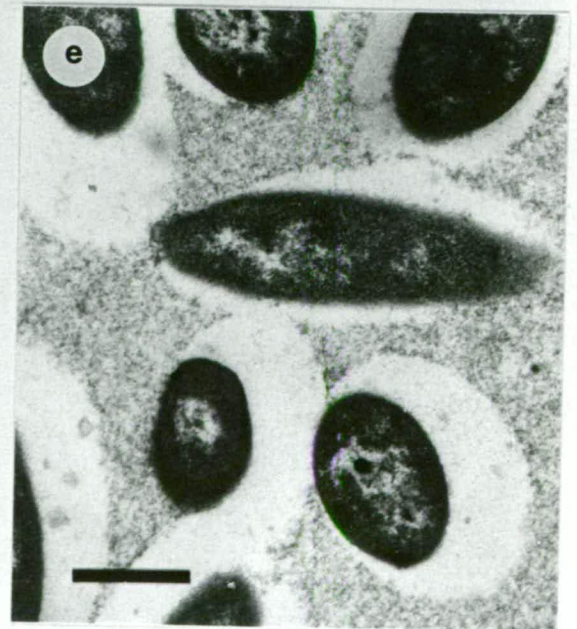
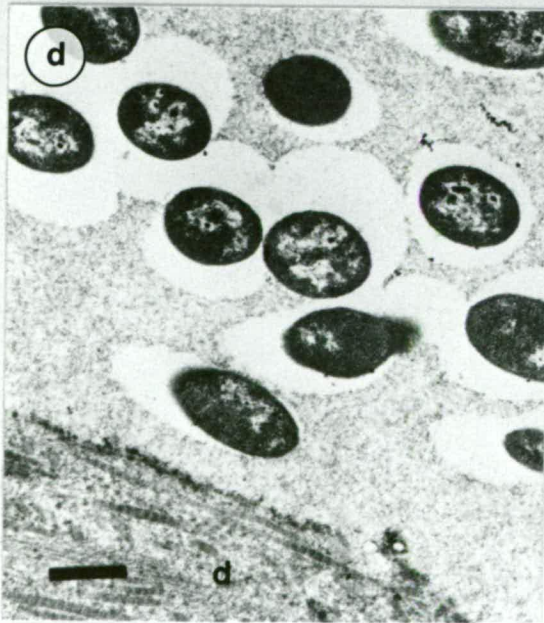
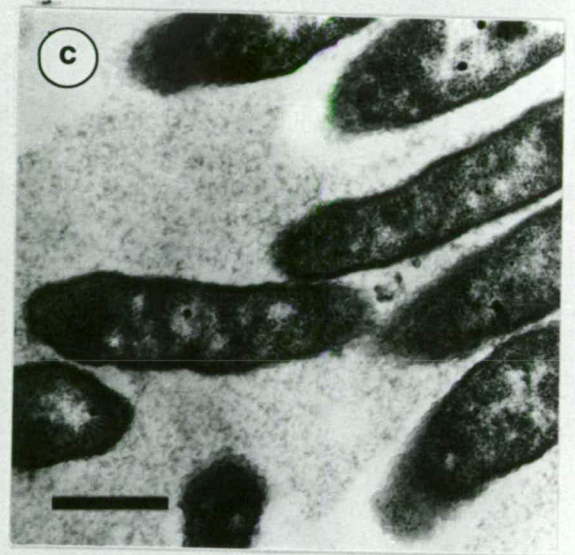
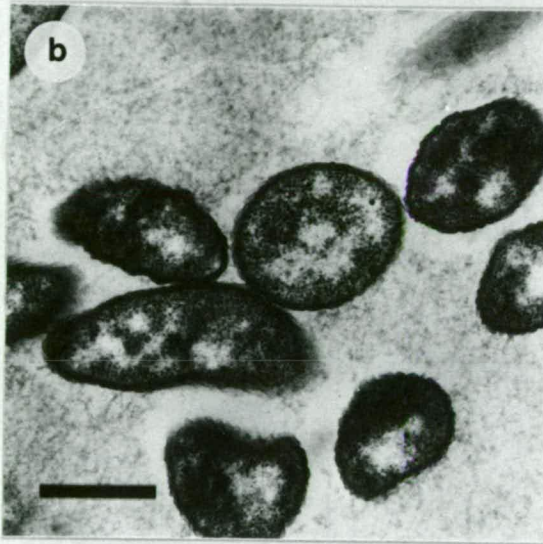


Figure 36. Microbial degradation of the skin from carcasses stored 16 days at 2°C.

- (a) and (b). Sections showing bacteria in an area of skin not covered by layer material. The hemispherical depressions (arrowed) under the bacteria, may indicate highly localised degradation of the skin substrate. Specimens treated with glutaraldehyde and alcian blue, and post fixed in osmium tetroxide. Sections stained with uranyl acetate/lead citrate. Bar = 0.5µm.
- (c) Section of a yeast cell growing within the layer material and partly embedded in the skin surface. The yeast cell seems to have degraded the skin substrate to create the depression. Specimen treated with glutaraldehyde, post fixed in osmium tetroxide and sections stained with permanganate/lead citrate. Bar - 1µm.
- (d) High magnification TEM of the yeast/skin contact point in (c). No localised disruption of the skin substrate or collagen fibres (arrowed) has occurred. Bar = 0.2µm.

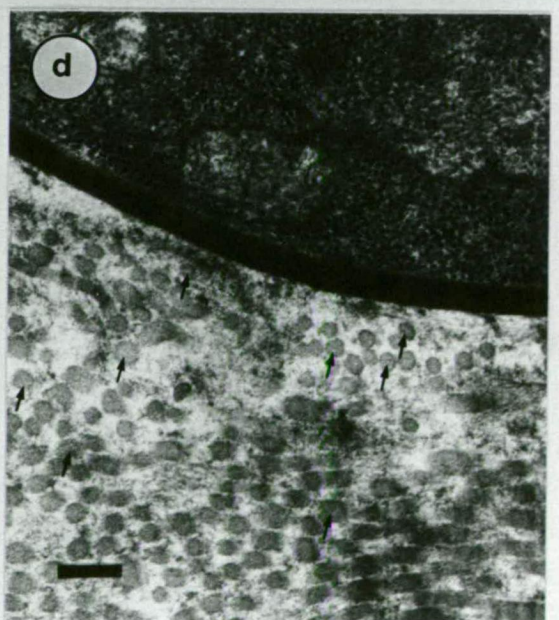
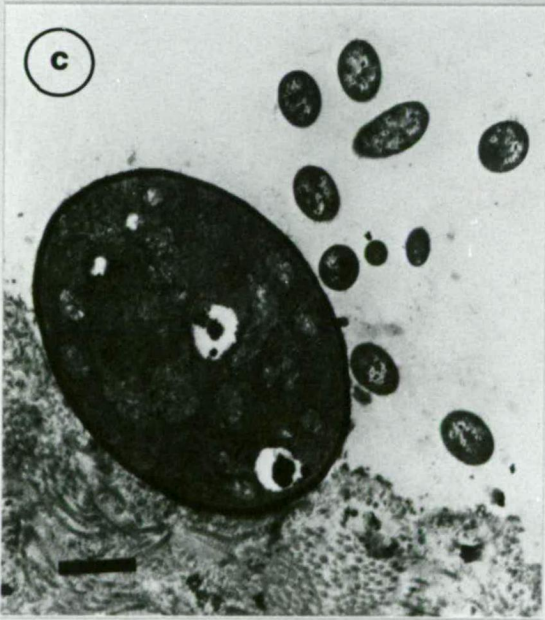
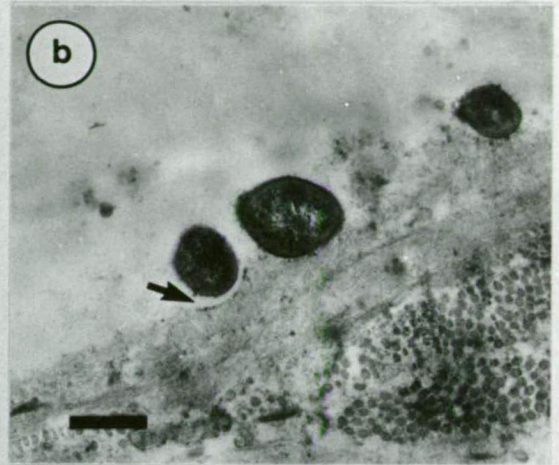
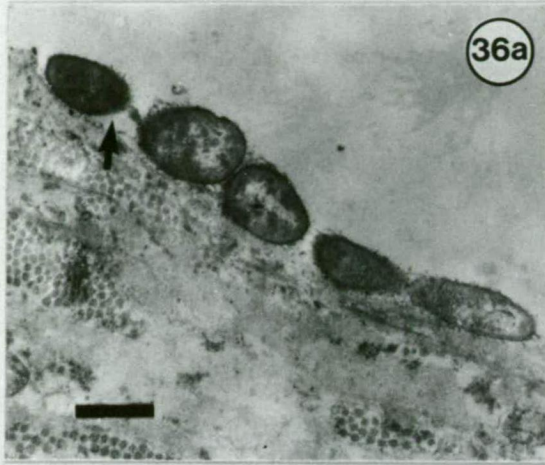


Figure 37. Fimbriae-like appendages of bacteria growing within the fluid layer on the skin of carcasses stored 16 days at 2°C. Skin samples treated with glutaraldehyde and alcian blue and post fixed in osmium tetroxide. All sections stained with uranyl acetate/lead citrate.

- (a) Low magnification TEM of fimbriate bacteria close to the skin surface.
Bar = 1 μ m.
- (b) High magnification TEM of (a) showing fimbriae (arrows) of gram -ve bacteria.
Bar = 0.2 μ m.
- (c) TEM of a chain of gram -ve bacteria on the skin surface. The bacteria may be connected by fimbriae-like structures (arrows). Bar = 0.2 μ m.

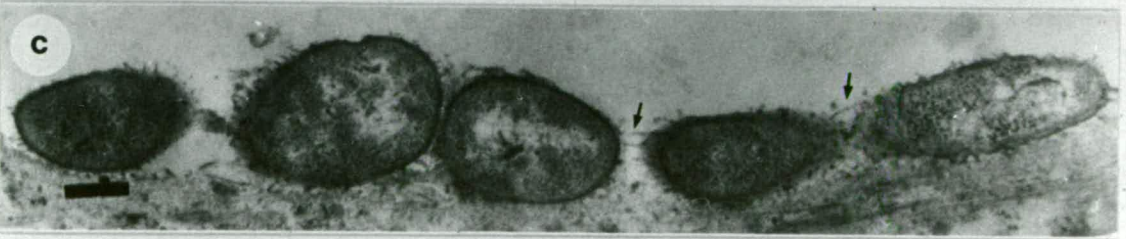
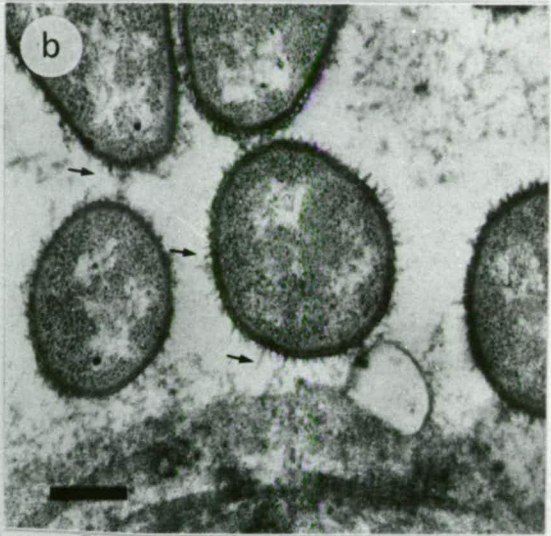
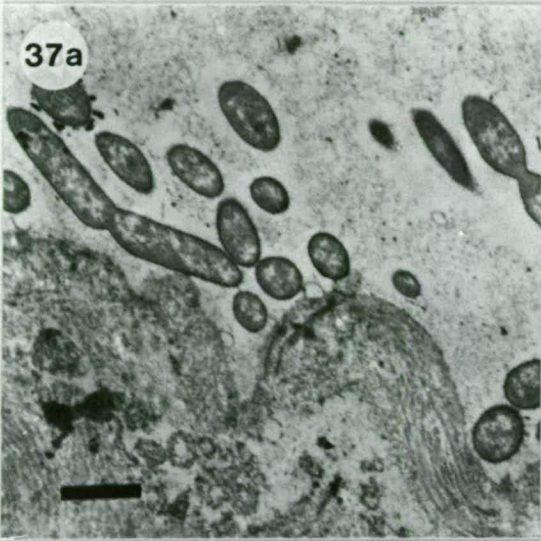


Figure 38. TEM of sections of bacteria in the fluid film of skin from a carcass stored 16 days at 2⁰C. Skin treated with a solution of glutaraldehyde containing alcian blue and post fixed with osmium tetroxide. Sections stained with uranyl acetate/lead citrate.

(a) Bacterium with fimbriae-like structures agglutinated by alcian blue. Also bacterium with normal fimbriae (arrowed).
Bar = 0.5 μ m.

(b) A bacterium with heavily stained zones around the cell wall exterior.
Bar = 0.2 μ m.

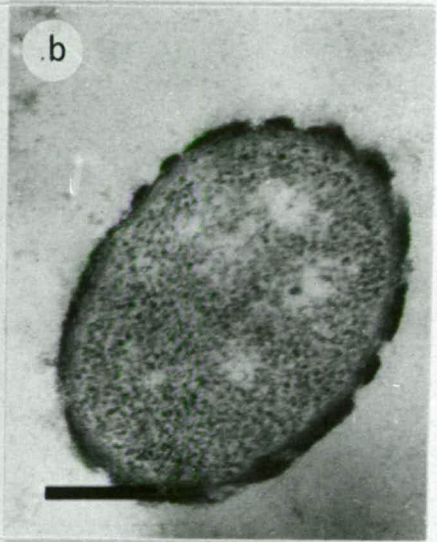
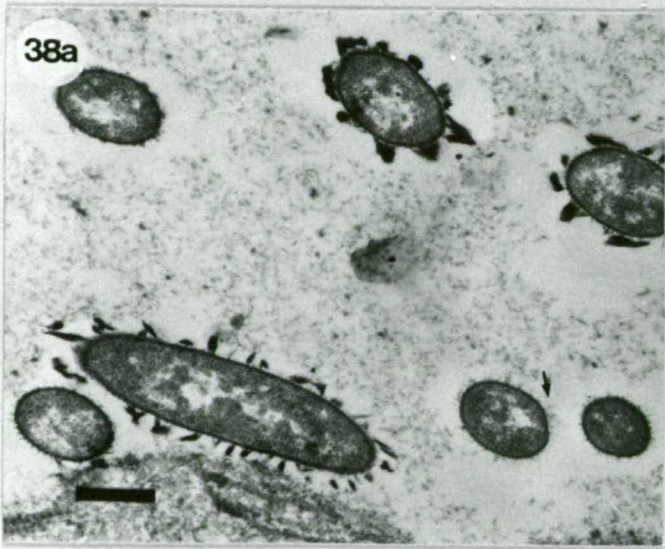


Figure 39. SEM of microorganisms on the surface of skin from broiler carcasses stored under high humidity conditions at 4, 10, 15 and 22⁰C until onset of spoilage. Specimens fixed in osmium tetroxide vapour.

- (a) Bacteria in the preserved layer material surface on skin from a carcass stored at 4⁰C. These bacteria are lying in pits and are connected to the layer material by coarse strands. Bar = 3 μ m.
- (b) As for (a) above except skin from a carcass stored at 10⁰C. Bar = 3 μ m.
- (c) and (d). Colonies of bacteria on skin from a carcass stored at 10⁰C. Bars = 200 μ m and 100 μ m respectively.
- (e) Bacteria in the preserved layer material surface on skin from a carcass stored at 15⁰C. Note the short rod shaped bacteria lying in pits in the surface. The bacteria are connected to the layer material by many thin strands. Bar = 3 μ m.
- (f) As for (e) except skin from a carcass stored at 22⁰C. Bar = 3 μ m.

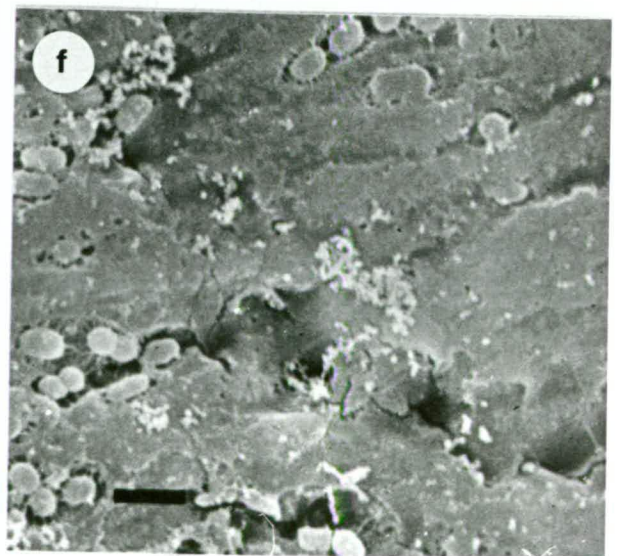
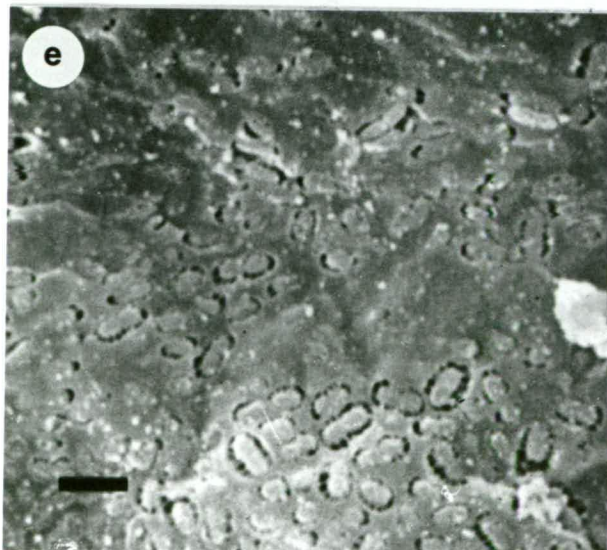
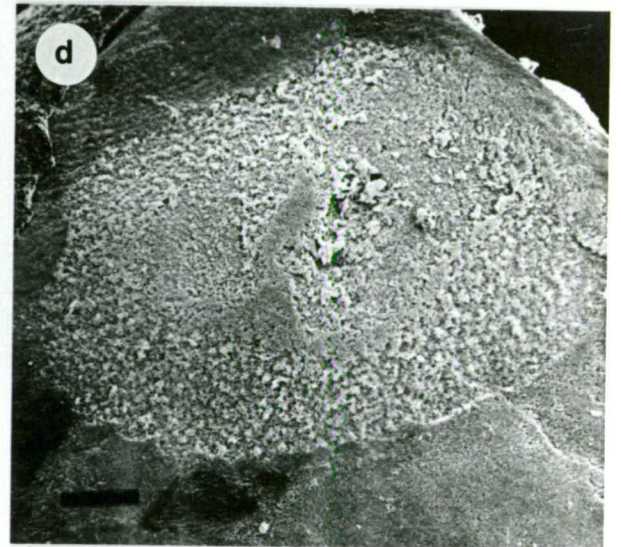
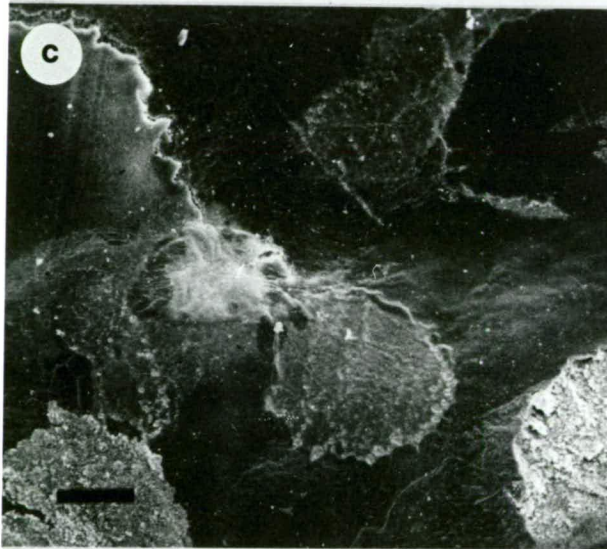
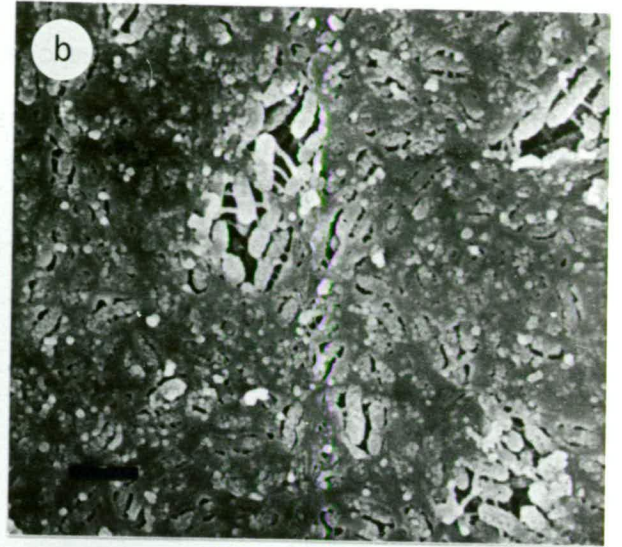


Figure 40. SEM of microorganisms on the surface of skin from broiler carcasses stored under high humidity conditions at 4⁰ and 10⁰C until onset of spoilage. Specimens fixed by immersion in a glutaraldehyde solution.

(a) Rod shaped bacteria in channels and on the skin surface of skin from a carcass stored at 4⁰C. The thin strands (arrowed) are flagella. Bar = 3µm.

(b) Rod shaped bacteria on the surface of skin from a carcass stored at 10⁰C.
Bar = 3µm.

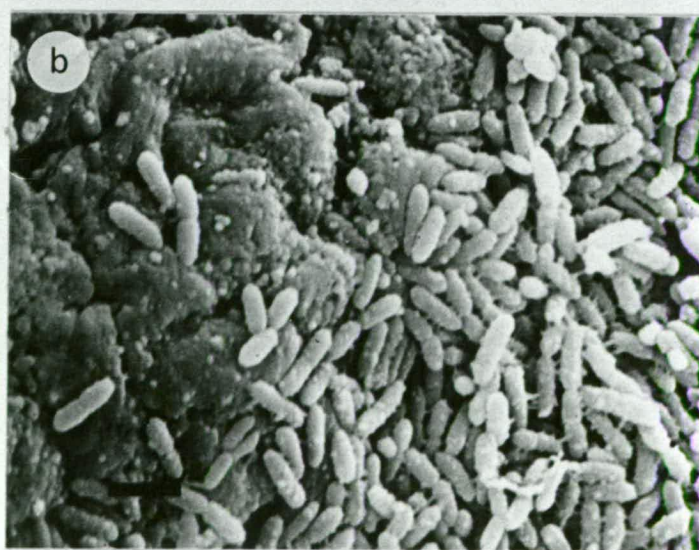


Figure 41. SEM of microorganisms on the surface of skin from carcasses stored under high humidity conditions at 15°C until onset of spoilage. Specimens fixed by immersion in a solution of glutaraldehyde.

(a) Low magnification micrograph of bacteria growing over the skin surface and down inside skin channels. Note the different morphological types present. Bar = 10 μ m.

(b) Short rod shaped bacteria on the skin surface. Bar = 3 μ m.

(c) Coccoid rod shaped bacteria and rod shaped bacteria associated with a channel in the skin surface. Bar = 3 μ m.

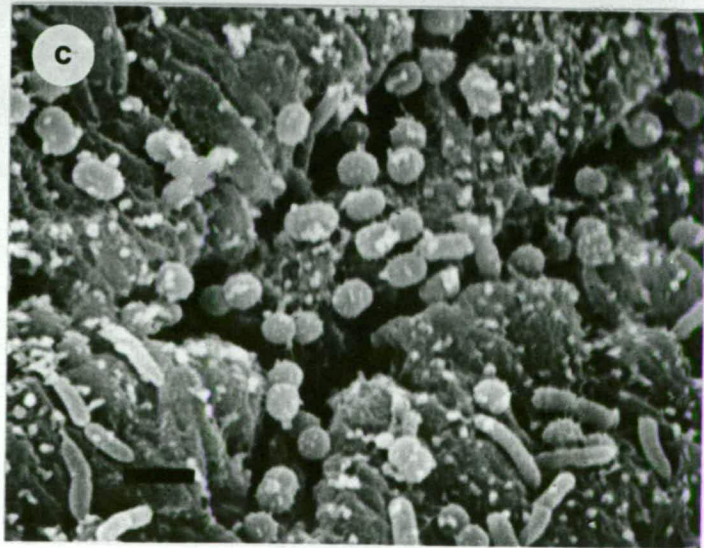
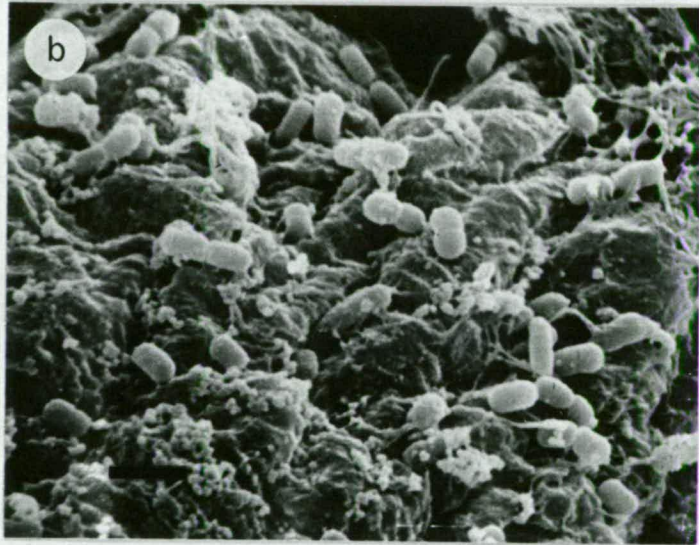
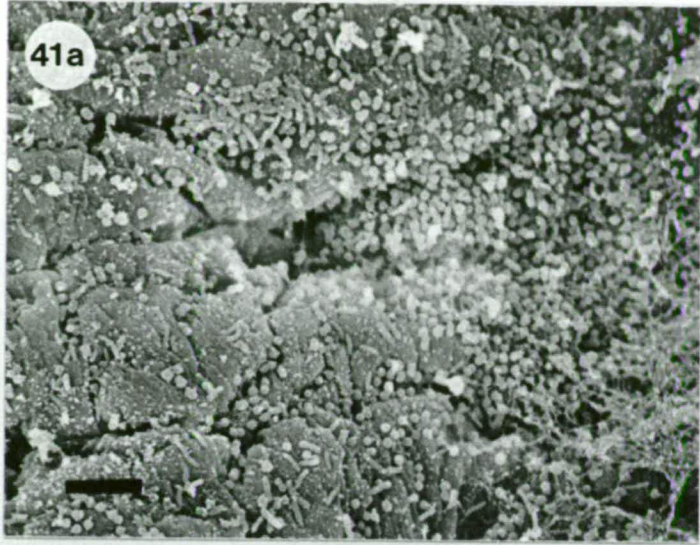


Figure 42. SEM of microorganisms on the skin surface of carcasses stored under high humidity conditions at 22°C until onset of spoilage. Specimens fixed by immersion in a solution of glutaraldehyde.

(a) Low magnification micrograph of bacteria in channels and on the surface of the skin. Bar = 10µm.

(b) Rod shaped bacteria growing in a channel in the skin surface. Note that several morphological types are intermingled. Bar = 10µm.

(c) Thick rod shaped and thin rod shaped bacteria on the skin surface. The thin rod shaped organisms possess flagella (arrows). Note that the two morphological types are intermingled. Bar = 3µm.

(d) Several different morphological types of bacteria on the skin surface. Bar = 3µm.



the fluid layer, discrete colonies of bacteria were always observed on the surface of skin from carcasses stored at 10°C (Figs. 39c, d). Other colony-like structures were observed on skin from carcasses stored at other temperatures when microbial growth in a specific area was sufficiently prolific to grow through the fluid layer surface.

The morphological types of bacteria present on the skin of broiler carcasses stored at 15 or 22°C was more varied than for carcasses stored at lower temperatures. Bacteria growing at 4°C or 10°C were morphologically similar to those observed on the skin of carcasses stored at 2°C, e.g. long rods (Figs. 39a, b; 40a, b). The morphological types encountered on carcasses stored at 15 or 22°C included: long rods (Figs. 41c; 42b, c, d); thick rods (Figs. 41b; 42b, c, d); short rods and coccoid rods (Figs. 39e, f; 41b, c; 42d). Different morphological types of bacteria were usually found intermingled (e.g. Figs. 41a; 42b, c, d), except where isolated groups of a particular morphological type were isolated.

Bacteria present on the surface of preserved layer material were associated with pits (Figs. 39a, b, e, f) similar to those observed for bacteria growing on the skin of carcasses stored at 2°C (Figs. 24; 25a, b). Usually, bacteria associated with a pit were connected to the layer material by thin strands. However, the short rods or coccoid rods associated with the surface of the layer material present on skin from carcasses stored at 15 or 22°C (Figs. 39e, f), were usually connected to their substrate by a large number of fine strands. Coarser fibrils (e.g. Figs. 39a, b) were only associated with other morphological types of bacteria.

(3) Carcasses stored at 2°C under drying conditions

The pattern of development of the spoilage microflora of the skin of carcasses stored at 2°C under drying conditions, was substantially different from microbial development on carcasses stored under moist conditions. The skin surface was dry to the touch and was covered in small colonies of microorganisms (Figs. 43a, b, c). Yeasts and bacteria were found growing under these conditions. Bacteria were also found growing within a layer of material over the skin surface (Figs. 44a, b, c, d). However, no microorganisms were found within dermal tissue.

Yeasts growing on the skin surface usually were associated with hemispherical depressions in the skin surface. These depressions indicate localised tissue breakdown (Fig. 44c, d). Bacteria growing within the film of layer material on the skin surface were always surrounded by cleared zones (Figs. 44a, b) similar to those around bacteria growing in the fluid film on the skin of carcasses stored under high humidity conditions (*cf.* Figs. 24; 25a, b).

Figure 43. Colonies of microorganisms present on the skin surface of carcasses stored 16 days under drying conditions at 2°C.

(a) SEM of small colonies of microorganisms on the skin surface. Specimen fixed in osmium tetroxide vapour. Bar = 200µm.

(b) As in (a) above. Bar = 100µm.

(c) Light micrograph of a section through a colony of bacteria growing on the skin surface. Note that the colony does not seem to have penetrated the skin surface. Specimen treated with glutaraldehyde and alcian blue, and post fixed in osmium tetroxide. Section stained with crystal violet. Bar = 100µm.

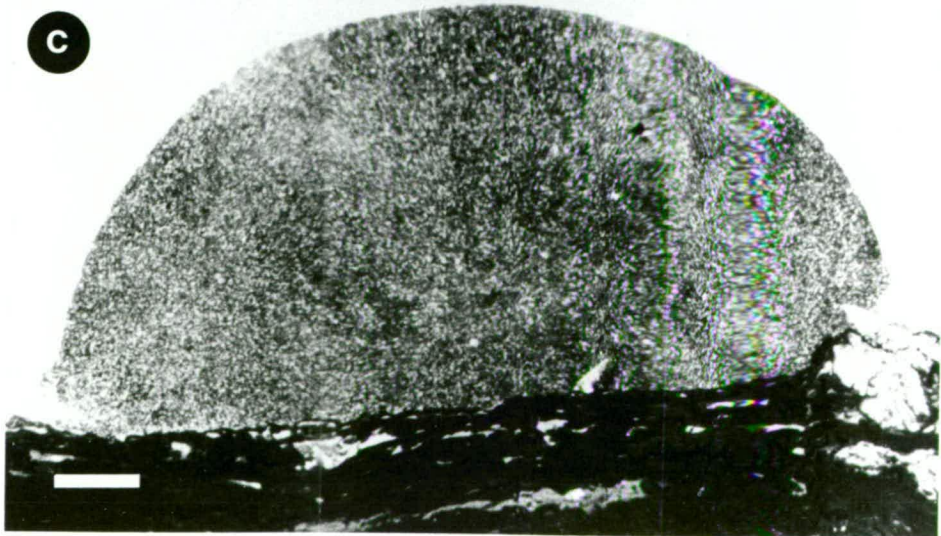
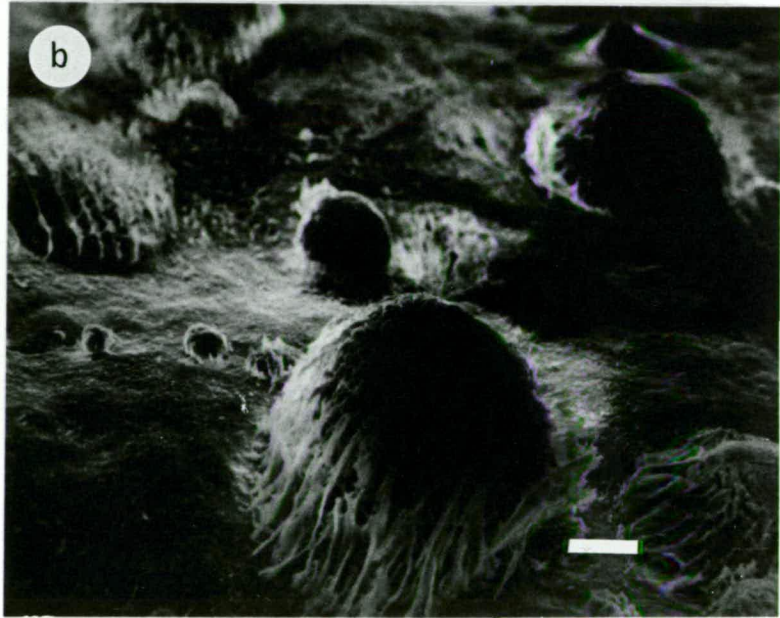
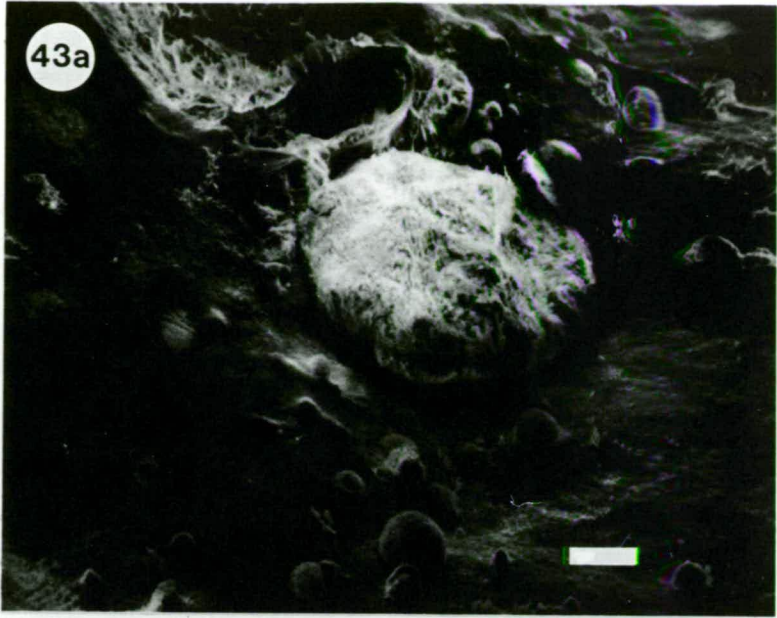
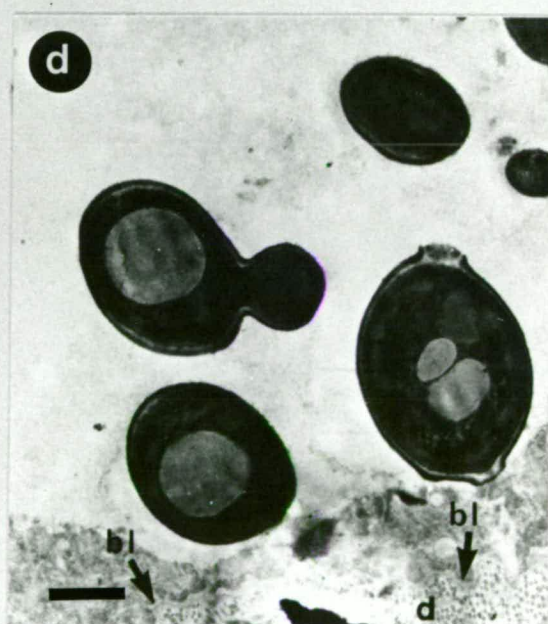
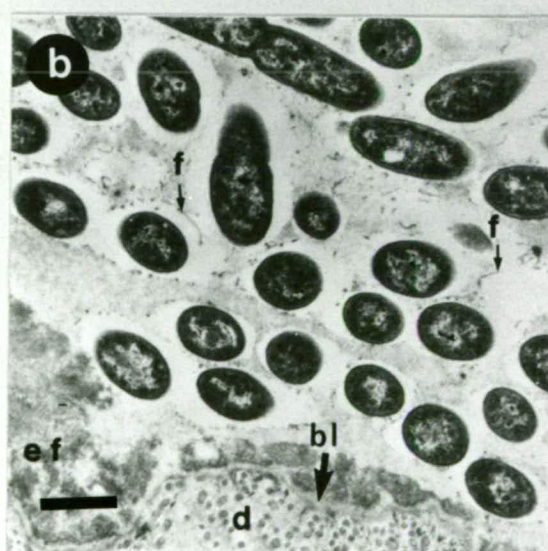
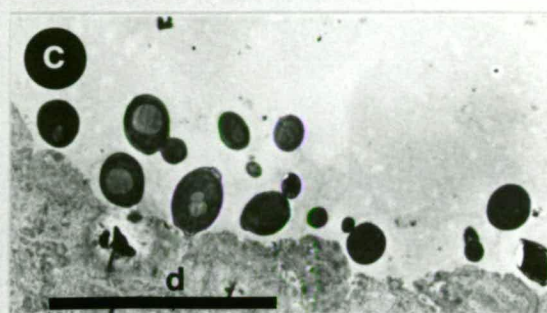
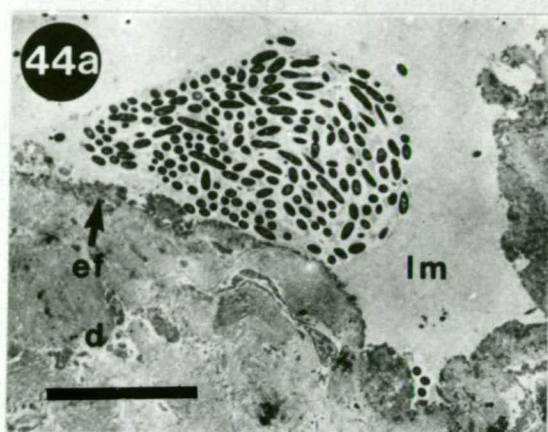


Figure 44. Microorganisms on the skin surface of broiler carcasses stored under drying conditions for 16 days at 2⁰C. Skin specimens treated with glutaraldehyde and alcian blue and post fixed in osmium tetroxide. Sections stained with uranyl acetate/lead citrate.

- (a) A section of a colony of bacteria within preserved layer material (lm) in a channel on the skin surface (d). Note the intact epidermal fragments (ef). Bar = 10µm.
- (b) High magnification TEM of (a) showing the relationship of the bacteria with the skin surface (d). Note the intact basal lamina (bl) and epidermal tissue fragments (ef). The bacteria do not seem to have degraded nearby skin tissue. Bacteria in the layer material are surrounded by a cleared zone. The thin strands within the layer material are flagella (f). Bar = 1µm.
- (c) A microcolony of yeasts on the skin surface (d). Note the hemispherical depressions in the skin surface below individual yeast cells which may indicate degradation of the skin substrate. Bar = 10µm.
- (d) High magnification TEM of (c) showing yeasts lying on epidermal fragments connected to the dermal tissue (d) by the intact basal lamina (bl). Bar = 5µm.



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. SEM/TEM were also used to establish the mode and location of growth of the spoilage microflora on the skin of carcasses stored at chill temperatures. This is a novel approach for examination of these aspects of the microbial ecology of microorganisms associated with flesh foods.

(a) Contamination of Broiler Carcass Skin During Processing

Before processing, broilers carry up to 10^6 microorganisms per 16cm^2 of skin. The majority of the bacteria are *Micrococcus* spp. belonging to Baird-Parker's subgroups 5 and 6 (Baird-Parker 1966) 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; hence it is probable they are part of a transitional skin microflora. The gram positive cocci, however, must be regarded as a more permanent or normal skin microflora because they are present in high numbers and are located in specific sites on the skin.

The nature and origin of the clumps of particulate material enclosing the micrococci is unknown, but may represent accumulations of lipid and other materials exuded from lysed corneous cells. Matoltsy (1969) 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 (1972) noted the skin of the chicken secretes a lipoid or sebaceous material similar to that produced by the oil gland. These workers demonstrated lipid granules on the skin surface of poultry. On the basis of their results, they suggested that the entire avian skin is a secretory organ providing its own requirement for sebaceous material. Microscopic evidence from the present study suggested a lipid component within the clumps of particulate matter (Figs. 11a, b) and showed the presence of other materials which could be stained with uranyl acetate and/or lead citrate. 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 non-processed 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 (Barnes and Thornley, 1966; Barnes, 1976; Daud *et al.* 1979), but in each study, the proportion of each particular type present on the skin was different. Based on results reported by McMeekin and Thomas (1978), 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 a locational basis. It is interesting that micrococci (presumably those normally removed from the skin of non-processed carcasses during plucking) were

found on the skin of processed carcasses (Table 5). Similar observations have been reported by Barnes and Thornley (1966) and Daud *et al.* (1979), while van Schothorst *et al.* (1972) and Mulder *et al.* (1978) 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 non-processed 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 occurred at several stages during processing. Similar trends have been reported previously (Surkiewicz *et al.*, 1969; Mead and Thomas, 1973a; Mead *et al.*, 1975; Mulder *et al.*, 1976; Reddy *et al.*, 1978; and others). However, numbers of psychrotrophic contaminants increased at all stages of processing examined, especially during the immersion washing and chilling stages. Knoop *et al.* (1971) and Lahellec *et al.* (1972) 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 (Barnes 1960; Clark 1968; Notermans *et al.* 1977). Plucking, however, was the first stage of processing to contribute to psychrotrophic contamination of carcasses (Table 6a). Since

water inputs were virtually free of these organisms, plucking machine surfaces must have acted as a source of contamination. Mead (1976) 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.

Psychrotrophic bacteria are important because they represent the potential spoilage microflora of finished carcasses stored at refrigeration temperatures. Numbers of these organisms present on carcass surfaces should be kept as low as possible at all stages of processing to ensure maximum product shelf life. Generally, chlorination of water used during processing is ineffective in eliminating psychrotrophic bacteria contaminating carcasses at any stage of processing (Barnes, 1965; Patterson 1968c) and this is not surprising considering the greater chlorine resistance of poultry spoilage pseudomonads (Mead *et al.*, 1975). Adequate sanitation and cleaning of equipment surfaces is essential to aid reduction of psychrotrophic contaminants during processing.

Clark (1968) and Thomson *et al.* (1974) noted 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 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. The skin surface of immersion chilled carcasses appears swollen (Fig. 6a) compared to skin taken from carcasses immediately after plucking (Fig. 12c). This change presumably occurs because the skin cells absorb water during the chilling process, but more experimental evidence is required to confirm this inference. In particular, a SEM examination of freshly plucked skin soaked in water for various times might provide this information.

The skin surface of broiler carcasses was found to be covered by a liquid film after plucking and all subsequent procedures where carcasses were transiently immersed in processing waters. In this study, excised skin samples were treated with osmium tetroxide vapour or glutaraldehyde solutions to prepare skin for microscopic examination with or without the organic components present within the film. Vapour fixation stabilised these components *in situ*, whereas skin immersed in a glutaraldehyde solution rinsed these materials from the surface before adequate fixation could occur. Haggis and Phipps-Todd (1977) noted a similar rinse effect when thawing frozen chicken erythrocytes in a glutaraldehyde solution. On thawing, soluble proteins diffused away from the cells before adequate

fixation occurred and hence some erythrocytes were completely visible within the fibrin gel. Although freeze drying was found to preserve the skin fluid layer components *in situ*, the technique was inappropriate because lipid material exuded from the subcutaneous adipose tissue onto the skin surface during the drying process. This problem did not occur during critical point drying which was therefore preferred to freeze drying.

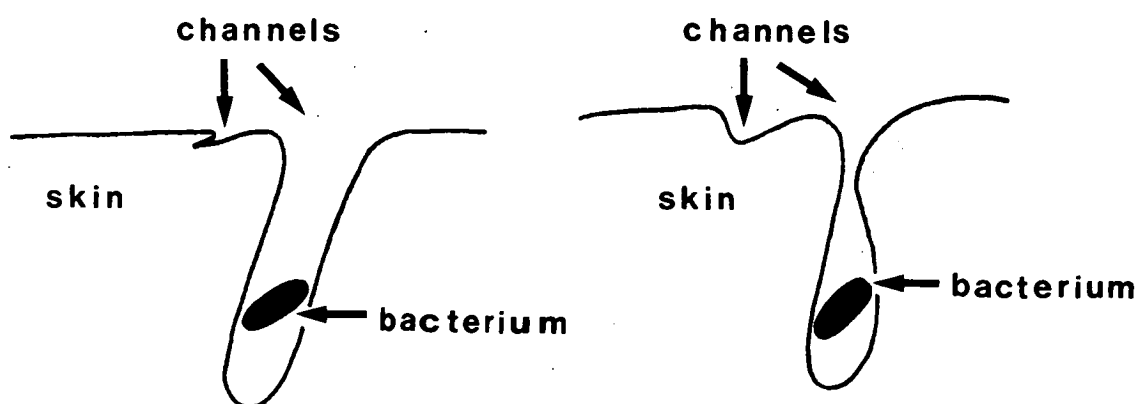
The gelation of osmium tetroxide vapour stabilised components of the liquid film by ethanol, is indicative of protein denaturation by this coagulative fixative (Hayat, 1970). This unexpected secondary fixation undoubtedly facilitated more complete preservation of the fluid film components than would occur had a non-reactive dehydrating agent such as acetone been used. Ethanol gelation, as well as uranyl acetate/lead citrate staining of the preserved components, suggested protein was a major component of the fluid film. Electrophoretic studies confirmed these observations and showed serum proteins were present. Serum albumin as well as some amino acids were found in samples of the liquid layer on processed carcasses as well as in immersion chiller water, suggesting that chiller water was the major source of the fluid layer. However, SEM/TEM examination of the preserved components of the liquid film on stored carcasses showed these materials increased in concentration during storage; these observations were confirmed by the electrophoresis and TLC studies. During storage, water soluble materials diffuse from the dermal tissue into the liquid layer on the skin surface. Therefore, the dermis may contribute soluble materials

to the liquid layer after transient immersion of the carcass in chiller water. Although the nature of the fluid film components of plucked uneviscerated carcasses was not determined, it is reasonable to infer these would be similar to materials present in the liquid film covering the skin of chilled carcasses. The increased concentration of components present (as suggested by the microscopic data) may be explained by tissue damage and the limited component dilution which occurs during plucking as compared to that which occurs during immersion chilling.

The skin microtopography and the presence of the liquid film on the skin of carcasses after plucking and other washing stages, are major determinants of the mechanism(s) of contamination. Microorganisms are located within the liquid film and some may occupy capillary-sized spaces in the skin surface. Bacteria located in these channels will not be as easily removed as those more superficially located in the film on the skin surface. These observations explain, in part, several points outlined in the literature review, e.g. that only a partial reduction in contamination may be effected by various cleaning procedures (Mead and Thomas, 1973b; Notermans and Kampelmacher, 1975b) and that viable counts obtained by maceration of skin samples are always greater than those obtained by swabs and rinses (Avens, 1968; Avens and Miller, 1970a; Patterson, 1972; Notermans *et al.*, 1975a, b). In addition, microscopic evidence presented here supports the conclusion reported by McMeekin and Thomas (1978) that bacteria contaminating the skin of processed carcasses, are located within a water film. Suggested roles of acidic

mu copolysaccharides in attachment of bacteria to the skin surface (Notermans and Kampelmacher, 1974, 1975b; Vanderzant et al., 1976) are not appropriate, but more microscopic evidence is needed to elucidate any possible role of these bridging substances in attachment. Attached bacteria described by Notermans and Kampelmacher (1975, 1975b) may in fact represent organisms lodged in channels and crevices in the skin surface, whereas bacteria more superficially located in the liquid film correspond to the water film microflora described by Notermans and Kampelmacher (1975b).

Mechanisms of contamination outlined above may be complicated by changes in the microtopography of the skin surface which occur during immersion cleaning and chilling. Increased skin cell turgidity, due to uptake of water, causes skin swelling which may trap bacteria already located in the deeper channels and crevices and render them even less accessible to cleaning practices, e.g.



BEFORE IMMERSION

AFTER IMMERSION

Skin swelling may also provide access to smaller channels and crevices. However, more experimental data is needed to confirm this aspect of contamination. Confirmatory evidence may be obtained by a series of experiments designed to compare retention of bacteria by skin samples allowed to swell to different degrees in suspensions of bacteria. It would be especially useful to combine such experiments with detailed microscopic examinations of the histological/microbiological relationships.

From a microbiological viewpoint, scalding and plucking processes are extremely important because significant cross-contamination can occur at this stage. 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 to unattached bacteria (Notermans and Kampelmacher, 1975a). Chlorination of water supplies used by plucking machines is usually ineffective in reducing the bacterial load of the skin of carcasses during plucking as well as cross-contamination (Mead *et al.*, 1975). The reasons for these observations are as yet unknown. However, Notermans and Kampelmacher (1975a) have suggested the location of bacteria within the skin surface may protect attached cells from serious heat damage. Microscopic data presented in this study lends support to this argument, and it seems reasonable that bacteria located deep in channels will be protected from heat damage as well as being difficult to remove. The presence of organic matter within the liquid film may explain why all

skin bacteria are not destroyed by chlorine or other antibacterial agents. Detailed microscopic examination of the relationship of contaminant bacteria with the skin surface before and after scalding and plucking processes may provide clues to explain these observations. Also, it would be interesting to compare changes in the skin structure which result from various scald treatments and relate this data to the bactericidal effects of those treatments and their effect on subsequent contamination and development. Increased skin damage (Clark, 1968), less cross-contamination (Mulder and Dorresteijn, 1977), greater reductions in numbers of viable bacteria (Mead, 1976) and shorter shelf-life (Ziegler *et al.*, 1955; Essary *et al.*, 1958) have been associated with high scald water temperatures. In general, the converse holds for soft scald treatments. Only this study has provided microscopic data to relate changes in the skin structure, as a result of a scald treatment, to some of these parameters.

In this study, SEM was used as an aid to evaluate the stomacher as a means of recovering microorganisms from chicken skin. Bacteriological counts indicated that stomaching skin for 5 minutes consistently recovered bacteria, although an analysis of variance showed counts obtained after 1, 2, 3, 5 or 10 minutes of stomacher action, were not significantly different. The decision to stomach skin samples for 5 minutes was therefore supported by microscopic observation, i.e. lipid was released from the skin adipose after 3-5 minutes and most bacteria, epidermal fragments and other debris were removed from all sites on the skin of fresh or spoiled carcasses, whilst the skin

was left substantially intact. Also, counts of bacteria after stomaching for 5 minutes were not significantly different from those obtained by blending, a result which agrees with data presented by Emswiler *et al.* (1977). Therefore, stomaching is a useful alternative to blending techniques when maximum recovery of microorganisms from broiler skin is required. Bacteria not removed from spoiled skin by stomacher action were held to the surface by extracellular material (Fig. 16 inset). However, since only a few attached bacteria were observed, these organisms represented an insignificant proportion of the total number of bacteria enumerated ($\approx 10^9$ cells/16cm² of skin).

Microscopy has not been used previously as an aid to evaluation of sampling techniques. Other workers have used only counts of bacteria to compare various methods (Barnes and Shrimpton 1958; Mallman *et al.* 1958; Fromm 1959; Kinsely and Mountney 1966; Avens 1968; Surkiewicz *et al.* 1969; Avens and Miller 1970a; Patterson 1972; Mead and Thomas 1973a, b; Cox and Blankenship 1975; Notermans, Kampelmacher and van Schothorst 1975a, b; Cox *et al.* 1976; Emswiler *et al.* 1977; Cox and Bailey 1978), and no attempt was made to establish the location and relationship of the bacteria with the skin. It seems obvious that the histological/microbiological relationships will be prime determinants in selecting a method of sampling. Hence combined microscopy and normal microbiological studies would be extremely useful in explaining differential results obtained by other methods such as swabs and whole bird rinses.

(b) Growth of Microorganisms on the Skin of Broiler Carcasses

Increases in numbers of microorganisms on breast or leg skin of carcasses stored at 2°C followed a typical growth curve. The 4-8 day lag period noted for the TVC (Figs. 19; 20), represented the time required for the number of psychrotrophic microorganisms to exceed numbers of viable microorganisms originally on the skin. After 8 days of storage the TVC represented the number of psychrotrophic organisms present on the skin. This point is further illustrated by a comparison of the composition of the microflora isolated by the TVC and the psychrotrophic plate count methods (Tables 9a, b; 10a, b). As has been shown in previous studies (Barnes 1976; Barnes *et al.* 1978; Daud *et al.* 1979), psychrotrophic *Pseudomonas* Groups I and II were the predominant types isolated from the skin of poultry carcasses stored at 2°C. *Acinetobacter/Moraxella*, *Flavobacterium/Cytophaga* types and coryneform-like bacteria were also isolated as a small fraction of the spoilage microflora. Yeasts were found growing on the skin, but in low numbers compared to numbers of bacteria and hence remained an insignificant proportion of the microflora. Similarly, yeasts have been found to fail to compete with psychrotrophic pseudomonads when turkey carcasses were stored at temperatures of 0°C or above (Barnes *et al.* 1978). The ability of yeasts to grow competitively on flesh foods depends on intrinsic factors such as the a_w of the food substrate (Ingram 1951). Under conditions of reduced a_w and the consequent reduction in the competitive ability of spoilage pseudomonads, yeast spoilage has been noted for meat held at -5°C (Haines 1931; Walker and Ayres 1970) and turkey carcasses stored at -2°C (Barnes *et al.* 1978). The rapid reduction in numbers of viable coliforms

organisms present on skin from stored carcasses is not unexpected and similar observations have been reported (Barnes *et al.* 1978). However, psychrotrophic enteric types have been isolated as a small fraction of the spoilage microflora of chill stored broiler skin (Daud *et al.* 1979) and breast and leg muscle (McMeekin 1975, 1977), although none were isolated in this study.

It is interesting that *Alteromonas putrefaciens* was not isolated from the skin of spoiling broiler carcasses. This organism has previously been noted as a significant proportion of the spoilage microflora of chicken carcasses (Barnes and Thornley 1966), chicken leg and breast muscle (McMeekin 1975, 1977) and fish (Levin 1968; Herbert *et al.* 1971). The reason for this observation is not evident and warrants further investigation.

Differences in the microflora of spoiled chicken leg and breast muscle attributable to pH of the growth substrate have been reported (Barnes and Impey 1968; McMeekin 1975, 1977). No differences in the spoilage microflora of intact breast or leg skin were observed and considering the similarity in the pH of leg and breast skin rinse water (Fromm and Monroe 1965), this is not surprising. These results demonstrate pH of the underlying muscle does not exert any significant selective influence upon the ultimate skin spoilage microflora.

Barnes and Impey (1968) and Barnes *et al.* (1973) used TTC and ultraviolet light to locate areas of growth of microorganisms on skin from broiler carcasses stored at 1°C. Growth occurred mainly in feather follicle shafts and only during the later stages of spoilage were bacteria growing all over the skin surface. Detailed microscopic studies used in this report,

showed the majority of microorganisms growing on either leg or breast skin, from carcasses stored at 2⁰, 4⁰, 10⁰, 15⁰ and 22⁰C under high humidity conditions, were located within the liquid film on the skin surface and deep within channels and feather follicle shafts. Colony-like formations were observed where bacteria grew through the surface of the liquid layer. Discrete colonies of microorganisms were only found on the skin of carcasses stored under drying conditions, although bacteria did grow within the more viscous liquid film present on the skin of those carcasses. Dermal skin tissue acted as an effective barrier against penetration and invasive growth by bacteria. These results suggest reasons why Barnes and co-workers did not detect growth in areas other than feather follicles until late spoilage. The location of microorganisms and the organic load of the surface liquid film would have severely limited the efficacy of their methods to detect sites of active growth. Bacteria deep in the channels in the skin would not be detected by either the TTC or the ultraviolet fluorescence technique and the liquid film may quench the penetration of ultraviolet radiation. However, the microscopic data does support the observation that bacteria only grew all over the skin surface in the final stages of spoilage (Barnes and Impey 1968). Bacteria grew first within the liquid layer confined by the channels on the skin but later proliferated through the liquid film over the skin surface. Growth of microorganisms on leg skin was not significantly different from growth on breast skin as was reported by Barnes and Impey (1968).

Although the microscopic techniques used during this study allowed observation of the distribution and growth of microorganisms on skin, the method only provided specific information about these organisms when their numbers exceeded $\approx 10^6/16\text{cm}^2$ skin. These numbers usually occurred after carcasses were stored at 2°C for about 8 days. Observation of lower numbers of bacteria on skin was limited for several reasons:- low numbers of bacteria meant sites of growth were widely separated; bacteria grew within the liquid film on the skin surface and only a few organisms were located at the surface of the preserved film components of osmium tetroxide vapour fixed skin; both the bacteria and the liquid film were rinsed from skin treated in glutaraldehyde solutions. Therefore, only when growth was sufficiently prolific were bacteria observed in thin sections of the preserved layer material or on specimens prepared for SEM. The same considerations apply to studies of the mechanism of contamination during processing.

The types of microorganisms found by microscopic studies confirmed in part some of the cultural data. The majority of microorganisms observed in all sites examined on the skin of carcasses stored at 2°C were gram negative rod-shaped bacteria which correspond to the pseudomonads commonly isolated as the major spoilage type growing on skin. Yeasts and small gram positive, rod-shaped bacteria were sometimes found; the latter type probably represent the psychrotrophic coryneform-like bacteria isolated as a small fraction of the spoilage microflora. Yeasts found on skin from carcasses stored under humid conditions usually grew among bacteria within the liquid film

although individual colonies were found on dry stored carcasses. The fact that colonies of small coccoid rod-shaped, gram negative types such as *Acinetobacter* and *Moraxella* spp. were not located on skin of carcasses stored at 2°C suggested these organisms grew within confined areas; hence their probability of detection in thin sections would be low. A single colony of about 10^8 cells on a piece of skin would be detected as a small fraction of the total microflora, but may not be found in sections of skin. Conversely, because the pseudomonads grew throughout the liquid film and were the major spoilage types present, these organisms were always found in sections of skin from spoiled carcasses.

The increased preservation of liquid film components present on the skin of spoiling broiler carcasses may be explained in terms of the numbers of bacteria present within the film and the fixative solutions used. Large numbers of bacteria within the film increased the viscosity of this liquid and thus helped prevent removal of liquid film components during immersion fixation. A similar effect has been noted by Haggis and Phipps-Todd (1977). These workers noted that the presence of large numbers of erythrocytes in a fibrin gel helped to prevent rinsing away of protein before adequate fixation occurred. The use of the cationic dye Alcian blue 8GX in fixative solutions helped improve fixation and preservation of liquid film components because this dye can non-specifically bind negatively charged groups (Behnke, 1968). Alcian blue, therefore, undoubtedly bound to carboxyl groups associated with proteinaceous material

present in the liquid film and hence the electron dense granules noted in TEM's of thin sections of the preserved liquid film components. The difference in size of the granules observed between glutaraldehyde plus alcian blue fixed tissue (Fig. 23b) and osmium tetroxide vapour fixed tissue (Fig. 23c) therefore represents differential staining effects of alcian blue and uranyl acetate/lead citrate.

Attachment of bacteria to solid surfaces has been extensively studied. Investigations have involved biological surfaces (Brooker and Fuller 1975; Marshall *et al.* 1975; Fuller *et al.* 1978; Harper *et al.* 1978; McCowan *et al.* 1978; and others) and non-biological surfaces (Meadows 1971; Marshall *et al.* 1971a, b; Fletcher and Floodgate 1973; Hendriks 1974; Marshall 1975). Various modes of attachment have been reported and include fimbriae or pili (Duguid 1959; Fuerst and Hayward 1969; Duguid *et al.* 1976; Harper *et al.* 1978) and capsular polysaccharides (Zobel 1943; Jones *et al.* 1969). Most studies have reported attachment as an important survival mechanism for bacteria associated with surfaces and Costerton *et al.* (1978) have noted that attachment may enable bacteria to conserve and concentrate digestive enzymes and nutrient resources. In contrast, microscopic observations of bacteria on the skin of chill stored broiler carcasses have shown bacteria are not attached to the skin surface, but are free within the liquid film normally present on this surface after processing. Fimbriae or acidic mucopolysaccharide polymer related attachment to skin was not observed in any thin sections of skin examined. Only bacteria growing on carcasses stored at 15⁰ or 22⁰C appeared to be

attached to the skin surface, although a more detailed examination of bacteria on skin from carcasses stored at these temperatures is required. Fimbriae related attachment between bacterial cells may have allowed clumps of several bacteria to form within the liquid layer on the skin. However, as a general conclusion, the results clearly indicate an ecological niche different from that encountered in animal digestive tracts and on marine submerged surfaces. In these systems, attachment of bacteria to the surface involved has an obvious survival value, but spoilage bacteria present on broiler carcass skin are largely free within a stable nutritious film on a food surface where specific or non-specific attachment mechanisms appear to have no survival value.

(c) Metabolism of Microorganisms on the Skin of Chill Stored Broiler Carcasses

Although bacteria growing on broiler skin caused highly localised degradation of the fluid film components and, to some extent, the dermal skin surface, no gross skin degradation or invasion was noticed. The spoilage microflora grew entirely within the confines of the fluid film, including liquid within the feather follicle shafts. Degradation of the fluid film components was observed as cleared zones around thin sections of bacteria and as pits around bacteria on the surface of preserved fluid film components when skin specimens were examined by SEM. Similar zones of substrate digestion have been noticed around:- *Pseudomonas fragi* grown on pig muscle (Dutson *et al.* 1971); the organism responsible for Legionnaires' disease when

grown in egg yolk sac cells (Katz and Nash 1978) or *in vivo* in lung tissue (Rodgers *et al.* 1978); rumen bacteria digesting sloughing cells of the stratified squamous epithelium of the reticulo-rumen in cattle (McCowan *et al.* 1978); *Ruminococcus flavifaciens* digesting epidermal cell walls of *Lolium perenne* (Latham *et al.* 1978); rhizosphere bacteria degrading the mucigel layer around plant roots (Guckert *et al.* 1975). The proteolytic activity of many of the *Pseudomonas* spp. isolated from spoiling poultry and meat is well known (Jay 1967; Rey *et al.* 1969; Adamcic and Clark 1970a, b; Borton *et al.* 1970a, b; Barnes and Melton 1971; Rey and Kraft 1971; Tarrant *et al.* 1971; Dainty *et al.* 1975). Considering the proteinaceous nature of the liquid film on broiler skin, there seems little doubt that these cleared zones represent localized extracellular enzymatic digestion of some protein component(s) of this film. Lower molecular weight compounds produced by enzyme action are apparently not fixed *in situ* and are rinsed away during the wash and dehydration steps employed in preparation of the skin samples for electron microscopic examination. Hence the cleared zones around bacteria growing in the liquid film. The structure and function of the coarse strands of material connecting bacteria to the edges of these pits is not known. However, a more detailed microscopic study of these structures may provide more information.

Several ultrastructural features have been associated with extracellular enzyme production and excretion by bacteria. Gosh *et al.* (1968) demonstrated massive convolutions of the peripheral membrane and long tubules associated with protoplast cell membranes in penicillinase induced *Bacillus licheniformis*.

These structures were suggested to be involved in penicillinase secretion. Cell wall blebs or evaginations have been implicated in secretion of toxic materials from *Escherichia coli* (Knox *et al* 1966) and *Vibrio cholerae* (Chatterjee and Das 1967). More recently, Wiebe and Chapman (1968a, b) noted blebs were induced on marine pseudomonads and achromobacteria by certain nutritional and physiological conditions and Dutson *et al.* (1971) showed similar structures were produced by *Pseudomonas fragi* only when grown in muscle tissue. Dutson and co-workers postulated proteolytic enzymes, produced by bacteria, were secreted into these blebs which later separated from the cell wall and released their contents into the muscle tissue surrounding the bacteria. However, no such structures were found associated with any bacteria growing within the liquid film on the surface of spoiled broiler carcasses. Extracellular enzymes responsible for the cleared zones around bacteria in the preserved film must have originated from some other site on or in the bacterial cells. Pollock (1962), in a review of data related to extracellular enzymes, has pointed out the possibility of enzymes located on the exterior surface of bacterial cell membranes. Membrane-bound enzymes may explain the highly localized nature of fluid film component digestion, but more experimental work is needed to elucidate this aspect. Although the electrophoresis studies suggested serum albumin to be a major component of the liquid film it would also be useful to determine more precisely the actual fluid film component(s) which acted as the substrate.

Previous studies related to bacteria induced biochemical changes which occur during spoilage of poultry tissues and carcass

meats have emphasised conflicting data concerning the role of proteins and proteolysis in the spoilage process. A large number of authors have noted the proteolytic ability of a variety of bacterial types isolated from spoiling flesh foods. However, significant *in vivo* proteolysis has not been detected prior to the onset of spoilage (Jay 1967; Jay and Kontou 1967; Adamcic and Clark 1970a; Borton *et al.* 1970a, b; Hasegawa, Pearson, Price and Lechowich 1970; Hasegawa, Pearson, Price, Rampton and Lechowich 1970; Tarrant *et al.* 1971; Dainty *et al.* 1975). Other evidence suggests proteolysis does not occur until low molecular weight compounds present in the tissue are exhausted (Jay and Kontou 1967; Ockerman *et al.* 1969; Adamcic and Clark 1970a; Jay 1972) while Gill (1976) and Gill and Newton (1977) showed sufficient glucose, amino acids and other low molecular weight compounds are present at all stages of storage in sufficient quantities to enable continued growth. Dutson *et al.* (1971) used electron microscopy to reveal changes in muscle myofibrillar structure induced by bacteria. These changes were assumed to be related to proteolysis of structural proteins, but were not observed until after spoilage occurred. Similarly, Gill and Penney (1977) demonstrated penetration of proteolytic bacteria into spoiled beef muscle stored at 20⁰, 30⁰, 37⁰C.

Although marked changes in skin structure, which could be attributed to microbial growth and activity, were not observed in this study, degradation of liquid film components occurred before the onset of spoilage (Fig. 24). This evidence implies that proteins in the liquid film may be digested by bacterial enzymes at any stage of storage; an inference in conflict with

evidence presented by Adamcic and Clark (1970a). However, these workers were primarily concerned with biochemical changes associated with collagenolysis. Hence their analysis would have ignored proteolysis of serum proteins which have been shown to be present on skin by this study. Similarly other investigations reported only changes associated with sarcoplasmic and myofibrillar proteins (Borton *et al.* 1970a, b; Hasegawa, Pearson, Price and Lechowich 1970; Hasegawa, Pearson, Price, Rampton and Lechowich 1970; Tarrant *et al.* 1971; Dainty *et al.* 1975). All these studies detected changes in proteins only when numbers of spoilage bacteria were about 10^8 cells/g of tissue. Therefore, it is suggested that the analytical techniques used were not sufficiently sensitive to discern subtle changes brought about by lower numbers of bacteria. The microscopic techniques described in this study enabled the detection of substrate degradation on a single cell basis and hence represent an extremely sensitive tool for investigation of the activity of extracellular enzymes produced by bacteria growing on a test substrate.

Off odours and other changes associated with the spoilage of flesh foods stored at chill temperatures are caused by the growth of a restricted group of psychrotrophic bacteria (Ayres 1960; Barnes and Impey 1968; Herbert *et al.* 1971; McMeekin 1975, 1977). Pure cultures of these bacteria have been grown on sterile muscle preparations and consequent production of off odours used to indicate the ability of isolates to contribute to spoilage of the naturally contaminated food. In this study, the spoilage potential of psychrotrophic bacteria isolated from the skin of chill stored broiler carcasses, was characterized by

ability to produce off odour from chicken leg muscle, skin and artificial media. Techniques to obtain sterile muscle substrates are well known (McMeekin 1975, 1977), and the rationale for the use of intact muscle has been discussed previously (Herbert *et al.* 1971). Sterile intact skin samples are not readily obtained and microscopic data presented in this text demonstrate why sterile skin cannot be obtained without treatments which may damage this substrate. However, previous reports (Barnes 1960; Clark 1968; Notermans *et al.* 1977), including data presented here, have shown skin from carcasses immediately after scalding is free of viable psychrotrophic bacteria. Preliminary studies, not presented here, showed skin samples, from chilled carcasses, subjected to a 3 minute scald treatment at 62°C, did not develop off odour and remained virtually free of these organisms even after 1 month's storage at 2°C. Consequently, these skin preparations were used to test the ability of known off odour producing isolates, to produce off odour when grown on their original substrate. This methodology represents a new approach; other workers have simply used frozen crushed, irradiated skin preparations or extracts of skin collagen to assess the growth of spoilage bacteria (Clark 1968; Adamcic and Clark 1970a, b).

The incidence of spoilers during the course of spoilage of flesh foods is normally restricted to a small fraction of the microflora. Hence Adams *et al.* (1964) noted these remained less than 20% of the population growing in fish press juice. Herbert *et al.* (1971) showed that although numbers of bacteria causing off odour increased considerably during chill storage of cod muscle, spoilers never accounted for more than 20% of the

microflora. McMeekin (1977) noted similar results for spoilage of chicken leg muscle, although an apparent selection for spoilers was demonstrated in stored breast muscle (McMeekin 1975). The latter result was explained in terms of the isolation procedures employed and by postulating faster growth rates of the spoilers isolated (McMeekin 1977). Results presented here showed up to 40% of all isolates from breast and leg skin produced off odour when grown on chicken leg muscle and nearly all of these spoilers produced off odours when incubated on skin. This result is somewhat higher than that reported by Daud *et al.* (1979) who showed less than 19% of the microflora of skin could produce off odour from muscle tissue. However, less than 25% of all isolates produced DTNB positive odours from either substrate. Since DTNB positive head space vapours were characterised by a strong sulphide-like odour, these isolates probably represented the major spoilage types on poultry skin.

Pseudomonas groups I and II types were the major spoilage organisms isolated from either breast or leg skin of carcasses stored at 2°C. Overall, a greater proportion of *Pseudomonas* group I strains (60%) produced off odours compared to *Pseudomonas* group II (38%). Lerke *et al.* (1965) has characterized the spoilers of fish press juice and found an extremely close correlation between spoilage ability and taxonomic position. Herbert *et al.* (1971) and McMeekin (1977) associated fruity odours with *Pseudomonas* group II types and sulphide-like odours with *Pseudomonas* group I and III/IV types. In this study, members of both *Pseudomonas* group I and group II produced sulphide-like³ odours, although the latter group were mainly responsible for

fruity-type odours. Fruity odours produced by *Pseudomonas* group I were usually accompanied by a sulphide-like component. *Pseudomonas* group III/IV strains produced only sulphide-like odours. These results suggest there is no clear cut association between spoilage ability and taxonomic position.

Sulphide-like odours have been described in a variety of chill stored flesh foods; including fish (Chai *et al.* 1968; Herbert *et al.* 1971; Herbert *et al.* 1971; Levin 1968; Miller *et al.* 1973c), shellfish (Lapin and Koburger 1974), meat (McMeekin and Patterson 1975; Nicol *et al.* 1970) and poultry (McMeekin 1975, 1977; Freeman *et al.* 1976; Lee *et al.* 1979). The organism most commonly responsible has been *Alteromonas putrefaciens* which produces hydrogen sulphide and methanethiol by degradation of cysteine, cystine and methionine (Herbert and Shewan 1976). However, in this study, methanethiol was the major sulphur volatile responsible for the sulphide-like odours produced by strains of *Pseudomonas* groups I and II grown on chicken skin or leg muscle. Pure culture studies of isolates grown in methionine supplemented peptone broth supported previous evidence that methanethiol was produced from methionine (Segal and Starkey 1969; Herbert and Shewan 1975). The presence of dimethyl disulphide in headspace vapours containing methanethiol suggests oxidation of this thiol occurred. Miller *et al.* (1973a, c) identified both methanethiol and dimethyl disulphide when cultures of *Pseudomonas putrefaciens*, *Achromobacter*, *Pseudomonas fluorescens* and *Pseudomonas perolens* were grown in a sterile homogenate of fish muscle. Miller *et al.* (1973b) also reported identification of dimethyl disulphide produced from *Pseudomonas fragi* although

methanethiol was absent. Kadota and Ishida (1972) have noted that in instances where dimethyl disulphide was present but not methanethiol, it is likely that all the methanethiol was oxidized to the former compound. Isolates from chicken skin which produced methanethiol usually did not produce hydrogen sulphide from leg muscle, skin or even cystine supplemented peptone broth. In this respect these results differ from those of a study reported by Daud *et al.* (1979) although similar results have been reported for cultures examined by Freeman *et al.* (1976). These differing results exemplify the vagaries encountered when examining the spoilage potential of spoilage isolates.

Production of dimethyl sulphide by pure cultures grown on skin and muscle has also been noted for isolates grown on fish muscle (Herbert *et al.* 1975; Herbert and Shewan 1976). The mechanism of formation of this compound is not clear although it is possible that this may be produced by methylation of methanethiol, but more experimental work is required to identify the precursor of this compound.

Volatile sulphur compounds, such as methanethiol and hydrogen sulphide, produced by bacteria grown on muscle and skin, were present in amounts well above the odour threshold levels. These levels have been reported by Guadagni *et al.* (1963) to be 0.5 ppb for methanethiol and 40 ppb for hydrogen sulphide, and Herbert (1970) reported 0.02 ppb for methanethiol, all in aqueous solution. DTNB positive odours produced by isolates used in this study contained methanethiol at concentrations greater than 3.4 ppb. This gas concentration represented the lowest concentration of

methanethiol detectable by DTNB test strips described by McMeekin *et al.* (1978). The lowest DTNB or lead acetate positive methanethiol or hydrogen sulphide gas concentration was produced by a 19.73 ppm and 19.70 ppm aqueous solution respectively. Therefore, headspace concentrations represent only a small fraction of the total volatiles produced in the aqueous phase on the tissue substrate.

Addition of glucose to methionine supplemented peptone broth completely inhibited production of methanethiol by bacteria grown in this medium. Bacteria utilized glucose preferentially to methionine and only after glucose was exhausted was production of methanethiol detected. The concept of preferentially utilized substrates is well known for bacteria. Jacoby (1964) demonstrated pseudomonads utilized glucose preferentially in liquid culture, while Gill (1976) has reported an initial saccharolytic phase of bacterial growth on meat. Only after all glucose has been utilized may other less preferred substrates, such as amino acids, be metabolized. In this respect, Gill (1976) showed inhibition of off odour production by tissue glucose and similar results have been noted after addition of glucose to ground beef (Shelef 1977).

The higher proportion of isolates able to produce DTNB positive odours when grown in methionine supplemented peptone broth as compared to leg muscle, is difficult to explain. In the simplest terms, bacteria unable to produce organoleptically important quantities of methanethiol from muscle methionine, may be responding to the higher concentration of this substrate present in the broth culture solution. This explanation is substantiated by the similar proportion of strains able to produce

a DTNB positive reaction in peptone broth without added methionine compared to those grown on leg muscle. Herbert and Shewan (1976) have suggested the enzymes responsible for production of methanethiol from methionine, are constitutive in nature. If the effect of concentration of methionine on methanethiol production is not simply a direct effect upon the reaction rate, then it may be that the enzymes responsible are actually induced. It is evident from these studies that more work is needed to elucidate the kinetics of the processes involved. Initial steps might be to examine the effect of substrate concentration and determine whether in fact the enzymes responsible are inducible or constitutive. Further, these results cast serious doubt upon the validity of use of methionine or cyst(e)ine supplemented media for the evaluation of spoilage potential of isolates from stored flesh foods.

It is interesting that methanethiol was the principal spoilage odour produced by the majority of spoilage organisms isolated from chill stored poultry carcasses. Only a few organisms produced hydrogen sulphide in sufficient quantities to cause a positive lead acetate reaction. However, Herbert *et al.* (1971) reported hydrogen sulphide was the principal sulphur volatile produced by pseudomonads isolated from spoiling cod muscle. These organisms were only isolated on the basis of hydrogen sulphide production on peptone iron agar, whereas, in this study, all bacteria isolated from psychrotroph plate counts were tested for off odour production. McMeekin *et al.* (1978) have suggested peptone iron agar is limited in its usefulness in that it detects only hydrogen sulphide producing strains and is less sensitive than tests based on lead acetate. Indeed, peptone

iron agar has been noted not to detect *Alteromonas putrefaciens* or any other hydrogen sulphide producing microorganisms (Daud *et al.* 1979). Therefore, Herbert *et al.* (1971) would not have recovered strains of bacteria able to produce significant amounts of methanethiol, but unable to produce hydrogen sulphide in sufficient quantities to produce a black precipitate on this medium. For similar reasons Gillespie (1977) and McMeekin *et al.* (1978) have suggested the use of DTNB based test procedures to aid in isolation of off odour producing bacteria from stored flesh foods.

(d) Application of Microscopy to Other Areas of Food Microbiology

The combined use of microscopy and normal microbiological methods has proved an extremely useful technique for the examination of contamination and subsequent growth of bacteria on the skin of broiler carcasses. The technique has been used in a confirmatory role and to obtain new information. Logically, this approach could yield useful information about contamination and spoilage of other food substrates.

Aspects of contamination of various flesh food tissues, e.g. visceral linings of poultry carcasses, muscle surfaces (with or without fasciae) and other epithelial tissues, are not well known. Initial microbiological studies of a number of these substrates have been made, but the histology/microbiology relationships have not been examined. Blankenship *et al.* (1977) examined clostridial spores contaminating chicken skin and Butler *et al.* (1979) investigated various physical and chemical parameters controlling contamination of pork skin as well as the

surfaces of beef and lamb carcasses. Similarly, Firstenberg-Eden *et al.* (1978) used chicken and beef muscle while Notermans *et al.* (1979) examined cow's teat skin. All these studies concluded adhesive mechanisms explained increased retention of test bacteria by these surfaces, but no direct evidence was reported to substantiate this inference. The arguments for bacterial adhesion rested upon uncritical citations from a collection of papers by Notermans and Kampelmacher (Notermans and Kampelmacher 1974, 1975a, b). Indeed, on the basis of results presented in this text and elsewhere (McMeekin and Thomas 1978), the interpretations Notermans and Kampelmacher placed on their results are somewhat dubious and illfounded. Evidence for this criticism has already been discussed in the attached literature review and by McMeekin and Thomas (1978). Only Firstenberg-Eden *et al.* (1979) have presented microscopic data in an attempt to substantiate other microbiological data. These workers studied the attachment of test bacteria to the teats of cows. A series of scanning electron micrographs, said to demonstrate acidic mucopolysaccharide fibres and slime involved in attachment of bacteria to the skin, were presented. Inferences based on these micrographs are somewhat tenuous. Some ultrastructural features described as attachment fibres bear a striking resemblance to flagella shown in some micrographs presented in this text as well as that shown by Matsuguchi *et al.* (1977). The bacterial slimes shown may be explained as extracellular material produced around bacteria during liquid culture which is carried over in the washed cell suspensions used as the inocula.

Slime associated with washed cell suspensions has been previously reported (Tago and Aida, 1977). Further, the strength of attachment was said to be related to formation of extracellular fibres, yet microcolonies of a *Pseudomonas* spp. (strain EBT/2/143; Notermans *et al.* 1979), the most firmly attached species examined, showed an absence of these fibres. Obviously, the studies described need re-evaluation. Carefully designed detailed microscopic studies carried out in association with normal microbiological procedures might allow a more reasoned evaluation of the mechanism(s) of contamination of many of the substrates mentioned. Particularly, attention needs to be paid to the effects various processing and experimental procedures may have upon the appearance and microtopography of the surface under investigation, since changes may significantly effect or alter results obtained. In this respect, changes in the microtopography of chicken skin as a result of immersion chilling serves as a suitable example of an effect which must be considered as an important parameter of contamination during broiler processing. Similar effects may occur when other animal tissues are immersed in aqueous suspensions.


Adhesive mechanisms explaining attachment of various test bacteria to whole tissue substrates are not easily demonstrated. Frequently, the polysaccharide fibrils so often associated with bacterial attachment, are not resolved by the SEM and organic materials within liquid films associated with test surfaces may confuse the issue even further. This latter point is well illustrated in micrographs presented in this study (Figs. 5 and 6).

Bacteria in the liquid film on the skin of broiler carcasses can be fixed *in situ* within the fluid film components, but removal of this film by rinsing also removed most of the bacteria. Consequently, a more easily controlled system based upon suspensions of substrate cells may be used in place of whole tissue samples. Such a technique has already been described (Harper *et al.* 1978). The advantages of this system are inherent in the ability to examine attachment of bacteria to individual substrate cells, and when used in conjunction with SEM and TEM may provide useful information about attachment mechanisms. An obvious example for which this technique may be used is an examination of the possible attachment of salmonellae to poultry tissues. *Salmonella* serotypes may be examined for morphological structures commonly associated with bacterial adhesion (e.g. fimbriae and capsular glycocalyx) and the histological/microbiological relationship subsequently described in terms of index of adhesion and strength of attachment.

Preliminary observations of the growth of microorganisms on the skin of air chilled broiler carcasses have been made (McMeekin *pers. comm.*). These studies show the presence of layer material on the skin surface similar to that observed for immersion chilled carcasses. However, instead of developing within this material, the majority of microorganisms grow within discrete balls of material, on the skin surface, which act as foci for development of the spoilage microflora. The source of these balls of material and their relationship to contaminant psychrotrophic bacteria needs to be examined and answers may well be provided by further microscopic studies.

Growth and penetration of proteolytic bacteria into muscle tissue has been examined by light microscopy (Gill and Penney 1977). Unfortunately, the incubation temperatures selected did not include one in the chill range and detailed relationships of the bacteria with the invaded tissue were not examined. SEM and TEM could therefore be used to provide more basic information about the mechanism of muscle fibre disruption.

Other areas of food microbiology where a knowledge of the organism - substrate interaction will provide valuable information include:-

- (1) An investigation of the passage of potentially pathogenic microorganisms through the digestive tract of oysters.
- (2) Demonstration and localization of a commensal gut microflora in oysters.
- (3) An examination of the efficacy of various sampling and cleaning procedures.
- (4) Use of scanning  electron micrographs of microorganisms contaminating food and equipment surfaces, as a visual aid in the education of food industry employees.

However, whilst the use of SEM and TEM in food microbiology is rapidly gaining acceptance, there are a number of basic, albeit important points which should be made to workers intending to enter this field.

1. Microscopic examinations should be carried out in conjunction with normal microbiological procedures. The latter are often required to allow satisfactory interpretation of microscopic observations.

2. SEM/TEM examinations should be combined since this ensures a more correct interpretation of data. This point was especially evident in the identification of epidermal fragments on SEM's of processed skin (Figs. 6b and 13c).
3. Careful choice of preparative procedures and subsequent evaluation of the results are required to minimise the risk of artefacts and false interpretation of data.

CONCLUSIONS

1. Scanning and transmission electron microscopy used in conjunction with standard microbiological procedures allowed investigation of histological/microbiological relationships of broiler carcass skin sampled at key stages of processing and during storage at various temperatures.
2. The autochthonous skin microflora of breast and leg skin of the live bird were mainly *Micrococcus* spp. which were located in accumulations of sebum-like substances on the surface of the *stratum corneum*.
3. Scalding and plucking procedures removed the skin epidermis and exposed dermal tissue to colonization by microbial contaminants from the mechanical plucker or subsequent stages of processing.
4. Immersion washer and chiller water were the major sources of psychrotrophic microorganisms which contaminated the skin of processed broiler carcasses.
5. Dermal skin microtopography and a liquid film on the skin surface of carcasses, were implicated as major factors controlling contamination during processing.
6. The liquid film on the skin surface of fresh immersion chilled carcasses contained serum proteins and a number of amino acids. The amount of liquid and the concentration of the components increased during storage of broiler carcasses.

7. Exposure of skin to osmium tetroxide vapour allowed preservation of liquid film organic components and bacteria *in situ*, whereas immersion fixation rinsed these components from the skin and allowed unobstructed observation of the surface. Addition of alcian blue to solutions of glutaraldehyde improved preservation of liquid film components.
8. Microorganisms on the surface of skin from broiler carcasses stored under humid conditions, grew within the liquid film, deep in skin channels and in feather follicle shafts. Under drying storage conditions, microorganisms grew as colonies, although bacteria were found growing within the viscous fluid film on the skin of these carcasses.
9. Bacteria growing on the skin of chill stored carcasses were not attached to the skin surface by acidic mucopolysaccharide polymers or any other bridging substances. Attachment processes were found to be associated with bacteria growing on carcasses stored at 15⁰ and 22⁰C.
10. The dermal tissue acted as an effective barrier against invasive growth of bacteria. No gross skin degradation was observed on spoiled carcasses, although digestion of proteinaceous material within the liquid film by extracellular enzymes, produced by bacteria, was noted before onset of spoilage.

11. *Pseudomonas* groups I and II were major types of bacteria responsible for production of off odours on the skin of broiler carcasses stored at 2°C. The major volatile produced was methanethiol. Pure cultures of isolates producing off odours when grown on leg muscle also produced these odours when grown on skin preparations.
12. A higher proportion of psychrotrophic bacteria isolated from the skin of broiler carcasses stored at 2°C produced methanethiol in quantities sufficient to cause a positive reaction to DTNB when grown in methionine supplemented peptone broth than when grown on leg muscle. Addition of glucose to the methionine medium inhibited production of methanethiol.

BIBLIOGRAPHY

- ABBOTT, M.T., PEARSON, A.M., PRICE, J.F. and HOOPER, G.R. (1977). Ultrastructural changes during autolysis of red and white porcine muscle. *J. Food Sci.* 42, 1185.
- ADAMCIC, M., and CLARK, D.S. (1970a). Collagenolytic activity of pigmented pseudomonads. *Can. J. Microbiol.* 16, 709.
- ADAMCIC, M., and CLARK, D.S. (1970b). Bacteria induced biochemical changes in chicken skin stored at 5°C. *J. Food Sci.* 35, 103.
- ADAMCIC, M., CLARK, D.S. and YAGUCHI, M. (1970). Effect of psychrotolerant bacteria on the amino acid content of chicken skin. *J. Food Sci.* 35, 272.
- ADAMS, R., FARBER, L., and LERKE, P. (1964). Bacteriology of spoilage of fish muscle. II. Incidence of spoilers during spoilage. *Appl. Microbiol.* 12, 277.
- ADLER, J. (1973). A method for measuring chemotaxis and the use of the method to determine optimum conditions for chemotaxis by *Escherichia coli*. *J. Gen. Microbiol.* 74, 77.
- AVENS, J.S. (1968). Enumeration of bacteria on poultry carcass skin. *Poult. Sci.* 47, 1653.
- AVENS, J.S., and MILLER, B.F. (1970a). Quantifying bacteria on poultry carcasses. *Poult. Sci.* 49, 1309.
- AVENS, J.S., and MILLER, B.F. (1970b). Optimum skin blending method for quantifying poultry carcass bacteria. *Appl. Microbiol.* 20, 129.
- AVENS, J.S. and MILLER, B.F. (1972). Pasteurization of turkey carcasses. *Poult. Sci.* 51, 1781.

- AYRES, J.C. (1960). The relationship of organisms of the genus *Pseudomonas* to the spoilage of meat, poultry and eggs. *J. Appl. Bacteriol.* 23, 471.
- AYRES, J.C., OGILVY, W.S., and STEWART, G.F. (1950). Post-mortem changes in stored meats. 1. Microorganisms associated with development of slime on eviscerated cut up poultry. *Food Technol., Champaign*, 4, 199.
- BACLE, J.F. and BIGLAND, C.H. (1962). Avian mycologic survey in Alberta. *Am. Vet. Med. Assoc. J.* 141, 476.
- BAIRD-PARKER, A.C. (1963). A classification of micrococci and staphylococci based on physiological and biochemical tests. *J. Gen. Microbiol.* 30, 409.
- BAIRD-PARKER, A.C. (1966). Methods for classifying staphylococci and micrococci. In Gibbs, B.M. and Skinner, F.A. eds.: *Identification Methods for Microbiologists. Part A.* Academic Press, London.
- BAILEY, J.S., REAGAN, J.O., CARPENTER, J.A., and SCHULER, G.A. (1979). Microbiological condition of broilers as influenced by vacuum and carbon dioxide in bulk shipping packs. *J. Food Sci.* 44, 134.
- BALDOCK, J.D. (1974). Microbiological monitoring of the food plant. Methods to assess bacterial contamination on surfaces. *J. Milk Food Technol.* 37, 361.
- BARAN, W.L., KRAFT, A.A., and WALKER, A.W. (1970). The effects of carbon dioxide and vacuum packaging on colour and bacterial count of meat. *J. Milk Food Technol.* 33, 77.
- BARNES, E.M. (1960). Bacteriological problems in broiler preparation and storage. *Roy. Soc. Health J.* 80, 145.

- BARNES, E.M. (1965). The effect of chlorinating chill tanks on the bacteriological condition of processed chickens. Supplement au Bulletin d'Institut International du Froid, Commission 4, Karlsruhe Annexe 1965 - 1, 219.
- Cited in Mead, G.C., Adams, B.W. and Parry, R.T. (1975). The effectiveness of in plant chlorination in poultry processing. Br. Poult. Sci. 16, 517.
- BARNES, E.M. (1975). The microbiological problems of sampling a poultry carcass. Proc. 2nd European Symp. Poult. Meat, Oosterbeek, Netherlands.
- BARNES, E.M. (1976). Microbiological problems of poultry at refrigerator temperatures - a review. J. Sci. Fd. Agric. 27, 777.
- BARNES, E.M. and IMPEY, C.S. (1968). Psychrophilic spoilage bacteria of poultry. J. Appl. Bacteriol. 31, 97.
- BARNES, E.M. and IMPEY, C.S. (1970). The isolation and properties of the predominant anaerobic bacteria in the caeca of chickens and turkeys. Br. Poult. Sci. 11, 467.
- BARNES, E.M., IMPEY, C.S., and PARRY, R.T. (1973). The sampling of chickens, turkeys, ducks and game birds. In Board, R.G. and Lovelock, D.W. eds.: Sampling - microbiological monitoring of environments. Academic Press, London.
- BARNES, E.M., IMPEY, C.S., GEESON, J.D., and BUHAGIAR, R.W.M. (1978). The effect of storage temperature on the shelf life of eviscerated air chilled turkeys. Br. Poult. Sci. 19, 77.

- BARNES, E.M., MEAD, G.C., BARNUM, D.A., and HARRY, G.C. (1972).
The intestinal flora of the chicken in the period 2 to 6 weeks of age with particular reference to the anaerobic bacteria. *Br. Poult. Sci.* 13, 311.
- BARNES, E.M., and MELTON, W. (1971). Extracellular enzymic activity of poultry spoilage bacteria. *J. Appl. Bacteriol.* 34, 599.
- BARNES, E.M., and SHRIMPTON, D.H. (1958). The effect of tetracycline compounds on the storage life and microbiology of chilled eviscerated poultry. *J. Appl. Bacteriol.* 21, 313.
- BARNES, E.M., and THORNLEY, M.J. (1966). The spoilage flora of eviscerated chickens stored at different temperatures. *J. Food Technol.* 1, 113.
- BARRITT, M.M. (1936). The intensification of the Vogues-Proskauer reaction by the addition of α -naphthol. *J. Path. Bacteriol.* 42, 441.
- BEHNKE, O. (1968). Electron microscopical observations on the surface coating of human blood platelets. *J. Ultrastruct. Res.* 24, 51.
- BERNER, H. and SCHOLTYSEK, S. (1978). Möglichkeiten zur Verbesserung der Schlachthygiene beim Geflügel. *Fleischwirtschaft* 48, 422.
- BLANKENSHIP, L.C. (1977). Attachment characteristics of *Clostridium perfringens* spores to broiler carcass skin. *Abstr. Ann. Meet. Amer. Soc. Microbiol.* 77, 245.
- BLOOD, R.M., and JARVIS, B. (1974). Chilling of poultry: The effects of process parameters on the level of bacteria in spinchiller water. *J. Food Technol.* 9, 157.

- BORTON, R.J., BRATZLER, L.J., and PRICE, J.F. (1970a). Effects of four species of bacteria on porcine muscle. I. Protein solubility and emulsifying capacity. J. Food Sci. 35, 779.
- BORTON, R.J., BRATZLER, L.J., and PRICE, J.F. (1970b). Effects of four species of bacteria on porcine muscle. 2. Electrophoretic patterns of extracts of salt soluble protein. J. Food Sci. 35, 783.
- BROOKER, B.E. (1976). Cytochemical observations on the extracellular carbohydrate produced by *Streptococcus cremoris*. J. Dairy Res. 43, 283.
- BROOKER, B.E., and FULLER, R. (1975). Adhesion of lactobacilli to the chicken crop epithelium. J. Ultrastruct. Res. 52, 21.
- BRYAN, F.L., AYRES, J.C., and KRAFT, A.A. (1978a). Contributory sources of salmonellae on turkey products. Am. J. Epidemiol. 87, 578.
- BUCHLI, K., VAN SCHOTHORST, M., and KAMPELMACHER, E.H. (1966). Untersuchungen uber die hygienische Beschaffenheit von mit Wasser resp. Luft gekuhltem Schlachtflugel. Archiv. Lebensmittelhyg. 17, 97.
- BUTLER, J.L., STEWART, J.C., VANDERZANT, C., CARPENTER, Z.L., and SMITH, G.C. (1979). Attachment of microorganisms to pork skin and surfaces of beef and lamb carcasses. J. Food Prot. 42, 401.
- CANTY, A.J., KISHIMOTO, R., DEACON, G.B., and FARQUHARSON, G.J. (1976). Metal-sulphur stretching frequencies and structures of complexes of zinc, cadmium, and mercury with thiols. Inorg. Chim. Acta. 20, 161.

- CASTELL, C.H., and ANDERSON, G.W. (1948). Bacteria associated with the spoilage of cod fillets. J. Fish.Res. Bd. Can. 7, 378.
- CASTELL, C.H., and GREENOUGH, M.F. (1957). The action of *Pseudomonas* on fish muscle. 1. Organisms responsible for odours produced during incipient spoilage of chilled fish muscle. J. Fish. Res. Bd. Can. 14, 617.
- CASTELL, C.H., GREENOUGH, M.F., and DALE, J. (1959). The action of *Pseudomonas* on fish muscle. 3. Identification of the organisms producing fruity and oniony odours. J. Fish.Res. Bd. Can. 16, 13.
- CASTELL, C.H., and GREENOUGH, M.F. (1959). The action of *Pseudomonas* on fish muscle. 4. Relation between substrate composition and the development of odours by *Pseudomonas fragi*. J. Fish. Res. Bd. Can. 16, 21.
- CASTELL, C.H., and MAPPLEBECK, E.G. (1952). The importance of *Flavobacterium* in fish spoilage. J. Fish. Res. Bd. Can. 9, 148.
- CASTELL, C.H., RICHARDS, J.F., and WILMOT, I. (1949). *Pseudomonas putrefaciens* from cod fillets. J. Fish. Res. Bd. Can. 7, 430.
- CHAI, T., CHEN, C., ROSEN, A., and LEVIN, R.E. (1968). Detection and incidence of specific species of spoilage bacteria on fish. II. Relative incidence of *Pseudomonas putrefaciens* and fluorescent pseudomonads on haddock fillets. Appl. Microbiol. 16, 1738.
- CHATAGNER, F., and SAURET-IGNAZI, G. (1956). Cited in Herbert, R.A. and Shewan, J.M. (1975).
- CHATTERJEE, S.N., and DAS, J. (1967). Electron-microscopic observations on the excretion of cell-wall material by *Vibrio cholerae*. J. Gen. Microbiol. 49, 1.

- CHUNG, J.R., and KIM, I.S. (1976). Post mortem degradation of fish muscle proteins. 1. Nature of proteolysis and bacterial contribution. Bull. Korean Fish. Soc. 9, 120.
- CHUTE, H.L., O'MEARA, D.C., TRESNER, H.D., and LACOMBE, E. (1956). The fungus flora of chickens with infections of the respiratory tract. Amer. J. Vet. Res. 17, 763.
- CHUTE, H.L., O'MEARA, D.G., and BARDEN, E.S. (1962). A bibliography of avian mycosis. Misc. Publ. Maine Agric. Expt. Sta. No. 631.
- CLARK, D.S. (1965a). Method of estimating the bacterial population on surfaces. Can. J. Microbiol. 11, 407.
- CLARK, D.S. (1965b). Improvement of spray gun method of estimating bacterial populations on surfaces. Can. J. Microbiol. 11, 1021.
- CLARK, D.S. (1968). Growth of psychrotolerant pseudomonads and *Achromobacter* on chicken skin. Poult. Sci. 47, 1575.
- CLARK, D.S. (1970). Growth of psychrotolerant pseudomonads and achromobacteria on various chicken tissues. Poult. Sci. 49, 1315.
- CLARK, D.S., and BURKI, T. (1972). Oxygen requirements of strains of *Pseudomonas* and *Achromobacter*. Can. J. Microbiol. 18, 321.
- CLARK, D.S., and LENTZ, C.P. (1969a). Microbiological studies in poultry processing plants in Canada. Can. Inst. Food Technol. 2, 33.
- CLARK, D.S., and LENTZ, C.P. (1969b). The effects of carbon dioxide on the growth of slime producing bacteria on fresh beef. Can. Inst. Food Technol. 2, 72.
- CLARKE, J.T. (1964). Cited in Ivor Smith (ed.) (1969). Chromatographic and electrophoretic techniques. Volume II, p. 383. William Heinemann, U.K.
- CLARKE, P.H. (1953). Hydrogen sulphide production by bacteria. J. Gen. Microbiol. 8, 397.

- COCHRAN, W.G. and COX, G.M. (1957). Experimental Designs (2nd edn.)
Wiley International, New York.
- COOPER, J.E., and NEEDHAM, J.R. (1976). An investigation into
the prevalence of *S. aureus* on avian feet. Vet. Rec. 98, 172.
- COREY, R.R., and BYRNES, J.M. (1963). Oxytetracycline resistant
coliforms in commercial poultry products. Appl. Microbiol.
11, 481.
- COSTERTON, J.G., GEESEY, C.G., and CHENG, K.J. (1978). How
bacteria stick. Sci. Am. 238, 86.
- COX, N.A., and BLANKENSHIP, L.C. (1975). Comparison of rinse
sampling methods for the detection of *Salmonella* on eviscerated
carcasses. J. Food Sci. 40, 1333.
- COX, N.A., MERCURI, J., JUVEN, J., and THOMSON, J.E. (1975).
Enterobacteriaceae at various stages of poultry chilling.
J. Food Sci. 40, 44.
- COX, N.A., MERCURI, A.J., JUVEN, B.J., THOMSON, J.E., and CHEW, V.
(1973). The effects of succinic acid and heat on the
microbiological quality of poultry meat. Poult. Sci. 52, 2015.
- COX, N.A., MERCURI, A.J., JUVEN, B.H., THOMSON, J.E., and CHEW, V.
(1974). Evaluation of succinic acid and heat to improve
the microbiological quality of poultry meat. J. Food Sci.
39, 985.
- COX, N.A., MERCURI, A.J., THOMSON, J.E., and CHEW, V. (1976).
Swab and excised tissue sampling for total and *Enterobacteriaceae*
counts of fresh and surface frozen broiler skin. Poult. Sci.
55, 2405.
- COX, N.A., MERCURI, A.J., THOMSON, J.E., and GREGORY, D.W. (1974).
Quality of broiler carcasses as affected by hot water
treatments. Poult. Sci. 53, 1566.

- COYNE, F.P. (1932). The effect of carbon dioxide on bacterial growth with special reference to the preservation of fish. J. Soc. Chem. Ind. 50, 1197.
- DAINTY, R.H., SHAW, B.G., de BOER, K.A., and SCHEPS, E.S.J. (1975). Protein changes caused by bacterial growth on beef. J. Appl. Bacteriol. 39, 73.
- DAUD, H.B. (1978). Poultry spoilage, associated bacteria and storage temperature. Honours Thesis, University of Tasmania, Hobart, Tasmania.
- DAUD, H.B., McMEEKIN, T.A., and THOMAS, C.J. (1979). Spoilage association of chicken skin. Appl. Environ. Microbiol. 37, 399.
- DAVIES, W.L. and GILL, E. (1936). Investigations on fishy flavours. Chem. Ind. (London). 55, 1415.
- DEVRIESE, L.A., DEVOS, A.H., and van DAMME, L.R. (1975). Quantitative aspects of the *Staphylococcus aureus* flora of poultry. Poult. Sci. 54, 95.
- DOBSON, D.C. (1966). Influence of various management conditions on the incidence of breast blisters and staphylococcal synovitis in large type male turkeys grown both in confinement and on range. Poult. Sci. 45, 1080.
- DOUGHERTY, T.J. (1974). *Salmonella* contamination in a commercial poultry (broiler) processing operation. Poult. Sci. 53, 814.
- DUGUID, J.P. (1959). Fimbriae and adhesive properties in *Klebsiella* strains. J. Gen. Microbiol. 21, 271.
- DUGUID, J.P., DAREKAR, M.R., and WHEATER, D.W.F. (1976). Fimbriae and infectivity in *Salmonella typhimurium*. J. Med. Microbiol. 9, 459.

- DUTSON, T.R., PEARSON, A.M., PRICE, J.F., SPINK, G.C., and TARRANT, P.J.V. (1971). Observations by electron microscopy on pig muscle inoculated and incubated with *Pseudomonas fragi*. Appl. Microbiol. 22, 1152.
- DYE, M., and MEAD, G.C. (1972). The effect of chlorine on the viability of clostridial spores. J. Food Technol. 7, 173.
- ELLIOTT, R.P. (1969). U.S.D.A. worker talks about *salmonella* at processing plants. Mon. Bull. Min. Hlth. 20, 30.
- ELLIOTT, R.P., STRAKA, R.P., and GARABALDI, J.A. (1964). Polyphosphate inhibition of growth of pseudomonads from poultry meat. Appl. Microbiol. 12, 517.
- ELLMAN, G.L. (1959). Tissue sulphydryl groups. Arch. Biochem. Biophys. 82, 70.
- EMSWILER, B.S., PIERSON, C.J., and KOTULA, A.W. (1977). Stomaching vs. blending: a comparison of two techniques for the homogenisation of meat samples for microbiological analysis. Food Technol. 31, 40.
- ESSARY, E.O., MOORE, W.E.C., and KRAMER, C.Y. (1958). Influence of scald temperatures, chill times and holding temperatures on the bacterial flora and shelf life of freshly chilled tray-pack poultry. Food Technol. 12, 684.
- FANELLI, M.J., SADLER, W.W., and BROWNELL, J.R. (1970). Preliminary studies on persistence of salmonellae in poultry litter. Avian Dis. 14, 131.
- FAVERO, M.S., McDADE, J.J., ROBERTSON, J.A., HOFFMAN, R.K., and EDWARDS, R.W. (1968). Microbiological sampling of surfaces. J. Appl. Bacteriol. 31, 336.

- FIRSTENBERG-EDEN, R., NOTERMANS, S., THIEL, F., HENSTRA, S., and KAMPELMACHER, E.H. (1979). Scanning electron microscopic investigations into attachment of bacteria to teats of cows. *J. Food Prot.* 42, 305.
- FIRSTENBERG-EDEN, R., NOTERMANS, S., and VAN SCHOTHORST, M. (1978). Attachment of certain bacterial strains to chicken and beef meat. *J. Food Safety* 1, 217.
- FISHWICK, M.J., and LEA, C.H. (1969). Chemical and organoleptic changes in poultry meat resulting from the growth of psychrophilic spoilage bacteria at 1°C. 4. Changes in muscle lipid. *Br. Poult. Sci.* 10, 239.
- FLETCHER, M., and FLOODGATE, G.D. (1973). An electron-microscopic demonstration of an acidic polysaccharide involved in the adhesion of a marine bacterium to solid surfaces. *J. Gen. Microbiol.* 74, 325.
- FOSTER, R.D., and MEAD, G.C. (1976). Effect of temperature and added polyphosphate on the survival of salmonellae in poultry meat during cold storage. *J. Appl. Bacteriol.* 41, 505.
- FREEMAN, L.R., SILVERMAN, G.J., ANGELINI, P., MERRITT, Jr., C. and ESSELEN, W.B. (1976). Volatiles produced by microorganisms isolated from refrigerated chicken at spoilage. *Appl. Environ. Microbiol.* 32, 222.
- FROMM, D. (1959). An evaluation of techniques commonly used to quantitatively determine the bacterial population of chicken carcasses. *Poult. Sci.* 38, 887.
- FROMM, D., and MONROE, R.J. (1965). Some observations concerning the hydrogen ion variability of the chicken carcass surface during storage. *Poult. Sci.* 44, 325.

- FUERST, J.A., and HAYWARD, A.C. (1969). Surface appendages similar to fimbriae (pili) on *Pseudomonas* species. J. Gen. Microbiol. 58, 227.
- FULLER, R. (1973). Ecological studies on the lactobacillus flora associated with the crop epithelium of the fowl. J. Appl. Bacteriol. 36, 131.
- FULLER, R. (1975). Nature of the determinant responsible for the adhesion of lactobacilli to chicken crop epithelial cells. J. Gen. Microbiol. 87, 245.
- FULLER, R., and TURVEY, A. (1971). Bacteria associated with the intestinal wall of the fowl (*Gallus domesticus*). J. Appl. Bacteriol. 34, 617.
- FULLER, R., BARROW, P.A., and BROOKER, B.E. (1978). Bacteria associated with the gastric epithelium of neonatal pigs. Appl. Environ. Microbiol. 35, 582.
- GALTON, M.M., MACKEL, D.C., LEWIS, A.L., HAIRE, W.C., and HARDY, A.V. (1955). Salmonellosis in poultry processing plants in Florida. J. Vet. Res. 16, 132.
- GARDNER, G.A., and CARSON, A.W. (1967). Relationship between carbon dioxide production and growth of pure strains of bacteria on porcine muscle. J. Appl. Bacteriol. 30, 500.
- GARDNER, F.A., DENTON, J.H., and HATLEY, S.E. (1977). Effects of carbon dioxide environments on the shelf life of broiler carcasses. Poult. Sci. 56, 1715.
- GARG, D.N. and SETHI, M.S. (1969). Fungal flora of the avian respiratory tract. Poult. Sci. 48, 339.
- GERATS, G.E., and SNIJDERS, J.M. (1977). Assessment of bacterial counts in the meat processing industry. I. A review of the literature. Tijdschr. Diergeneesk. D. 102, 377.

- GHOSH, B.K., SARGENT, M., and LAMPDEN, J.O. (1968). Morphological phenomena associated with penicillinase induction and secretion in *Bacillus licheniformis*. *J. Bacteriol.* 96, 1314.
- GIBBS, P.A., PATTERSON, J.T., and THOMPSON, J.K. (1978). The distribution of *Staphylococcus aureus* in a poultry plant. *J. Appl. Bacteriol.* 44, 401.
- GIBBONS, R.J., and VAN HOUTE, J. (1975). Bacterial adherence in oral microbiol ecology. *Ann. Rev. Microbiol.* 29, 19.
- GILL, C.O. (1976). Substrate limitation of bacterial growth at meat surfaces. *J. Appl. Bacteriol.* 41, 401.
- GILL, C.O., and NEWTON, K.G. (1977). The development of aerobic spoilage flora on meat stored at chill temperatures. *J. Appl. Bacteriol.* 43, 189.
- GILL, C.O., and NEWTON, K.G. (1979). Spoilage of vacuum-packaged dark, firm, dry meat at chill temperatures. *Appl. Environ. Microbiol.* 37, 362.
- GILL, C.O., and PENNEY, N. (1977). Penetration of bacteria into meat. *Appl. Environ. Microbiol.* 33, 1284.
- GILLESPIE, N.C. (1977). A bacteriological and chemical examination of fish spoilage in Australia. pp. 301-317. *In* Reported Proceedings of the Australian Fish Exposition, Melbourne, 1977. Seminar session no. 4, Australian Government Publishing Service, Canberra.
- GLAUERT, A.M. (1965). Section staining, cytology, autoradiography and immunochemistry for biological specimens. *In* Kay, D.H. (ed.) *Techniques for Electron Microscopy*, p. 254. Blackwell Scientific Publications, Oxford.

- GLAUERT, A.M., and GLAUERT, R.H. (1958). Araldite as an embedding medium for electron microscopy. *J. Biophys. Biochem. Cytol.* 4, 191.
- GLICK, B., HOLBROOK, K.A., OLAH, I., PERKINS, W.D., and STINSON, R. (1978). A scanning electron microscopy study of the caecal tonsil: The identification of a bacterial attachment to the villi of the caecal tonsil and possible presence of symphatics in the caecal tonsil. *Poult. Sci.* 57, 1408.
- GORESLINE, H.E., and HAUGH, R.R. (1959). Approximation of surface areas of cut up chicken and use in microbiological analysis. *Food Technol.* 13, 241.
- GOTZE, U. (1976). Die Ursache der Staphylokokken kontamination bei der Gewinnung von Bratthanchin. *Fleischwirtschaft* 56, 707.
- GRIFFITHS, N.M., and LEA, C.H. (1969). Chemical and organoleptic changes in poultry meat resulting from the growth of psychrophilic spoilage bacteria at 1°C. 5. Effects on palatability. *Br. Poult. Sci.* 10, 243.
- GUADAGNI, D.G., BUTTERY, R.G., and OKANO, S. (1963). Odour thresholds of some organic compounds associated with food flavours. *J. Sci. Fd. Agric.* 14, 761.
- GUCKERT, A., BREISCH, H., and REISINGER, O. (1975). Interface sol-racine. I. Etude au microscope electronique des relations mucigel-argile-microorganismes. *Soil Biol. Biochem.* 7, 241.
- HAGBERG, M.M., BUSTA, F.F., ZOTTOLA, E.A., and ARNOLD, E.E. (1973). Incidence of potentially pathogenic microorganisms in further processes turkey products. *J. Milk Fd. Technol.* 36, 625.

- HAGGIS, G.H., and PHIPPS-TODD, B. (1977). Freeze fracture for scanning electron microscopy. *J. Microsc.* 111, 193.
- HAINES, R. B. (1931). The growth of microorganisms on chilled and frozen meat. *J. Soc. Chem. Ind.* 50, 223 T.
- HAINES, R.B. (1933a). The influence of carbon dioxide on the rate of multiplication of certain bacteria as judged by viable counts. *J. Soc. Chem. Ind.* 52, 13.
- HAINES, R.B. (1933b). The influence of carbon dioxide on the rate of multiplication of certain bacteria. *J. Soc. Chem. Ind.* 52, 325.
- HAMDY, M.K., BARTON, N.D., and BROWN, W.E. (1964). Source and portal of entry of bacteria found in bruised poultry tissue. *Appl. Microbiol.* 12, 464.
- HARPER, M., TURVEY, A., and BRAMLEY, A.J. (1978). Adhesion of fimbriate *Escherichia coli* to bovine mammary gland epithelial cells *in vitro*. *J. Med. Microbiol.* 11, 117.
- HARRY, E.G. (1967). Some characteristics of *Staphylococcus aureus* isolated from the skin and upper respiratory tract of domestic and wild (feral) birds. *Res. Vet. Sci.* 8, 490.
- HASEGAWA, T., PEARSON, A.M., PRICE, J.F., and LECHOWICH, R.V. (1970a). Action of bacterial growth on the sarcoplasmic and urea soluble proteins from muscle. 1. Effects of *Clostridium perfringens*, *Salmonella enteritidis*, *Achromobacter liquefaciens*, *Streptococcus faecalis* and *Kurthia zopfii*. *Appl. Microbiol.* 20, 117.
- HASEGAWA, T., PEARSON, A.M., PRICE, J.F., RAMPTON, J.H., and LECHOWICH, R.V. (1970b). Effect of microbial growth upon sarcoplasmic and urea soluble proteins from muscle. *J. Food Sci.* 35, 720.

- HAYAT, M.A. (1970). Principles and techniques of electron microscopy. Biological applications. Volume 1. Van Nostrand Reinhold Co., New York.
- HENDRIKS, C.W. (1974). Sorption of heterotrophic and enteric bacteria to glass surfaces in the continuous culture of river water. *Appl. Microbiol.* 28, 572.
- HERBERT, R.A. (1970). A study of the roles played by bacterial and autolytic enzymes in the production of volatile sulphur compounds in spoiling North Sea cod. Ph.D. Thesis, University of Aberdeen.
- HERBERT, R.A., HENDRIE, M.S., GIBSON, D.M., and SHEWAN, J.M. (1971). Bacteria active in the spoilage of certain seafoods. *J. Appl. Bacteriol.* 34, 41.
- HERBERT, R.A., and SHEWAN, J.M. (1975). Precursors of the volatile sulphides in spoiling North Sea cod (*Gadus morhua*). *J. Sci. Fd. Agric.* 26, 1195.
- HERBERT, R.A., and SHEWAN, J.M. (1976). Roles played by bacterial and autolytic enzymes in the production of volatile sulphides in spoiling North Sea cod (*Gadus morhua*). *J. Sci. Fd. Agric.* 27, 89.
- HERBERT, R.A., ELLIS, R.J., and SHEWAN, J.M. (1975). Isolation and identification of the volatile sulphides produced during chill storage of North Sea cod (*Gadus morhua*). *J. Sci. Fd. Agric.* 26, 1187.
- HUGH, R., and LEIFSON, E. (1953). The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. *J. Bacteriol.* 66, 24.

- HUHTANEN, C.N., and PENSACK, J.M. (1965). The development of the intestinal flora of the young chick. *Poult. Sci.* 44, 825.
- IDZIAK, E.S., and INCZE, K. (1968). Radiation treatments of food. I. Radurization of fresh eviscerated poultry. *Appl. Microbiol.* 16, 1061.
- INGRAM, M. (1951). The effect of cold on microorganisms in relation to food. *Proc. Soc. Appl. Bacteriol.* 14, 243.
- INGRAM, M. (1971). Microbial changes in food - general considerations. *J. Appl. Bact.* 34, 1.
- INGRAM, M., and DAINITY, R.H. (1971). Changes caused by microbes in spoilage of meats. *J. Appl. Bacteriol.* 34, 21.
- INGRAM, M., and THORNLEY, M.J. (1959). Changes in the spoilage pattern of chicken meat as a result of irradiation. *Int. J. Appl. Radiat. Isot.* 6, 122.
- ISLAM, M.N., GRAY, R.J.H., and GEISER, J.N. (1978). Development of antimicrobial agents for the extension of poultry shelf life. *Poult. Sci.* 57, 1266.
- ISLAM, M.N., and ISLAM, N.B. (1979). Extension of poultry shelf life by poly(Hexamethylenebiguanide hydrochloride). *J. Food. Prot.* 42, 416.
- JACOBY, G.A. (1964). The induction and repression of amino acid oxidation in *Pseudomonas fluorescens*. *Biochem. J.* 92, 1.
- JAY, J.M. (1967). Nature, characteristics and proteolytic properties of beef spoilage at low and high temperatures. *Appl. Microbiol.* 15, 943.
- JAY, J.M. (1972). Mechanism and detection of microbial spoilage in meats at low temperature : a status report. *J. Milk Food Technol.* 35, 467.

- JAY, J.M., and KONTOU, K.S. (1967). Fate of free amino acids and nucleotides in spoiling beef. *Appl. Microbiol.* 15, 759.
- JOCELYN, P.C. (1972). *Biochemistry of the -SH group*. Academic Press, London.
- JONES, H.C., ROTH, I.L., and SANDERS, W.M. (1969). Electron microscopic study of a slime layer. *J. Bacteriol.* 99, 316.
- JORDAN, F.T.W. (1954). Incidence of fungi in the lungs of poultry. *Brit. Vet. J.* 110, 25.
- JUVEN, B.J., COX, N.A., MERCURI, A.J., and THOMPSON, J.E. (1974). A hot acid treatment for eliminating salmonellae from chicken meat. *J. Milk Food Technol.* 37, 327.
- KADOTA, H., and ISHIDA, Y. (1972). Production of volatile sulfur compounds by microorganisms. *Ann. Rev. Microbiol.* 26, 127.
- KALAB, M. (1977). Milk gel structure. VI. Cheese texture and microstructure. *Milchwissenschaft* 32, 449.
- KATZ, S.M., and NASH, P. (1978). Legionnaires' disease: structural characteristics of the organism. *Science* 199, 896.
- KEEL, J.E., and PARMELEE, C.E. (1968). Improving the bacteriological quality of chicken fryers. *J. Milk Food Technol.* 31, 377.
- KIMBER, A.M., BROOKER, B.E., HOBBS, D.G., and PRENTICE, J.H. (1974). Electron microscope studies of the development of structure in Cheddar cheese. *J. Dairy Res.* 41, 389.
- KING, A.D., and NAGEL, C.W. (1967). Growth inhibition of a *Pseudomonas* by carbon dioxide. *J. Food Sci.* 32, 575.
- KING, E.O., WARD, M.K., and RANEY, D.E. (1954). Two simple media for the demonstration of pyocyanin and fluorescin. *J. Lab. Clin. Med.* 44, 301.
- KINSLEY, R.H., and MOUNTNEY, G.J. (1966). A comparison of methods used for the examination of poultry carcasses. *Poult. Sci.* 45, 1211.

KITCHELL, A.G., INGRAM, G.C., and HUDSON, W.R. (1973).

Microbiological sampling in abattoirs. In Board, R.G. and Lovelock, D.W. (eds.). Sampling - Microbiological Monitoring of Environments. Academic Press, London.

KLOSE, A.A., KAUFMAN, V.F., BAYNE, H.G., and POOLE, M.F. (1971).

Pasteurization of poultry meat by steam under reduced pressure. Poult. Sci. 50, 1156.

KNOOP, G.B., PARMELEE, C.E., and STADELMAN, W.J. (1971).

Microbiological characteristics of wet and dry chilled poultry. Poult. Sci. 50, 530.

KNOX, K.M., VESK, M., and WORK, E. (1966). Relation between

excreted lipopolysaccharide complexes and surface structures of lysine limited culture of *Escherichia coli*. J. Bacteriol. 92, 1206.

KOTULA, A.W. (1966). Variability in the microbiological samplings

of chickens by the swab method. Poult. Sci. 45, 233.

KOTULA, A.W., BANWART, G.J., and KINNER, J.A. (1967). Effect of

post-chill washing on the bacterial counts of broiler chickens. Poult. Sci. 46, 1210.

KOTULA, A.W., THOMSON, J.E., and KINNER, J.A. (1962). Bacterial

counts associated with chilling of fryer chickens. Poult. Sci. 41, 818.

KOVACS, N. (1956). Identification of *Pseudomonas pyocyanea* by

the oxidase reaction. Nature 178, 703.

KRAFT, A.A., and AYRES, J.C. (1961). Production of fluorescence

on packaged chicken. Appl. Microbiol. 9, 549.

KUSCH, D., and GOTZE, U. (1974). Untersuchungen an Staphylokokken

aus dem Auftauwasser von Gefriergeflügel. Fleischwirtschaft 12, 1960.

- LAAKSO, S. (1976). The relationship between methionine uptake and demethiolation in a methionine-utilizing mutant of *Pseudomonas fluorescens* UK 1. J. Gen. Microbiol. 95, 391.
- LAHELLEC, C. (1975). Influence of some processing factors on the hygienic quality of poultry meat. Paper 28 in The Quality of Poultry Meat. Proceedings of 2nd European Symposium on Poultry Meat Quality, Oosterbeek, Netherlands.
- LAHELLEC, C., MEURIER, C., and CATSARAS, M. (1972). The psychrotrophic flora of poultry carcasses. I. Development at various stages through a slaughter house. Ann. Rech. Vet. 3, 421.
- LAHELLEC, C., MEURIER, G.B., and CATSARAS, M. (1975). A study of 5290 strains of psychrotrophic bacteria isolated from chickens. J. Appl. Bacteriol. 38, 89.
- LANCY, P., and MURRAY, R.G.E. (1978). The envelope of *Micrococcus radiodurans*: isolation, purification and preliminary analysis of the wall layers. Can. J. Microbiol. 24, 162.
- LAPIN, R.M., and KOBURGER, J.A. (1974). Hydrogen sulphide production by *Pseudomonas putrefaciens* in shrimps experimentally packed in nitrogen. Appl. Microbiol. 27, 666.
- LATHAM, M.J., BROOKER, B.E., PETTIPHER, G.L., and HARRIS, P.J. (1978). *Ruminococcus flavefaciens* cell coat and adhesion to cotton cellulose and to cell walls in leaves of perennial ryegrass (*Lolium perenne*). Appl. Environ. Microbiol. 35, 156.
- LAW, B.M., and SHARPE, M.E. (1978). Formation of methanethiol by bacteria isolated from raw milk and Cheddar cheese. J. Dairy Res. 45, 267.

- LEA, C.H., PARR, L.J., and JACKSON, H.F. (1969a). Chemical and organoleptic changes in poultry meat resulting from the growth of psychrophilic spoilage bacteria at 1°C.
3. Glutamine, glutathione, tyrosine, ammonia, lactic acid, creatine, carbohydrate, haem-pigment and hydrogen sulphide. Br. Poult. Sci. 10, 229.
- LEA, C.H., STEVENS, B.J.H., and SMITH, M.J. (1969b). Chemical and organoleptic changes in poultry meat resulting from the growth of psychrophilic spoilage bacteria at 1°C. 1. Introduction and changes in free amino acids. Br. Poult. Sci. 10, 203.
- LEE, M.L., SMITH, D.L., and FREEMAN, L.R. (1979). High resolution gas chromatographic profiles of volatile organic compounds produced by microorganisms at refrigerated temperatures. Appl. Environ. Microbiol. 37, 85.
- LEISTNER, L. and SZENTKUTI, L. (1970). Zwei Methoden zur bakteriologischen Untersuchung von Schlachtflügel. Fleischwirtschaft 50, 81.
- LERKE, P., ADAMS, R., and FARBER, L. (1965). Bacteriology of spoilage of fish muscle. III. Characterization of spoilers. Appl. Microbiol. 13, 625.
- LEVIN, R.E. (1968). Detection and incidence of specific species of spoilage bacteria on fish. I. Methodology. Appl. Microbiol. 16, 1734.
- LILLARD, H.S. (1971). Occurrence of *Clostridium perfringens* in broiler processing and further processing operations. J. Food Sci. 36, 1008.
- LILLARD, H.S. (1973). Contamination of blood system and edible parts of poultry with *Clostridium perfringens* during water scalding. J. Food Sci. 38, 151.

- LILLARD, H.S. (1978a). Improving quality of bird chiller water for recycling by diatomaceous earth filtration and chlorination. *J. Food Sci.* 43, 1528.
- LILLARD, H.S. (1978b). Evaluation of broiler necks flumed with diatomaceous earth filtered chiller water. *J. Food Sci.* 43, 1532.
- LILLARD, H.S., KLOSE, A.A., HEGGE, R.I., and CHEW, Y. (1973). Microbiological comparison of steam (at sub-atmospheric pressure) and immersion scalded broilers. *J. Food Sci.* 38, 903.
- LOCKHEAD, A.G., and LANDERKIN, G.B. (1935). Bacteriological studies of dressed poultry. I. Preliminary investigations of bacterial action at chill temperatures. *Sci. Agric.* 15, 765.
- LOVETT, J., MESSER, J.W., and READ, R.B. (1971). Microflora of Southern Ohio poultry litter. *Poult. Sci.* 50, 746.
- LUCAS, A.M., and STETTENHEIM, P.R. (1972). Avian Anatomy. Integument. Agriculture Handbook 362, U.S. Dept. Agric., Washington, D.C.
- LUFT, J.H. (1971). Ruthenium red and ruthenium violet. I. Chemistry, purification, methods of use for electron microscopy, and mechanism of action. *Anat. Rec.* 171, 347.
- MCCOWAN, R.P., CHENG, K.J., BAILEY, C.B.M., and COSTERTON, J.W. (1978). Adhesion of bacteria to the epithelial cell surfaces within the reticulo-rumen of cattle. *Appl. Environ. Microbiol.* 35, 149.
- McMEEKIN, T.A. (1975). Spoilage association of chicken breast muscle. *Appl. Microbiol.* 29, 44.

- McMEEKIN, T.A. (1976). Potential use of dipslides for microbiological quality control in the food industry. *Food Technol. Aust.* 28, 129.
- McMEEKIN, T.A. (1977). Spoilage association of chicken leg muscle. *Appl. Environ. Microbiol.* 33, 1244.
- McMEEKIN, T.A., GIBBS, P.A., and PATTERSON, J.T. (1978). Detection of volatile sulphide-producing bacteria isolated from poultry processing plants. *Appl. Environ. Microbiol.* 35, 1216.
- McMEEKIN, T.A., and PATTERSON, J.T. (1975). Characterization of hydrogen sulphide producing bacteria isolated from meat and poultry plants. *Appl. Microbiol.* 29, 163.
- McMEEKIN, T.A., and THOMAS, C.J. (1978). Retention of bacteria on chicken skin after immersion in bacterial suspensions. *J. Appl. Bacteriol.* 45, 383.
- MACKEY, B.M., and DERRICK, C.M. (1979). Contamination of the deep tissues of carcasses by bacteria present on the slaughter instruments or in the gut. *J. Appl. Bacteriol.* 46, 355.
- MALLMAN, W.L., DAWSON, L.E., SULTZER, B.M., and WRIGHT, H.S. (1958). Studies on microbiological methods for predicting shelf life of dressed poultry. *Food Technol.* 12, 122.
- MANNING, D.J. (1974). Sulphur compounds in relation to Cheddar cheese flavour. *J. Dairy Res.* 41, 81.
- MARSHALL, K.C. (1975). Clay mineralogy in relation to survival of soil bacteria. *Ann. Rev. Phytopathol.* 13, 357.
- MARSHALL, K.C., CRUICKSHANK, R.H., and BUSHBY, H.V.A. (1975). The orientation of certain root-nodule bacteria at interfaces, including legume root hair surfaces. *J. Gen. Microbiol.* 91, 198.

- MARSHALL, K.C., STOUT, R., and MITCHELL, R. (1971a). Mechanism of the initial events in the sorption of marine bacteria to surfaces. *J. Gen. Microbiol.* 68, 337.
- MARSHALL, K.C., STOUT, R., and MITCHELL, R. (1971b). Selective sorption of bacteria from seawater. *Can. J. Microbiol.* 17, 1413.
- MAST, M.G., and MacNEIL, J.H. (1978). Use of glutaraldehyde as a disinfectant in immersion chilling of poultry. *Poult. Sci.* 57, 681.
- MATOLTSY, A.G. (1969). Keratinization of the avian epidermis: An ultrastructural study of the newborn chick skin. *J. Ultrastruct. Res.* 29, 438.
- MATSUGUCHI, M., TAKEYA, K., UMEDA, A., AMAKO, K., WATABE, T., and AITA, S. (1977). Optimal condition for observing bacterial flagella by the scanning electron microscope. *J. Electron Microsc.* 26, 343.
- MAY, K.N. (1961). Skin contamination of broilers during commercial evisceration. *Poult. Sci.* 40, 531.
- MAY, K.N. (1974). Chilling of poultry meat. Changes in microbiological numbers during final washing and chilling of commercially slaughtered broilers. *Poult. Sci.* 53, 1282.
- MAY, K.N., IRBY, J.D., and CARMON, J.L. (1962). Shelf life and bacterial counts of excised poultry tissue. *Food Technol.* 16, 66.
- MEAD, G.C. (1975). Hygiene aspects of the chilling process. *Proc. 2nd Eur. Poult. Meat Symp.*, Oosterbeek, Netherlands, 35 (1).
- MEAD, G.C. (1976). Microbiology of the poultry carcass and the processing plant. *J. Roy. Soc. Health* 96, 164.

- MEAD, G.C., ADAMS, B.W., and PARRY, R.T. (1975). The effectiveness of in-plant chlorination in poultry processing. Br. Poult. Sci. 16, 517.
- MEAD, G.C., and IMPEY, C.S. (1970). The distribution of clostridia in poultry processing plants. Br. Poult. Sci. 11, 407.
- MEAD, G.C., and THOMAS, N.L. (1973a). Factors affecting the use of chlorine in the spinchilling of eviscerated poultry. Br. Poult. Sci. 14, 99.
- MEAD, G.C., and THOMAS, N.L. (1973b). The bacteriological condition of eviscerated chickens processed under controlled conditions in a spinchilling system and sampled by two different methods. Br. Poult. Sci. 14, 413.
- MEADOWS, P.S. (1971). The attachment of bacteria to solid surfaces. Arch. Mikrobiol. 75, 374.
- MERCURI, A.J., and KOTULA, A.W. (1964). Relation of 'breast swab' to 'drip' bacterial counts in tray packed chicken fryers. J. Food Sci. 29, 854.
- MILLER, III, A., SCANLAN, R.A., LEE, J.S., LIBBEY, L.M., and MORGAN, M.E. (1973a). Volatile compounds produced in sterile fish muscle (*Sebastes melanops*) by *Pseudomonas perolens*. Appl. Microbiol. 25, 257.
- MILLER, III, A., SCANLAN, R.A., LEE, J.S., and LIBBEY, L.M. (1973b). Identification of the volatile compounds produced in sterile fish muscle (*Sebastes melanops*) by *Pseudomonas fragi*. Appl. Microbiol. 25, 952.
- MILLER, III, A., SCANLAN, R.A., LEE, J.S., and LIBBEY, L.M. (1973c). Volatile compounds produced in sterile fish muscle (*Sebastes melanops*) by *Pseudomonas putrefaciens*, *Pseudomonas fluorescens* and an *Achromobacter* species. Appl. Microbiol. 26, 18.

- MORRIS, T.G., and AYRES, J.C. (1960). Incidence of salmonellae on commercially processed poultry. *Poult. Sci.* 39, 1131.
- MOSSEL, D.A.A. (1971). Physiological and metabolic attributes of microbial groups associated with foods. *J. Appl. Bacteriol.* 34, 96.
- MOSSEL, D.A.A. (1977). *Microbiology of Foods*. University of Utrecht, Netherlands.
- MOUNTNEY, G.J., and O'MALLEY, J. (1965). Acids as poultry meat preservatives. *Poult. Sci.* 44, 582.
- MULDER, R.W.A.W., and DORRESTEIJN, L.W.J. (1977). Hygiene beim Brichen von Schlachtgeflügel (Hygiene during the scalding process of broilers). *Fleischwirtschaft* 57, 2220.
- MULDER, R.W.A.W., DORRESTEIJN, L.W.J., HOFMANS, G.J.P., and VEERKAMP, C.H. (1976). Experiments with continuous immersion chilling of broiler carcasses according to the code of practice. *J. Food Sci.* 41, 438.
- MULDER, R.W.A.W., DORRESTEIJN, L.W.J., and VAN DER BROEK, J. (1978). Cross contamination during the scalding and plucking of broilers. *Br. Poult. Sci.* 19, 61.
- MULDER, R.W.A.W., NOTERMANS, S., and KAMPELMACHER, E.H. (1977). Inactivation of salmonellae on chilled and deep frozen broiler carcasses by irradiation. *J. Appl. Bacteriol.* 42, 179.
- MULDER, R.W.A.W., and VEERKAMP, C.H. (1974). Improvement of poultry slaughter house hygiene as a result of cleaning before cooling. *Poult. Sci.* 53, 1690.
- MULLER, G., and AFIFI, S.A. (1976). Direct bacteriological examination of food by means of the fluorescence bacterioscopy. *Jena Rev.* 251.

- NÄGEL, C.W., SIMPSON, K.L., NG, H., VAUGHN, R.H., and STEWART, G.F. (1960). Microorganisms associated with spoilage of refrigerated poultry. *Food Technol. Champaign* 14, 21.
- NICOL, D.J., SHAW, M.K., and LEDWARD, D.A. (1970). Hydrogen sulphide production by bacteria and sulfmyoglobin formation in prepacked chilled beef. *Appl. Microbiol.* 19, 937.
- NOTERMANS, S., FIRSTENBERG-EDEN, R., and VAN SCHOTHORST, M. (1979). Attachment of bacteria to teats of cows. *J. Food Prot.* 42, 228.
- NOTERMANS, S., HINDLE, V., and KAMPELMACHER, E.H. (1976). Comparison of cotton swabs vs. alginate swab sampling methods in the bacteriological examination of broiler chickens. *J. Hyg.* 77, 205.
- NOTERMANS, S., JEUNICK, J., VAN SCHOTHORST, M., and KAMPELMACHER, E.H. (1973). Comparative investigations into possible cross contamination in the spin chiller and during spray chilling. *Fleischwirtschaft* 53, 1450.
- NOTERMANS, S., and KAMPELMACHER, E.H. (1974). Attachment of some bacterial strains to the skin of broiler chickens. *Br. Poult. Sci.* 15, 573.
- NOTERMANS, S., and KAMPELMACHER, E.H. (1975a). Heat destruction of some bacterial strains attached to broiler skin. *Br. Poult. Sci.* 15, 351.
- NOTERMANS, S., and KAMPELMACHER, E.H. (1975b). Further studies on the attachment of bacteria to skin. *Br. Poult. Sci.* 16, 487.
- NOTERMANS, S., KAMPELMACHER, E.H., and VAN SCHOTHORST, M. (1975a). Studies on sampling methods used in the control of hygiene in poultry processing. *J. Appl. Bacteriol.* 39, 55.

NOTERMANS, S., KAMPELMACHER, E.H., and VAN SCHOTHORST, M. (1975b).

Studies on different sampling methods for the determination of bacterial counts of frozen broilers. J. Appl. Bacteriol. 39, 125.

NOTERMANS, S., VAN LEUSDEN, F.M., VAN SCHOTHORST, M., and

KAMPELMACHER, E.H. (1975). Salmonella-contaminatie van stachtkuikens tijdens het stachtproces in enkele pluimveeslachterijen. Tijdschr. Diergeneesk. D. 100, 259.

NOTERMANS, S., VAN LEUSDEN, F.M., and VAN SCHOTHORST, M. (1977).

Suitability of different bacterial groups for determining faecal contamination during post-scalding stages in the processing of broiler chickens. J. Appl. Bacteriol. 43, 383.

OCHI, Y., MITUOKA, T., and SEGA, T. (1964). Untersuchungen über die Darmflora des Huhn III Mitterlung: die Entwicklung der Darmflora von Kuber bis zum Huhn. Backteriol. Parisitenk. 193, 80.

OCKERMAN, H.W., CAHILL, V.R., WEISER, H.H., DAVIS, C.E., and

SIEFKER, J.R. (1969). Comparison of sterile and inoculated beef tissue. J. Food Sci. 34, 93.

OGILVY, W.S., and AYRES, J.C. (1951). Post-mortem changes in

stored meats. II. The effect of atmospheres containing carbon dioxide in prolonging the storage life of cut up chicken. Food Technol. 5, 97.

PARTMANN, W. (1966). Untersuchungen zur Beurteilung autolytischer

und bakterieller einflüsse auf den Fischverdef. Z. Lebensmitt. u.-Forsch. 129, 205.

PATON, A.M., and JONES, S.M. (1973). The observation of

microorganisms on surfaces by incident fluorescence microscopy. J. Appl. Bacteriol. 36, 441.

- PATTERSON, J.T. (1968a). Chlorination of water used for poultry processing. Br. Poult. Sci. 9, 129.
- PATTERSON, J.T. (1968b). Bacterial contamination of processed poultry. Br. Poult. Sci. 10, 89.
- PATTERSON, J.T. (1968c). Bacterial flora of chicken carcasses treated with high concentrations of chlorine. J. Appl. Bacteriol. 31, 544.
- PATTERSON, J.T. (1971a). Microbiological aspects of poultry processing. Br. Poult. Sci. 12, 197.
- PATTERSON, J.T. (1971b). Microbiological assessment of surfaces. J. Food Technol. 6, 63.
- PATTERSON, J.T. (1972). Microbiological sampling of poultry carcasses. J. Appl. Bacteriol. 35, 569.
- PATTERSON, J.T. (1975). The effects of various treatments on the microbial flora of whole poultry carcasses with particular reference to *Staphylococcus aureus* contamination. Br. Poult. Sci. 16, 307.
- PATTERSON, J.T., and STEWART, D.J. (1962). Bacteriology of processed broilers. 1. Methods of Examination. Res. Exptl. Rec. Min. Agric. N.I. 11, 57.
- PERIC, M., ROSSMANITH, E., and LEISTNER, L. (1971a). Untersuchungen über die Beeinflussung des Oberflächenkeimgehaltes von Schlachthähnchen durch die Spinchiller Kühlung. Fleischwirtschaft 51, 216.
- PERIC, M., ROSSMANITH, E., and LEISTNER, L. (1971b). Verbesserung der mikrobiologischen Qualität von Schlachthähnchen durch die Spruh Kühlung. Fleischwirtschaft 52, 574.
- PERRY, G.A., LAWRENCE, R.L., and MELNICK, D. (1964). Extension of poultry shelf life by processing with sorbic acid. Food Technol. 18, 891.

- PIETSZCH, O., and LEVETZOW, R. (1974). The problem of salmonellosis of poultry as related to poultry chilling. *Fleischwirtschaft* 54, 76.
- POLLOCK, M.R. (1962). "Exoenzymes". In *The Bacteria: A Treatise on structure and function*. IV. The physiology of growth, 121-178. Edited by Gunsalus, I.C. and Stanier, R.Y. Academic Press, New York and London.
- RACCACH, M., and BAKER, R.C. (1978). Lactic acid bacteria as an antispoilage and safety factor in cooked, mechanically deboned poultry meat. *J. Food Prot.* 41, 703.
- RACCACH, M., and BAKER, R.C. (1979). The effect of lactic acid bacteria on some properties of mechanically deboned poultry meat. *Poult. Sci.* 58, 144.
- RACCACH, M., BAKER, R.C., REGENSTEIN, J.M., and MULNIX, E.J. (1979). Potential application of microbial antagonism to extended storage stability of a flesh type food. *J. Food Sci.* 44, 43.
- REDDY, K.V., KRAFT, A.A., BASIAK, R.J., MARION, W.W., and HOTCHKISS, D.K. (1978). Effect of pinchilling and freezing on bacteria on commercially processed turkeys. *J. Food Sci.* 43, 334.
- REISS, J. (1977). Scanning electron microscopy of mouldy bread. *Mikroskopie* 33, 68.
- REY, C.R., and KRAFT, A.A. (1971). Effect of freezing and packaging methods on the survival and biochemical activity of spoilage organisms on chicken. *J. Food Sci.* 36, 454.
- REY, C.R., KRAFT, A.A., SEALS, R.G., and BIRD, E.W. (1969). Influence of temperature on some biochemical characteristics of *Pseudomonas* associated with spoilage of poultry. *J. Food Sci.* 34, 279.

- REYNOLDS, E.S. (1963). The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* 17, 208.
- RHODES, D.N. (1965). The radiation pasteurization of broiler carcasses. *Br. Poult. Sci.* 6, 265.
- RODGERS, F.G., MACRAE, A.D., and LEWIS, M.J. (1978). Electron microscopy of the organism of Legionnaires' disease. *Nature* 272, 825.
- ROSKEY, C.T., and HAMDY, M.K. (1975). Persistence of *Staphylococcus*. Bruised tissue microenvironment affecting persistence of *Staphylococcus aureus*. *J. Food Sci.* 40, 496.
- ROTH, L.A., and CLARK, D.S. (1972). Studies on the bacterial flora of vacuum packaged fresh beef. *Can. J. Microbiol.* 18, 1761.
- RUIZ-HERRERA, J., and STARKEY, R.L. (1969). Dissimilation of methionine by fungi. *J. Bacteriol.* 99, 544.
- SALANITRO, J.P., BLAKE, I.G., and MUIRHEAD, P.A. (1974). Studies on the caecal microflora of commercial broiler chickens. *J. Appl. Microbiol.* 28, 439.
- SALANITRO, J.P., FAIRCHILDS, I.G., and ZGORNICKI, Y.D. (1974). Isolation, culture characteristics and identification of anaerobic bacteria from the chicken caecum. *J. Appl. Microbiol.* 27, 678.
- SALANITRO, J.P., BLAKE, I.G., MUIRHEAD, P.A., MAGLIO, M., and GOODMAN, J.R. (1978). Bacteria isolated from the duodenum, ileum and caecum of young chicks. *Appl. Environ. Microbiol.* 35, 782.

- SANDERS, D.H., and BLACKSHEAR, C.D. (1971). Effect of chlorination in the final washer on bacterial counts of broiler chicken carcasses. *Poult. Sci.* 57, 422.
- SATO, T., and SHAMOTO, M. (1973). A simple rapid polychrome stain for epoxy embedded tissue. *Stain Technol.* 48, 223.
- SEEKINS, J.G., GUENTHER, E. and WALTER, W.G. (1958). A comparison of six methods for determining numbers of bacteria on poultry meat under three types of handling. *Proc. Montana Acad. Sci.* 18, 13.
- SEGAL, W., and STARKEY, R.L. (1969). Microbial decomposition of methionine and identity of the resulting sulphur products. *J. Bacteriol.* 93, 908.
- SHANK, J.L., and LUNDQUIST, B.R. (1963). The effect of packaging conditions on the bacteriology, colour and flavor of table ready meats. *Food Technol.* 17, 1163.
- SHARF, J.M. (ed.) (1966). Recommended methods for the microbiological examination of foods. pp. 119-125. American Public Health Association, Inc., New York.
- SHARPE, E.M., LAW, B.A., and PHILLIPS, B.A. (1976). Coryneform bacteria producing methanethiol. *J. Gen. Microbiol.* 94, 430.
- SHARPE, E.M., LAW, B.A., PHILLIPS, B.A., and PITCHER, D.G. (1977). Methanethiol production by coryneform bacteria: Strains from dairy and human skin sources and *Brevibacterium linens*. *J. Gen. Microbiol.* 101, 345.
- SHELEF, L.A. (1977). Effect of glucose on the bacterial spoilage of beef. *J. Food Sci.* 42, 1172.
- SHEWAN, J.M. (1976). The biochemistry and microbiology of low temperature spoilage. *Food Technol. Aust.* 28, 409.

- SHEWAN, J.M., HOBBS, G., and HODGKISS, W.J. (1960a). A determinative scheme for the identification of certain genera of bacteria with special reference to the *Pseudomonadaceae*. J. Appl. Bacteriol. 23, 379.
- SHEWAN, J.M., HOBBS, G., and HODGKISS, W.J. (1960b). The *Pseudomonas* and *Achromobacteria* groups of bacteria in the spoilage of marine white-fish. J. Appl. Bacteriol. 23, 463.
- SHEWAN, J.M., and JONES, J.R. (1957). Chemical changes occurring in cod muscle during chill storage and their possible use as objective indices of quality. J. Sci. Fd. Agric. 8, 491.
- SHRIMPTON, D.H., and BARNES, E.M. (1960). A comparison of oxygen permeable and impermeable wrapping materials for the storage of chilled eviscerated poultry. Chem. Ind. 1492.
- SILLIKER, J.H., ANDREWS, H.P., and MURPHY, J.F. (1957). A new non-destructive method for the bacteriological sampling of meats. Food Technol. 11, 317.
- SIMMONS, G.C., and BYRNES, R.V. (1972). The origin of salmonellas in chickens and chicken carcasses. Aust. Vet. J. 48, 186.
- SIMMONSEN, B. (1971). Methods for determining the microbial counts of ready to cook poultry. Worlds Poult. Sci. J. 27, 368.
- SMITH, E.C. (1934). The cold storage of poultry. Part I. Gas storage of chickens. J. Soc. Chem. Ind. 53, 345 T.
- SMITH, H.W. (1965). Observations on the flora of the alimentary tract of animals and factors affecting its composition. J. Path. Bacteriol. 89, 95.
- SMITH, H.W., and CRABB, W.E. (1960). The effect of diets containing tetracyclines and penicillin on the *Staphylococcus aureus* flora of the nose and skin of pigs, chickens and their attendants. J. Path. Bacteriol. 79, 243.

- SPENCER, J.V., and SMITH, L.E. (1962). The effect of chilling fryer chickens in a solution of polyphosphate upon moisture uptake, microbial spoilage, tenderness, juiciness and flavour. *Poult. Sci.* 41, 1685.
- SRIVASTAVA, S.K., SINGH, V.B., and SINGH, N.P. (1972). Bacterial flora of the poultry environment. *Ind. J. Microbiol.* 12, 7.
- STANSBY, M.E. (1962). Speculations on fishy odours and flavours. *Food Technol.* 16, 28.
- STEINHAUER, J.E., and BANWART, G.J. (1964). The effect of food grade polyphosphates on the microbial population of chicken meat. *Poult. Sci.* 43, 618.
- STEVENS, B.J.H., and LEA, C.H. (1969). Chemical and organoleptic changes in poultry meat resulting from the growth of psychrophilic spoilage bacteria at 1°C. 2. Changes in nucleotides. *Br. Poult. Sci.* 10, 219.
- STOTT, J.A., HODGSON, J.E., and CHANEY, J.C. (1975). Incidence of salmonellae in animal feed and the effect of pelleting on content of *Enterobacteriaceae*. *J. Appl. Bacteriol.* 39, 41.
- STRAKA, R.P., and STOKES, J.L. (1957). Rapid destruction of bacteria in commonly used diluents and its elimination. *Appl. Microbiol.* 5, 21.
- SURKIEWICZ, B.F., JOHNSTON, R.W., MORAN, A.B., and KRUMM, G.W. (1969). Bacteriological survey of chicken eviscerating plants. *Food Technol.* 23, 1066.
- TAGO, Y., and AIDA, K. (1977). Extracellular mucopolysaccharide closely related to bacterial floc formation. *Appl. Environ. Microbiol.* 34, 308.

- TAMURA, K., HECHELMANN, H., and LEISTNER, L. (1971). Nachweiss von enteropathogen *Escherichia coli* - Stamme bei Gefluegel und Fleisch. *Fleischwirtschaft* 51, 1639.
- TARRANT, P.J.V., PEARSON, A.M., PRICE, J.F., and LECHOWICH, R.V. (1971). Action of *Pseudomonas fragi* on the proteins of pig muscle. *Appl. Microbiol.* 22, 224.
- TAYLOR, M.H., HELBACKA, N.V., and KOTULA, A.W. (1966). Evacuated packaging of fresh broiler chickens. *Poult. Sci.* 45, 1207.
- THOMAS, C.J., McMEEKIN, T.A., and BALIS, C. (1977). Retention of bacteria in liquid films at agar surfaces. *Appl. Environ. Microbiol.* 34, 456.
- THOMAS, N.L. (1977). The continuous chilling of poultry in relation to E.E.C. requirements. *J. Food Technol.* 12, 99.
- THOMSON, J.E., BANWART, G.J., SANDERS, D.H., and MERCURI, A.J. (1967). Effect of Cl_2 , antibiotics, β propiolactone, acids, and washing on *Salmonella typhimurium* on eviscerated fryer chickens. *Poult. Sci.* 46, 146.
- THOMSON, J.E., BAILEY, J.S., and COX, N.A. (1979). Phosphate and heat treatments to control *Salmonella* and reduce spoilage and rancidity on broiler carcasses. *Poult. Sci.* 58, 139.
- THOMSON, J.E., COX, N.A., and BAILEY, J.S. (1977). Control of *Salmonella* and extension of shelf life of broiler carcasses with a glutaraldehyde product. *J. Food Sci.* 42, 1353.
- THOMSON, J.E., COX, N.A., WHITEHEAD, W.K., and MERCURI, A.J. (1974). Effect of hot spray washing on broiler carcass quality. *Poult. Sci.* 53, 946.

- THOMSON, J.E., COX, N.A., WHITEHEAD, W.K., MERCURI, A.J., and JUVEN, B.J. (1975). Bacterial counts and weight changes of broiler carcasses chilled commercially by water immersion and air-blast. *Poult. Sci.* 54, 1452.
- THORNLEY, M.J., INGRAM, M., and BARNES, E.M. (1960). The effects of antibiotics and irradiation on the *Pseudomonas* - *Achromobacter* flora on chilled poultry. *J. Appl. Bacteriol.* 23, 487.
- TIMMS, L. (1968). Observations on the bacterial flora of the alimentary tract in three age groups of normal chickens. *Brit. Vet. J.* 124, 470.
- TUNG, M.A., GARLAND, M.R., and GILL, P.K. (1979). A scanning electron microscope study of bacterial invasion in hens' egg shell. *J. Inst. Can. Sci. Technol. Aliment.* 12, 16.
- UMEMOTO, Y., SATO, Y., and KITO, J. (1978). Direct observation of fine structures of bacteria in ripened Cheddar cheese by electron microscopy. *Agric. Biol. Chem.* 42, 227.
- VALLEY, G. (1928). The effect of carbon dioxide on bacteria. *Q. Rev. Biol.* 3, 209.
- VAN SCHOTHORST, M., NOTERMANS, S., and KAMPELMACHER, E.H. (1972). Einige hygienische aspekte der Geflügelschlachtung (Hygiene in poultry slaughter). *Fleischwirtschaft* 52, 749.
- VAN SCHOTHORST, M., NORTHOLT, M.D., KAMPELMACHER, E.H., and NOTERMANS, S. (1976). Studies on the estimation of the hygienic condition of frozen broiler chickens. *J. Hyg.* 76, 57.
- VANDERZANT, C., CARPENTER, Z.L., and SMITH, G.C. (1976). Sampling carcasses and meat products. Paper presented at 29th Annual Reciprocal Meat Conference of the American Meat Science Association.

- VEERKAMP, C.H., and HOFMANS, G.J.P. (1973). New development in poultry processing; simultaneous scalding and plucking. *Poult. Int.* 12, 16.
- VEERKAMP, C.H., MULDER, R.W.A.W., and GERRITS, A.R. (1972). Kühlung und Reinigung von Schlachtgeflügel. *Fleischwirtschaft* 52, 612.
- WAHBECK, C.J., PARMELEE, C.E., and STADELMAN, W.J. (1968a). Carbon dioxide preservation of fresh poultry. *Poult. Sci.* 47, 468.
- WAHBECK, C.J., SCHWALL, D.V., GRAUCHO, G.M., HECK, H.C., and ROGERS, A.B. (1968b). *Salmonella* and total count reduction in poultry treated with hypochlorite solutions. *Poult. Sci.* 47, 1090.
- WIEBE, W.J., and CHAPMAN, G.B. (1968a). Fine structure of selected marine pseudomonads and achromobacters. *J. Bacteriol.* 95, 1862.
- WIEBE, W.J., and CHAPMAN, G.B. (1968b). Variation in the fine structure of a marine achromobacter and a marine pseudomonad grown under selected nutritional and temperature regimes. *J. Bacteriol.* 95, 1874.
- WALKER, H.W., and AYRES, J.C. (1956). Incidence and kinds of microorganisms associated with commercially dressed poultry. *Appl. Microbiol.* 4, 345.
- WALKER, H.W., and AYRES, J.C. (1959). Microorganisms associated with commercially processed turkeys. *Poult. Sci.* 38, 1351.
- WALKER, H.W., and AYRES, J.C. (1970). Yeasts as spoilage organisms. In *The Yeasts*, 3rd ed. pp. 463-527. Eds. Rose, A.H., and Harris, J.S. Academic Press, London.

- WELLS, F.E., SPENCER, J.V., and STADELMAN, W.J. (1958). Effect of packaging materials and techniques on the shelf life of fresh poultry meat. *Food Technol.* 12, 425.
- WELLS, F.E., and STADELMAN, W.J. (1958). The development of yeasts on antibiotic treated poultry. *Appl. Microbiol.* 6, 420.
- WHITEHEAD, W.K., CHILDS, R.E., THOMSON, J.E., and COX, N.A. (1972). Design of a poultry final washer. Paper No. 72 - 894 presented at Winter Meeting of American Society of Agricultural Engineers, Chicago, Illinois.
- WILKERSON, W.B., AYRES, J.C., and KRAFT, A.A. (1961). Occurrence of enterococci and coliform organisms on fresh and stored poultry. *Food Technol.* 15, 286.
- WILLIAMS, M.L.B. (1967). A new method for evaluating surface contamination of raw meat. *J. Appl. Bacteriol.* 30, 498.
- WOODROFFE, R.C.S., and SHAW, D.A. (1974). Natural control and ecology of microbial populations on skin and hair. In Skinner, F.A., and Carr, J.G. (eds.). *The Normal Microbial Flora of Man*. Soc. Appl. Bacteriol. Symp. Ser. No. 3, Academic Press, London.
- YACOWITZ, H., PANSY, F., WIND, S., STRANDER, H., SASSAMEN, H.L., PAGANO, J.F., and TREJO, W.H. (1957). Use of nystatin (mycostatin) to retard yeast growth on chlortetracycline treated chicken meat. *Poult. Sci.* 36, 843.
- YNDESTAD, M., UNDERAL, B., and NORDAL, J. (1972). The effects of ultraviolet radiation on bacterial counts and shelf life of chicken carcasses. *Acta Agric. Scan.* 22, 169.
- ZIEGLER, F., SPENCER, J.V., and STADELMAN, W.J. (1954). A rapid method for determining spoilage in fresh poultry meat. *Poult. Sci.* 33, 1253.

- ZIEGLER, F., and STADELMAN, W.J. (1955). The effect of different scald water temperatures on the shelf life of fresh non-frozen fryers. *Poult. Sci.* 34, 237.
- ZOBELL, C.E. (1943). The effect of solid surfaces upon bacterial activity. *J. Bacteriol.* 46, 39.

APPENDICES

APPENDIX 1Media(a) Nutrient Agar

Oxoid Peptone Bacteriological (Code L37)	5g
Bonox	3g
Agar	15g
Water	1l

Ingredients dissolved, dispensed in 200ml quantities and autoclaved at 120°C @ 15 psi for 15 mins.

(b) Glucose Phosphate Peptone Water

Oxoid Bacto-Peptone (Code L37)	5g
K ₂ HPO ₄	5g
Water	1l

Solution autoclaved at 120°C @ 15 psi for 15 mins, cooled and filter sterilized glucose solution added to give a final concentration of 0.5% (w/v) glucose.

(c) Glucose Tryptone Water

Oxoid Tryptone (Code L42)	10g
Glucose	5g
Bromocresol Purple (0.4% w/v aqueous)	5ml
Water	1l

Solution dispensed and autoclaved at 120°C @ 15 psi for 15 mins.

Appendix 1. (Continued)(d) Sabouraud Agar

Glucose	40g
Oxoid Peptone Bacteriological (Code L37)	10g
Agar	15g
Water	1 litre

All ingredients were dissolved, the pH adjusted to 5.6.

The mixture was dispensed in 180ml volumes and autoclaved at 120°C @ 15 psi for 15 mins. Chloramphenicol (Boehringer Mannheim GmbH, West Germany) was added to molten agar (at 45°C) to inhibit growth of bacteria. 20ml of a filter sterilised aqueous solution of chloramphenicol (2.00mg/ml, w/v) was added to 180ml of cooled medium to give a final concentration of 200mg/l.

APPENDIX 2Electrophoresis(a) Gel Buffer (pH 8.7 at 25°C)

Tris Base	4.598
Citric acid	0.525g
Water	1 litre

(b) Tank Buffer (pH 8.8 at 25°C)

Boric acid	7.22g
Sodium tetraborate	15.75
Water	1 litre

(c) Acrylamide Gels (6%)

Cyanogum 41 (B.D.H.)	6g
N.N.N.'N.'-tetramethylethylenediamine	0.10ml
Ammonium persulphate	0.10g
Gel buffer	100ml

After mixing, the solution was poured into gel formers (75 x 75 x 3mm inside dimensions; forming 10 wells 4 x 1.5mm and 1.5mm in depth), allowed to gel. All acrylamide gel slabs were stored in plastic bags at 4°C until required.

(d) Protein Stains

1. Amido Black - general protein stain

Amido black	0.3g
Methanol	30ml
Glacial acetic acid	10ml
Water	60ml

Gels were stained in this solution for at least 2 hours then washed in 7% (v/v) acetic acid until unbound stain was removed from the gel. Protein stains dark blue.

2. Protein bound carbohydrate - Periodic acid - Schiff Method (Clarke, 1964).

Periodic acid	1ml
Acetic acid	3ml
Water	96ml

Gels were fixed and preleached in 7% (v/v) acetic acid for at least 1 hour prior to immersion in the above solution. Preleaching was required to remove excess Tris which may be oxidized to a Schiff positive aldehyde during the acid treatment step. Leached gels were immersed in the acid solution for 1 hour followed by 1 hour immersion in commercial Schiff's reagent (Bio Science Laboratories Pty. Ltd., Melbourne, Australia). Stained gels were stored in 1% (w/v) aqueous sodium metabisulphite. Protein bound carbohydrate stains brilliant red.

3. Oil red O for lipoprotein (Lancy and Murray, 1978). Gel slabs were fixed and stained in 10% (v/v) trichloro acetic acid containing 30% (v/v) methanol saturated with oil red O for 2 hours at room temperature. The fixed gels were rinsed and contrast enhanced overnight at room temperature in 9% (v/v) acetic acid containing 30% methanol saturated with oil red O. Gels were destained in 7% (v/v) acetic acid containing 5% (v/v) methanol until the background gel was clear. Lipoprotein stains red.

APPENDIX 3

Recovery of Microorganisms from Broiler Carcass Skin by the Stomacher

(a) Effect of time of stomacher action on the number of microorganisms recovered (data for Table 7).

REPLICATE	TOTAL VIABLE COUNTS/16cm ² BREAST SKIN				
	TIME OF STOMACHER ACTION (MIN)				
	1	2	3	5	10
1	2.26 x 10 ⁵ *(5.35)	8.35 x 10 ⁴ (4.92)	2.43 x 10 ⁵ (5.39)	1.86 x 10 ⁵ (5.27)	2.88 x 10 ⁵ (5.46)
2	7.20 x 10 ⁴ (4.85)	3.42 x 10 ⁵ (5.53)	2.57 x 10 ⁵ (5.40)	2.23 x 10 ⁵ (5.35)	2.20 x 10 ⁵ (5.34)
3	7.40 x 10 ⁴ (4.97)	3.16 x 10 ⁵ (5.50)	4.45 x 10 ⁵ (5.65)	2.34 x 10 ⁵ (5.37)	1.55 x 10 ⁵ (5.19)
4	1.16 x 10 ⁵ (5.06)	7.55 x 10 ⁴ (4.88)	7.75 x 10 ⁴ (4.89)	1.51 x 10 ⁵ (5.18)	3.75 x 10 ⁴ (4.58)

*Numbers in brackets are total viable counts expressed as logarithms to base 10.

The log values of the above total count data were analysed as a randomized block design (Cochran and Cox 1957).

The times of stomaching were the treatments compared.

A table of the analysis of variance is presented below.

TABLE OF ANALYSIS OF VARIANCE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F RATIO
TREATMENT	4	0.2008	0.0502	0.58 n.s.
ERROR	15	1.3026	0.0868	
TOTALS	19	1.5034		

n.s. = not significant at the 95% confidence level.

(b) Comparison of numbers of microorganisms recovered by the stomacher and the blender techniques (data for Table 8).

SAMPLE	TOTAL VIABLE COUNTS/16cm ² BREAST SKIN	
	STOMACHER	ATOMIX BLENDER
1	4.95×10^4 *(4.69)	2.95×10^4 (4.47)
2	3.00×10^5 (5.47)	1.09×10^5 (5.04)
3	1.45×10^4 (4.16)	3.00×10^3 (3.48)
4	7.60×10^4 (4.88)	6.00×10^3 (3.78)
5	1.15×10^4 (4.06)	4.35×10^4 (4.64)
6	2.50×10^4 (4.40)	8.30×10^5 (5.92)
7	4.25×10^4 (4.63)	3.10×10^4 (4.49)
8	1.06×10^5 (5.03)	9.60×10^4 (4.98)
9	2.10×10^4 (4.32)	1.21×10^5 (5.08)
10	5.20×10^4 (4.72)	9.70×10^4 (4.99)
11	3.65×10^4 (4.56)	8.60×10^4 (4.93)
12	3.90×10^5 (5.59)	1.10×10^5 (5.04)
13	1.62×10^5 (5.21)	1.34×10^5 (5.13)
14	6.50×10^4 (4.81)	5.10×10^4 (4.71)
15	1.50×10^6 (6.18)	4.80×10^5 (5.68)
16	8.90×10^4 (4.95)	2.40×10^5 (5.38)
17	9.30×10^5 (5.97)	4.00×10^5 (5.06)
18	1.22×10^5 (5.09)	3.00×10^4 (4.48)

*Numbers in brackets represent the total viable counts expressed as logarithms (to base 10).

The log values of the above total count data were analysed as a randomized block design (Cochran and Cox 1957). The stomacher and the blender techniques were the two treatments compared.

A table of analysis of variance is presented below.

TABLE OF ANALYSIS OF VARIANCE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F RATIO
TREATMENTS	1	0.0576	0.0576	0.167 n.s.
ERROR	34	11.7356	0.3452	
TOTALS	35	11.7932		

n.s. = not significant at the 95% confidence limit.

Appendix 4. (a) Numbers of various types of
bacteria present on breast skin
of immersion chilled carcasses
stored for periods up to 16 days
at 2°C. (Data for Fig. 19.)

Appendix 4(a).

DAYS CARCASS STORED AT 20°C	0	4	8	12	16
TOTAL VIABLE COUNT PER 16cm ² SKIN	4.83 x 10 ⁵ *(5.68)	4.60 x 10 ⁵ (5.66)	1.04 x 10 ⁷ (7.02)	7.30 x 10 ⁷ (7.86)	2.57 x 10 ⁹ (9.41)
NUMBER OF PSYCHROTROPHS PER 16cm ² OF SKIN	6.00 x 10 ³ (3.78)	1.20 x 10 ⁵ (5.08)	3.16 x 10 ⁶ (6.50)	3.60 x 10 ⁷ (7.56)	2.04 x 10 ⁹ (9.31)
COLIFORM COUNT PER 16cm ² SKIN	<1 x 10 ²	<1 x 10 ²	<2 x 10 ² (2.0)	<1 x 10 ²	nd
NUMBER OF YEAST PROPAGULES PER 16cm ² SKIN	nd	4.00 x 10 ³ (3.60)	6.15 x 10 ⁴ (4.79)	3.35 x 10 ⁵ (5.53)	2.70 x 10 ⁴ (4.43)

*Numbers in parentheses are the numbers of organisms enumerated expressed as logarithms (to base ten).

nd = none detected.

Appendix 4. (b) Numbers of various types of
bacteria present on leg skin of
immersion chilled broiler carcasses
stored for periods up to 16 days at
2°C. (Data for Fig. 20.)

Appendix 4(b).

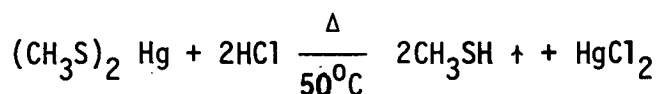
DAYS CARCASS STORED AT 2°C	0	4	8	12	16
TOTAL VIABLE COUNT PER 16cm ² SKIN	4.00 x 10 ⁵ *(5.60)	7.85 x 10 ⁵ (5.89)	4.63 x 10 ⁶ (6.67)	2.06 x 10 ⁷ (7.31)	1.29 x 10 ⁹ (9.11)
NUMBER OF PSYCHROTROPHS PER 16cm ² SKIN	6.00 x 10 ³ (3.78)	6.10 x 10 ⁴ (4.79)	5.01 x 10 ⁶ (6.70)	2.10 x 10 ⁷ (7.32)	1.00 x 10 ⁹ (9.00)
COLIFORM COUNT PER 16cm ² SKIN	5.25 x 10 ³ (3.72)	1.1 x 10 ³ (3.04)	2 x 10 ² (2.30)	1.5 x 10 ² (2.18)	nd
NUMBER OF YEAST PROPAGULES PER 16cm ² SKIN	nd	2 x 10 ³ (3.30)	7.05 x 10 ⁴ (4.85)	5.20 x 10 ⁴ (4.72)	4.50 x 10 ⁴ (4.65)

*Numbers in parentheses are the numbers of organisms enumerated expressed as logarithms (to base ten).

nd = none detected.

Appendix 5.(a) Minimum concentration of methanethiol detected by DTNB paper

Methanethiol was released from mercuric mercaptide by heating under acid conditions.



The lowest concentration of methanethiol detected by DTNB paper was produced from 2.40×10^{-7} g of mercuric mercaptide.

∴ Weight of methanethiol produced, assuming 100% yield

$$= \frac{2.40 \times 10^{-7} \times 2 \times 48.11}{294.8} = 7.83 \times 10^{-8} \text{ g}$$

and the volume of this weight of gas at STP

$$= \frac{7.838 \times 10^{-8} \times 22400}{48.11} \text{ ml} = 3.650 \times 10^{-6} \text{ ml}$$

Now, since this volume of gas occupied a vessel of 106ml volume, then the gas concentration is

$$= \frac{3.65 \times 10^{-6} \times 10^6}{106} \text{ ppm (v/v)} = 0.034 \text{ ppm (v/v)}.$$

Thus the minimum methanethiol gas concentration detectable by DTNB test strips is 0.034 ppm (v/v).

Appendix 5. (Continued)

(b) Concentrations of solutions of methanethiol and hydrogen sulphide required to produce a positive headspace reaction to DTNB or lead acetate papers

The concentration of hydrogen sulphide or methanethiol aqueous solutions was determined by reaction of these gases with DTNB in solution. The absorbance of the coloured complex was measured spectrophotometrically at a wavelength of $412\mu\text{m}$, and the concentration of the gas determined.

$$\text{i.e. } C = \frac{A}{E_m} \quad (\text{for } 1 \text{ cm path length})$$

where C = the concentration

A = the absorbance of the coloured anion

E_m = molar extinction coefficient (13600/M/cm)

The absorbance of the complexed hydrogen sulphide and methanethiol solutions which produced the lowest gas concentration detectable by DTNB or lead acetate test strips was 0.120.

$$\therefore C = \frac{0.12}{13600} \text{ M} = 8.80 \times 10^{-6} \text{ M}$$

Now, for hydrogen sulphide, the weight of gas present in solution is

$$34.06 \times 8.80 \times 10^{-6} \text{ g/l} = 3 \times 10^{-4} \text{ g/l of solution.}$$

This weight of gas occupies $\frac{3.00 \times 10^{-4} \times 22400}{34.06}$ ml/litre of solution

$$= 1.973 \times 10^{-1} \text{ ml/l of solution}$$

$$\equiv 19.73 \text{ ppm (v/v)}$$

Thus the concentration of hydrogen sulphide gas in solution required to produce a minimum headspace concentration detectable by lead acetate and DTNB test papers is 19.73 ppm (v/v).

Similarly, 4.23×10^{-4} g methanethiol per litre of solution (equivalent to 19.70 ppm (v/v)) produced the minimum headspace concentration detectable by DTNB test papers.