Microbiology of the Avian Egg

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Preface

There is a marked and most unfortunate dichotomy in the studies of avian eggs and hence in the application of new findings in commerce. Thus over the past twenty years there has been a renewed interest in the contributions of various parts of an egg to embryo development. This is best illustrated by those studies that have explored the diffusion of respiratory gases across the shell and at long last have provided a fundamental definition of a previously nebulous term, porosity. The activity in this general area has led in the past four years to the publication of three major books dealing with many aspects of egg structure, function and embryogenesis. When browsing over these books, two developments are evident. First, the advantages that are to be gained by comparative studies. Thus it is now common to see within a single book articles concerned with the eggs of a range of avian species as well as those of reptiles. Second, it is evident that zoologists and physiologists as well as those employed in large breeding firms are all contributing to an improvement of our knowledge of the egg's role in the breeding biology of birds.

Comparative studies are a very uncommon feature of studies concerned with bacterial infection of eggs. Moreover there is as yet little effort made to link studies of bacterial contamination of eggs with a fundamental aspect of eggs, the mechanisms allowing embryogenesis to occur without interference from saprophytic microorganisms that could colonize the yolk and deny the embryo its principal reserve of nutrients. Empirical observations together with laboratory studies in the period 1900–1960 provided an adequate basis for the mass production, distribution and marketing of eggs without fear of a high incidence of addling. The inadequacies of these data have been cruelly exposed by the current 'epidemic' of salmonellosis associated with egg products. Indeed this book was prepared with the objective of providing both an overview of current knowledge and a platform upon which to build future studies. The editors wish to thank all the contributors for their co-operation in producing this book.

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Hen's egg shell structure and function

S.E. SOLOMON, M.M. BAIN, S. CRANSTOUN AND V. NASCIMENTO

The formation of the physical barrier to bacterial ingress into the egg takes more than 20 h. During this time the yolk and albumen move from the isthmus region of the oviduct, in which the paired shell membranes are elaborated, to the tubular shell gland and shell gland pouch where mineralization proceeds (Arias *et al.*, 1993). The latter process is preceded by the addition of plumping fluid to the albumen. This increases the total volume of the latter and renders the flaccid shell membranes taut. Calcium salts from the oviducal fluid in which the egg is bathed, precipitate onto selected (nucleation) sites on the outer shell membrane. These sites are referred to as the mammillary knobs and the whole as the mammillary layer. The true shell consists of five morphologically distinct regions which, proceeding outwards from and including the mammillary knob layer, are designated the cone, palisade, vertical crystal layer and cuticle (Figure 1.1).

1.1 THE CUTICLE

Simons (1971) noted that cuticle thickness on the eggs of domestic hens varies from 0.5 to 12.8 µm over the surface of the same egg and has an effective lifespan of 96 h after oviposition (Vadehra *et al.*, 1970). The cuticle-less egg is not an unusual phenomenon (Sparks, 1985) and the patchy distribution of cuticle, which Board and Halls (1973) reported on 8% of all brown eggs studied in their experiments, is according to Alls *et al.* (1964) the result of varying pressure within the shell gland pouch. (Figures 1.2–1.4)

The present authors propose that cuticular variation is the norm, with age, strain and environment all exerting a profound effect on the degree of cuticular coverage. Assessment of the latter using the dye 'Pea Green' is both subjective and open to criticism as to its accuracy; thus eggs rendered
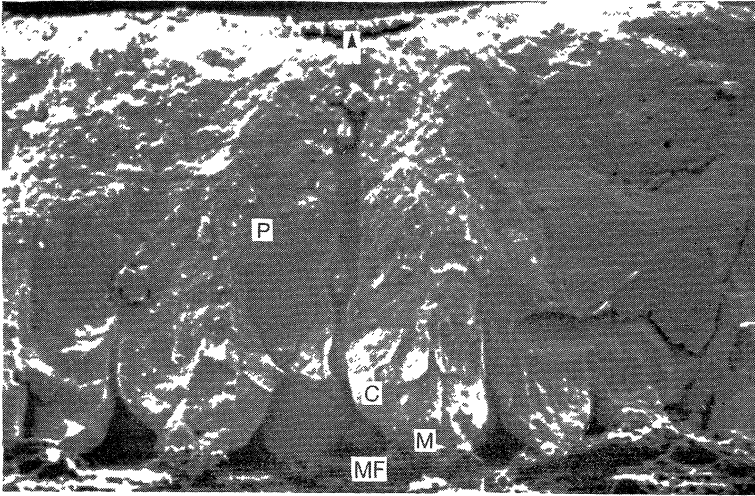
Figure 1.1 Transverse section through the shell. The cuticle can be seen at the opening of the pore canal (arrow). P, palisade layer; C, cone layer; M, mammillary layer; MF, membrane fibres. Scanning electron micrograph. Shell fragments dislodged during the fracture process have accumulated in the pore canal. ×240.

green by the dye have subsequently been observed to have no cuticle when viewed at ultrastructural level (unpublished results), neither can the dye reveal minor variations in cuticle thickness and the microscopic abrasions which negate its role as an effective barrier.
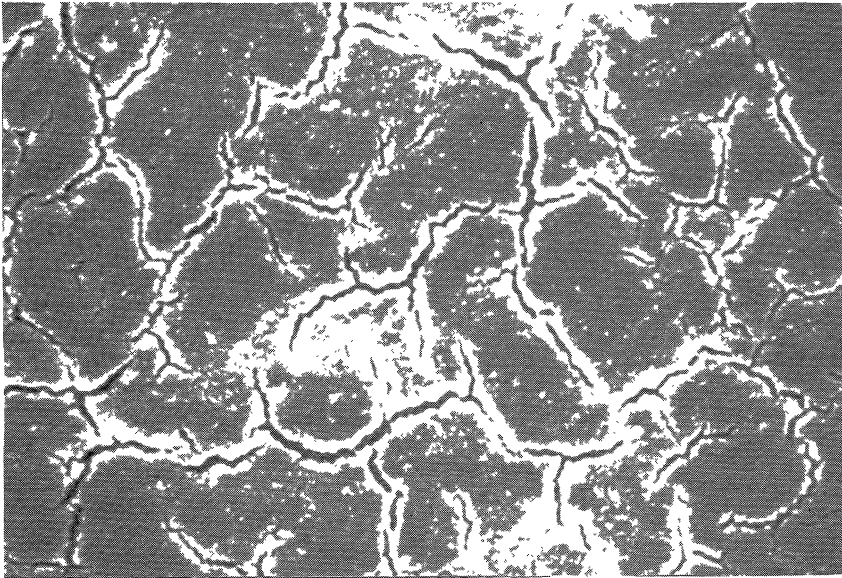
Figure 1.2 SEM image of the outer surface of the cuticle, illustrating its highly fissured appearance. ×1080.
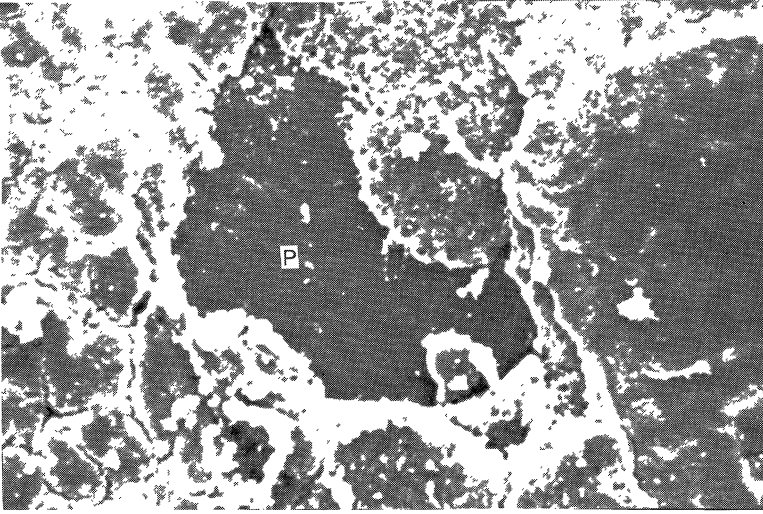
Figure 1.3 Part of the cuticle is missing, and the palisade layer (P) exposed. ×965.

The presence of the pigment, protoporphyrin, is unequivocal on the cuticular surface of the brown egg. However, it is not confined to the cuticular complex but also occurs in association with the upper palisade and vertical crystal layers of the shell. Protoporphyrin is also present in white eggs. As the egg leaves the cloaca, the cuticle is moist and immature (Sparks, 1985). As the egg cools, the cuticle hardens and so achieves maturity.

Theoretically the cuticle subserves a number of diverse functions, varying
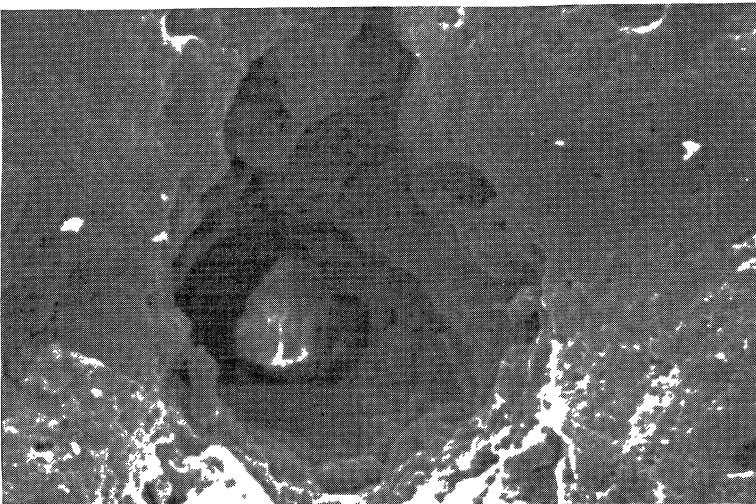
Figure 1.4 This shell had no cuticle. Note the pore canal. ×965.

from reducing water loss to the first line of defence against bacterial penetration. The biological role of the cuticle is discussed in Chapter 2. With reference to penetration, it has been observed to block the external surface of pores. According to Wedrel *et al.* (1974), the cuticle consists of 85–87% protein, 3.5–4.4% carbohydrate, 2.5–3.5% fat and 3.5% ash. It is assumed to derive in part from the secretions of the non-ciliated cells lining the shell gland pouch. Following oviposition such cells are devoid of their normal complement of electron dense granules (Solomon, 1991).

1.2 THE PORES

The shell of the hen's egg is permeated by a variable number of pores ranging from 7000 to 17 000 (Tyler, 1953; Simkiss, 1968), with the greatest number occurring at the equator or blunt pole of the egg. Not all pores extend through the entire depth of the shell and their aetiology is still uncertain. According to Schmidt (1966) these openings arise as a result of the incomplete fusion of cones. This theory was developed by Tullett (1975), who found a positive linear correlation (r=0.918) between the number of mammillae 0.25/m^2 and the number of pores/mm^2. He hypothesized that the packing of mammillae was crucial to pore formation. Despite the fact that such openings breach the integrity of the shell, there is still doubt as to whether they represent the sole portal of entry for microorganisms. Fromm and Munroe (1960) correlated porosity with bacterial penetration; Reinke and Baker (1966) refuted this view. Nascimento (1993) has demonstrated a positive correlation between specific shell defects and bacterial transfer, namely the presence of aberrant crystal forms such as aragonite, cubic calcite and the rounded type 'B' bodies which are characteristic of the eggs of both young and 'stressed' birds. His data suggest that pores play a minor role in this process.

1.3 THE TRUE SHELL

Immediately beneath the cuticular layer, the calcite crystals assume a vertical orientation (Figure 1.5). This narrow band overlies the polycrystal-line columns of the palisade which form the bulk of the true shell. During the growth period the former interlock (Figure 1.6). The earlier they fuse, the greater is the effective thickness of the shell (Bain, 1991). In common with other calcified tissues, an organic matrix (Figure 1.7) is present. It appears to be unevenly distributed throughout the true shell, its concentration increasing to a maximum two-thirds of the way through the thickness of the shell and rapidly decreasing thereafter (Cooke and Balch, 1970;
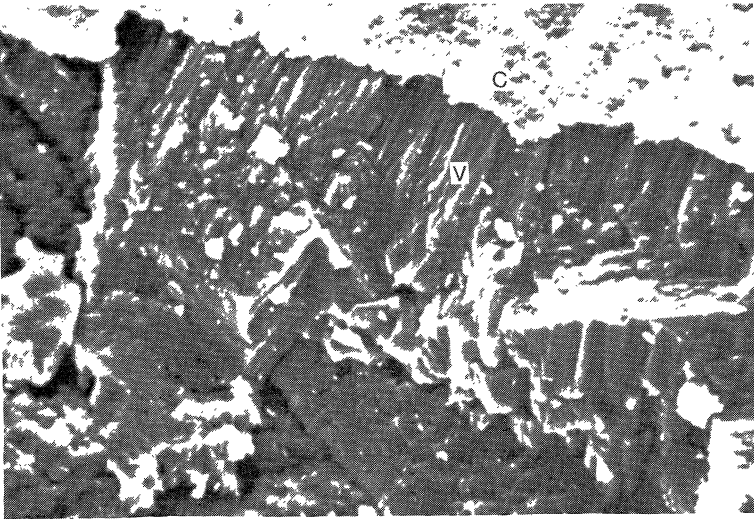
Figure 1.5 The vertical crystal layer (V) overlaid by cuticle (C). ×3750.

Simons, 1971). The matrix, a protein/polysaccharide complex with calcium binding properties, is formed from peptides synthesized in the liver (Eckert *et al.*, 1986). In 1980 Krampitz *et al.* isolated the calcium-binding polypeptide, ovocalcin, from the matrix. (Figure 1.7).

The palisade layer when considered at the ultrastructural level is characterized by the presence of vesicular holes which vary both in number and size (Bain, 1990). Peterson and Tyler (1967) and Simons (1971) proposed a
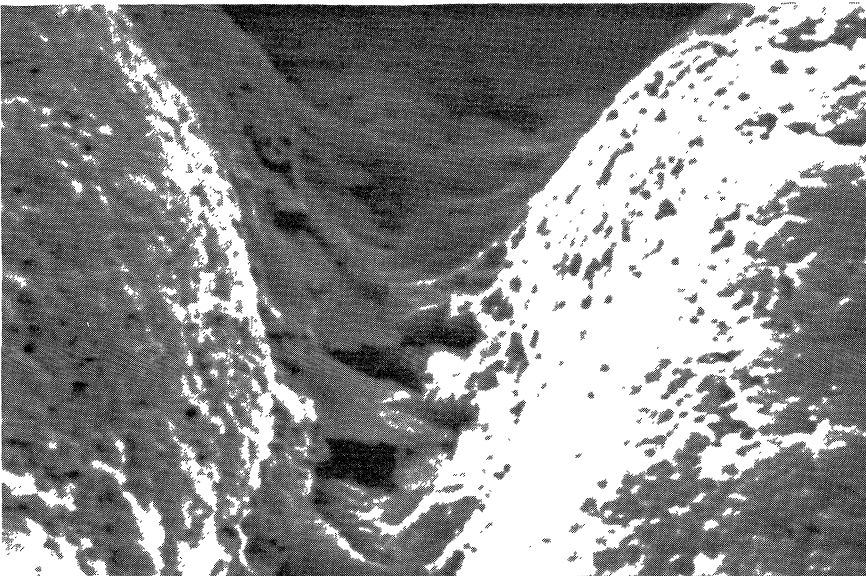
Figure 1.6 Interlocking palisade columns viewed from mammillary surface of the shell. ×8440.
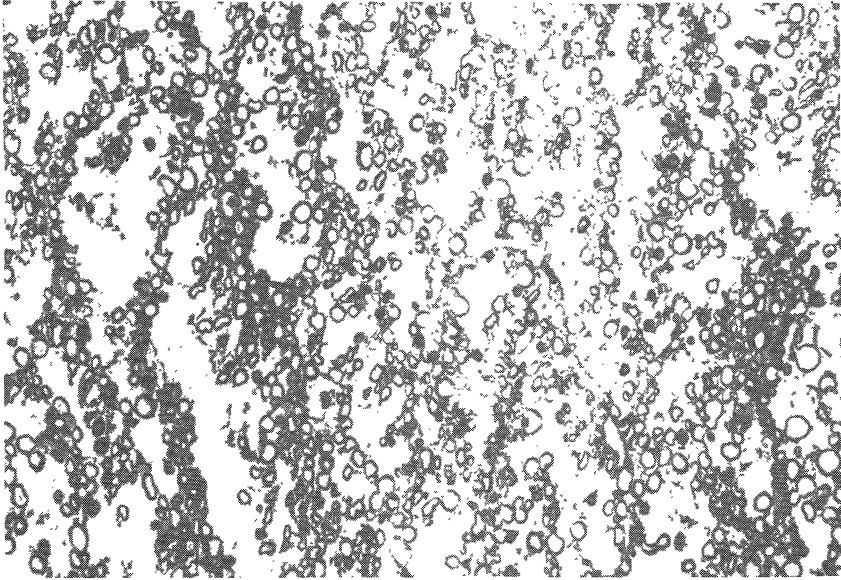
Figure 1.7 The organic matrix can be studied by electron microscopy by first decalcifying the shell with EDTA.(TEM) ×6750.

correlation between the arrangement of vesicular holes and the distribution of organic matrix. The latter author thus subdivided the palisade layer into an inner spongy layer and outer compact region. According to Bain (1990) this abrupt division does not occur. She substantiated her hypothesis by the observation that, when shell samples are chemically thinned, they display only a moderate increase in non-destructive deformation. If the shell were clearly divisible into a compact and spongy zone, then a more
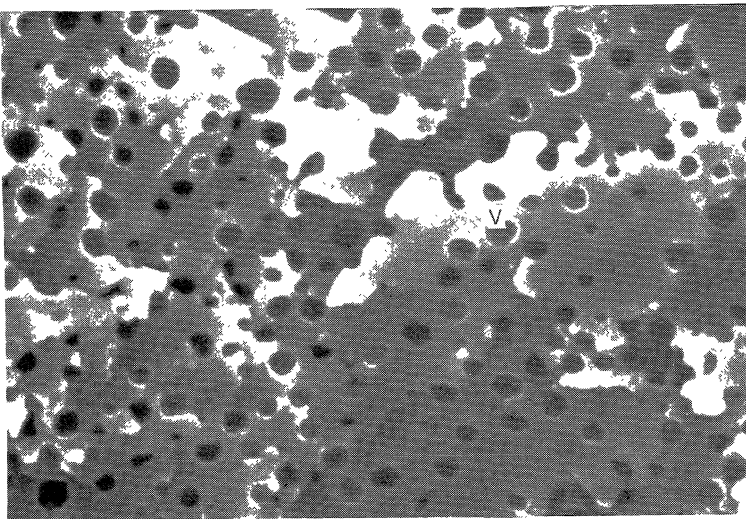
Figure 1.8 Vesicular holes (V) give the palisade layer its characteristic 'spongy' appearance when viewed in transverse section. ×7540.
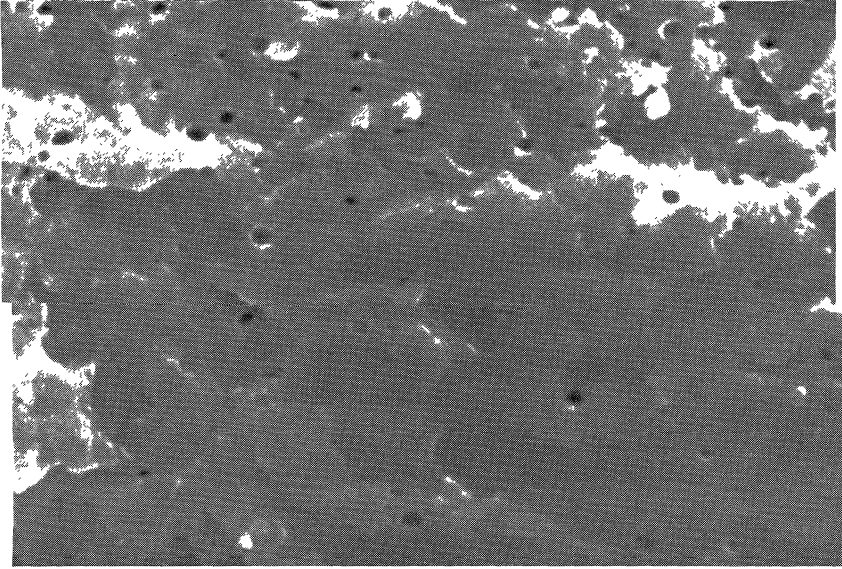
Figure 1.9 The size and number of vesicles vary within the palisade layer. In general the outer palisade layer has a more compact appearance. ×8440.

abrupt change in stiffness might have been anticipated as the more porous material became exposed (Figures 1.8 and 1.9).

Variations from the 'norm' do occur within the palisade layer; these are generally the 'knock-on' effect of deficiencies at the nucleation surface. The initial bonding between the shell membranes and the first
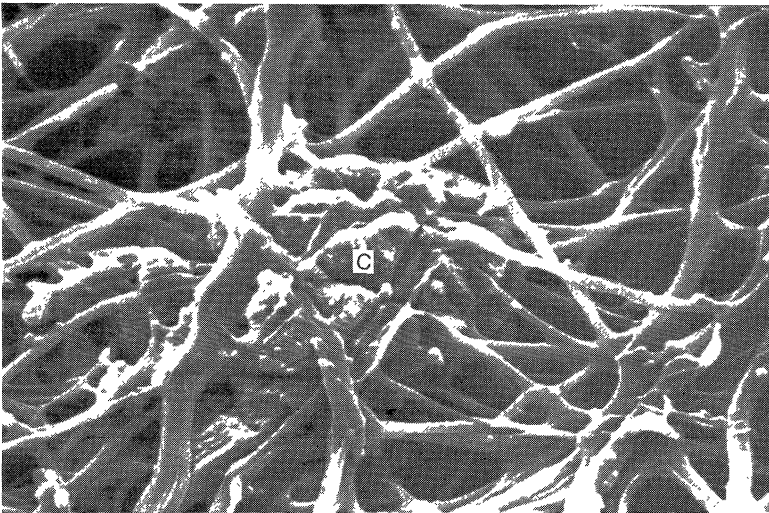
Figure 1.10 The inner surface of the shell. The crystals of the cone tip (C) penetrate the outer shell membrane. ×1880.
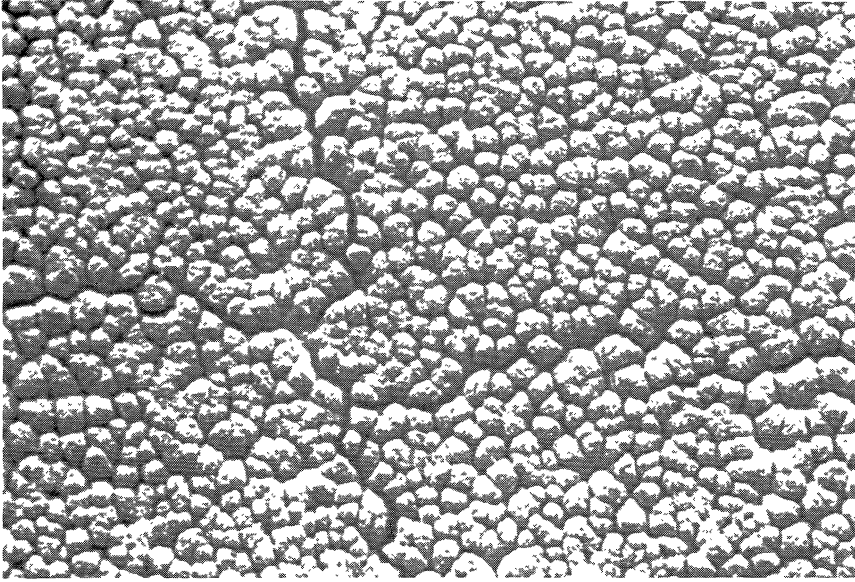
Figure 1.11 Crack lines induced by loading tend to follow paths of alignment. ×540.

crystals to precipitate is critical to the formation of the succeeding layers (Figure 1.10); indeed as will be described subsequently, when a crack occurs, it does so in the first instance at the level of the mammillary layer. Thus the organization of the calcite crystals at this level will not only dictate the origin of the crack site, but determine whether or not the crack will propagate or be checked in its progress through the shell.

Ultrastructural analyses of the eggshells of wild birds and pure lines of domesticated breeds, serve to illustrate that a basic pattern exists with respect to the construction of the shell and its prime function as a source of calcium and magnesium for embryonic development. Variations from the desired pattern do of course occur and there is preliminary evidence from analyses of the eggs of broiler breeders to correlate the inclusion of aberrant crystal

Table 1.1 Structural variations in the eggshell which alter resistance to bacterial penetration

Decrease resistance	Increase resistance
Late fusion	Early fusion
Type Bs	Good cap formation (i.e., close binding between organic and inorganic fractions of shell)
Type As	Cuffing
Aragonite	Confluent mammillae
Pitting: depressions, erosions, pin holes	A high mammillary density
Alignment of mammillae	
Cubics	
Changed membrane	
A low mammillary density	
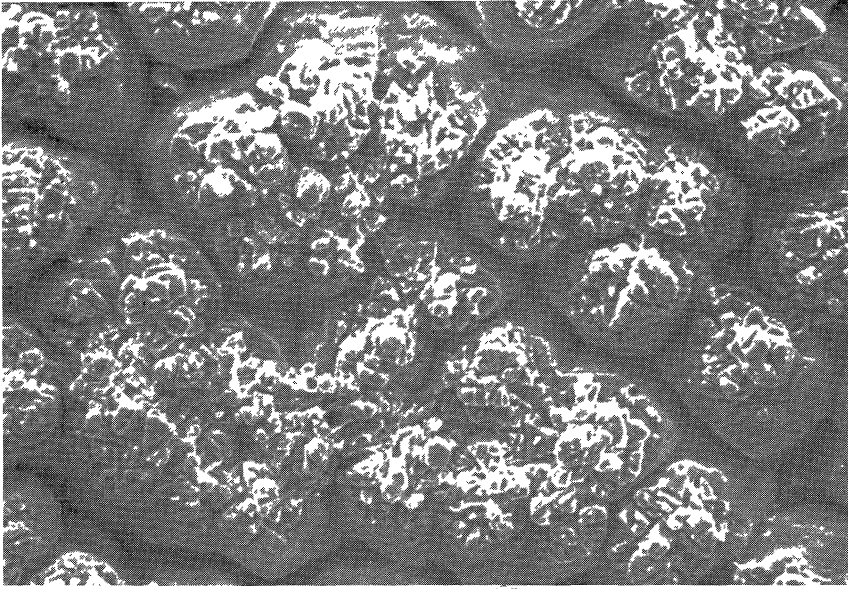
Figure 1.12 Early fusion of adjacent palisade columns, increases the effective thickness of the shell. ×540.

forms with failure to hatch (Roberts *et al.* 1992). The ten structural variations observed by the present authors, in the eggs of commercial layers in the UK, Canada and Australia are illustrated in Figures 1.11–1.20.

This can be broadly grouped into features which reflect changes in the
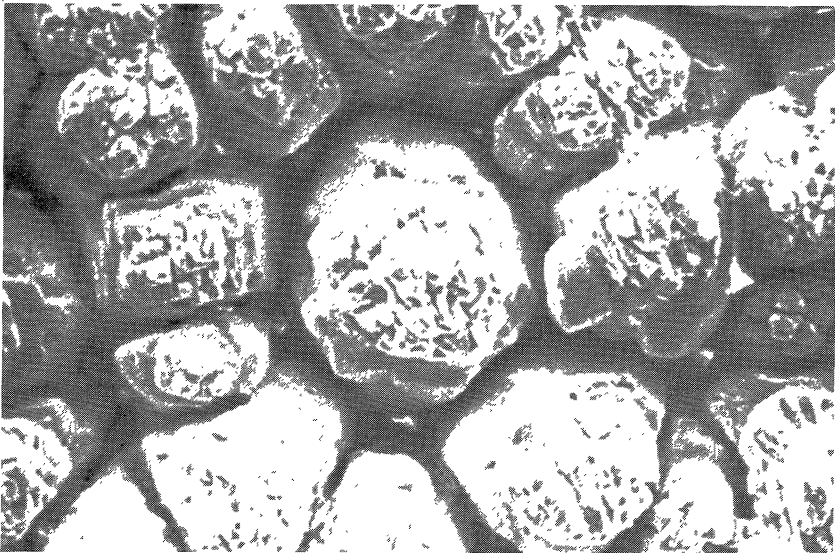
Figure 1.13 Late fusion of palisade columns. ×540.
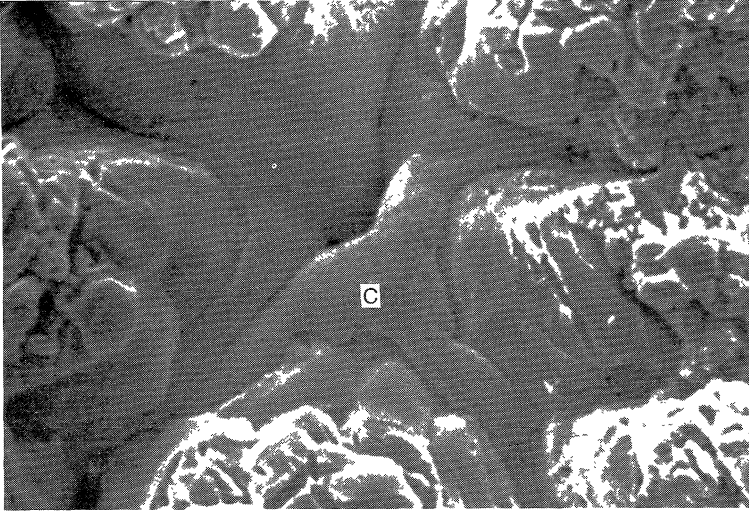
Figure 1.14 The extra cuff of calcium carbonate (C) serves to fill in the spaces between adjacent palisade columns. ×965.

rate of mineralization, i.e. are the result of altered conditions (organic and/or inorganic), in the shell gland pouch and those which have their origin proximal to the pouch region (Table 1.1). The latter is best illustrated with reference to the effect of infectious bronchitis on oviduct architecture and function.

Jones and Jordan (1970) observed the anterior portion of the oviduct to be most severely affected. Crinion *et al.* (1971a, b) correlated the production
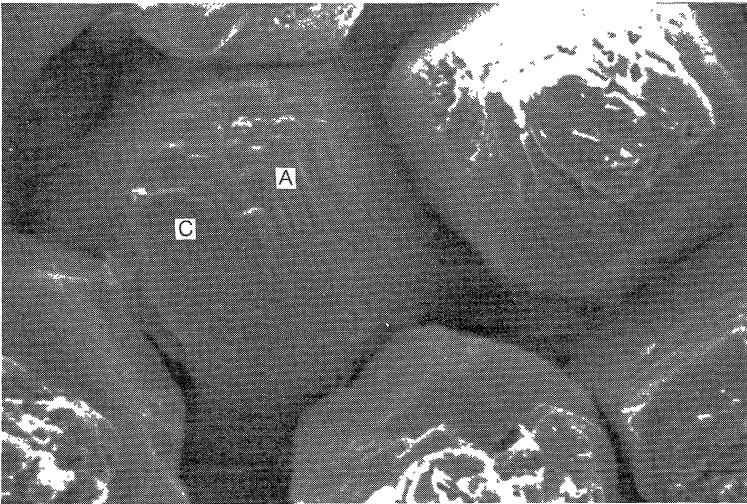
Figure 1.15 A type 'A' body displaying no obvious membrane attachment area on the cap (C) although continued mineralization has given rise to cone and palisade column at this point. ×1880.
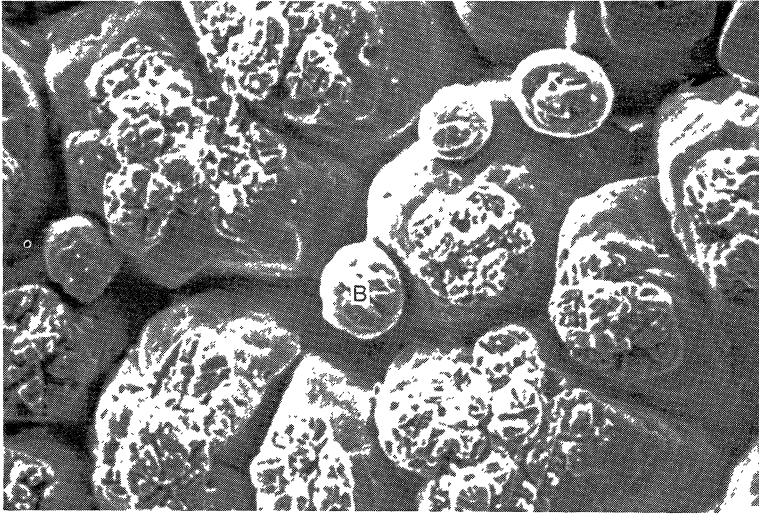
Figure 1.16 Rounded type 'B' bodies display evidence of minimal contact with the membrane fibres. They do not contribute to the thickness of the palisade layer and nucleate on the sides of more typical mammillae. ×480.

of poor quality albumen and shell with glandular hyperplasia in the magnum and regions distal to it. In the presence of a watery albumen mass, the shell membranes are irregularly disposed and in consequence the nucleation sites are altered. Whether this alteration is physical and/or
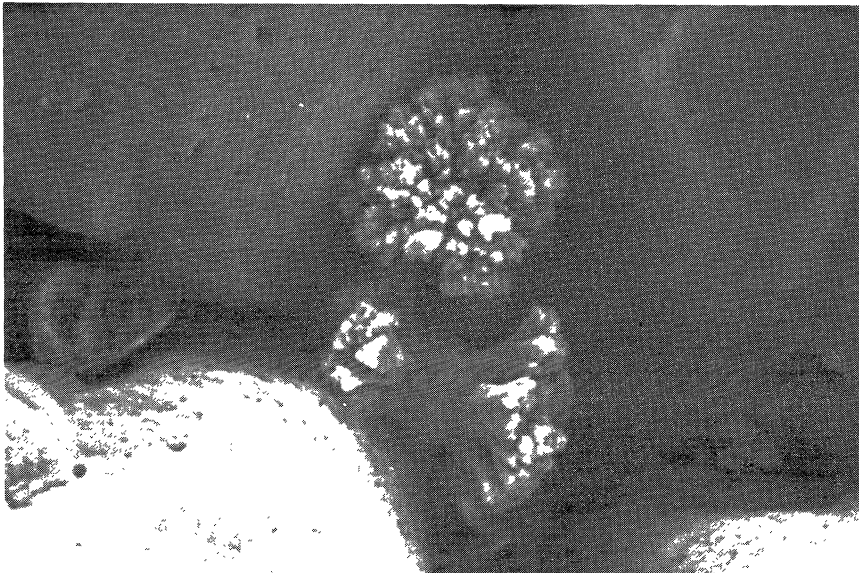
Figure 1.17 Aragonite crystals in the intermammillary space. ×2100.
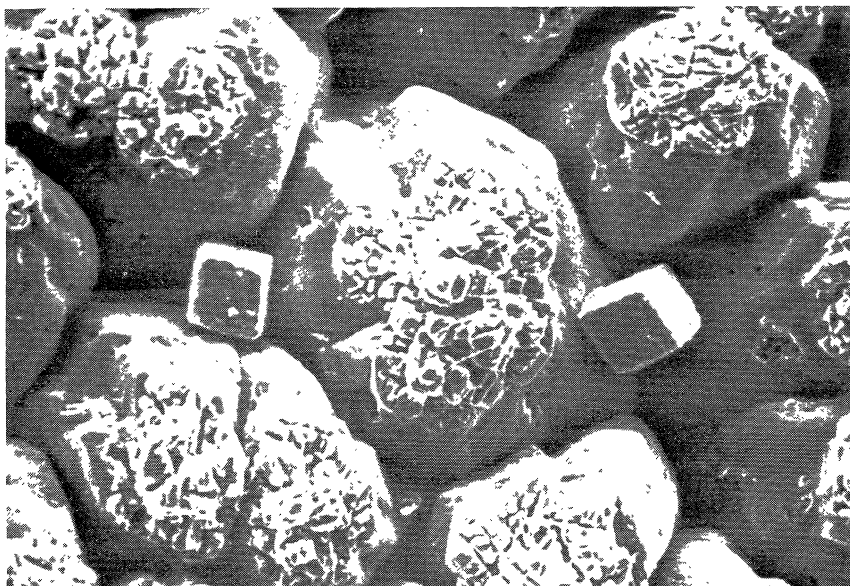
Figure 1.18   Cubic calcite confirmed by infrared analysis. ×1080.
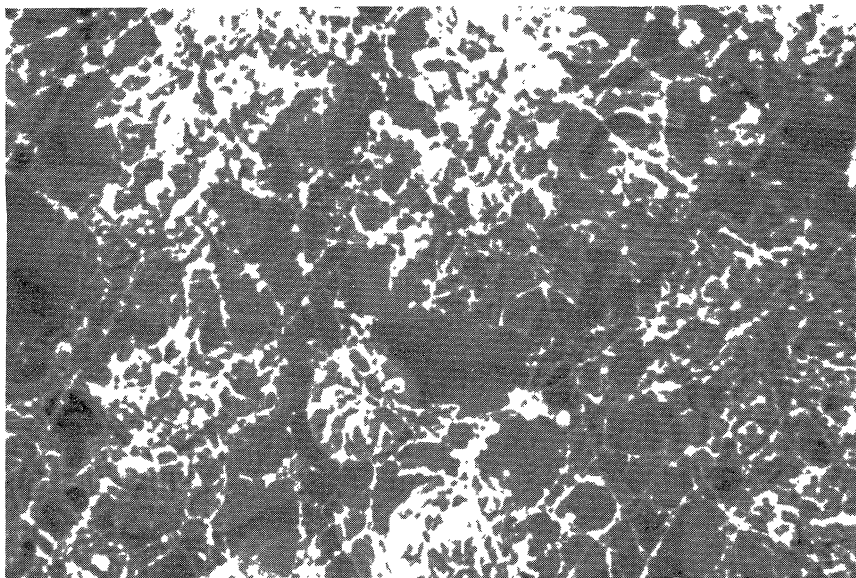
Figure 1.19   The process of plasma etching normally removes the organic membrane fibres, thereby exposing the mammillary layer. In the presence of sulphur-rich membrane fibres, normal ashing times are inadequate to dissociate the two. ×540.
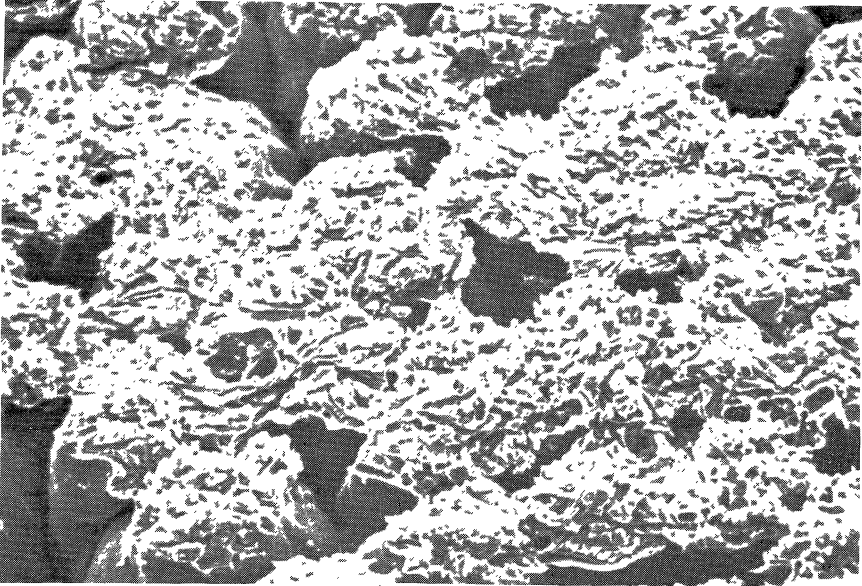
Figure 1.20 Confluence. The mammillary caps are fused, making it difficult to distinguish individual bodies. Such fusion occurs when nucleation sites are clumped. ×540.

chemical is still a matter of conjecture, but the end result is disorganization in the deeper layers of the shell.

The eggshell can contain any of the three morphological forms of calcium
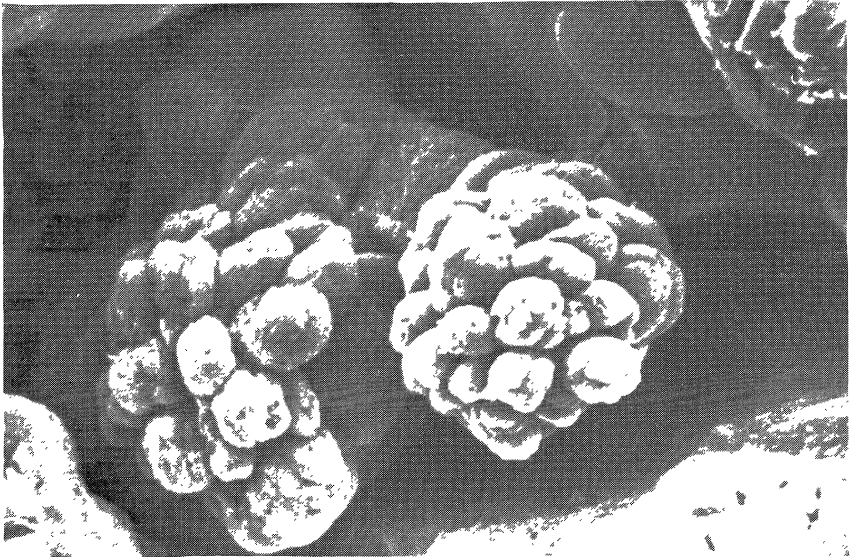
Figure 1.21 Aragonite can assume a variety of crystal forms, namely grape-like crystals observed in the intermammillary region and identified by infrared analysis. ×2100.
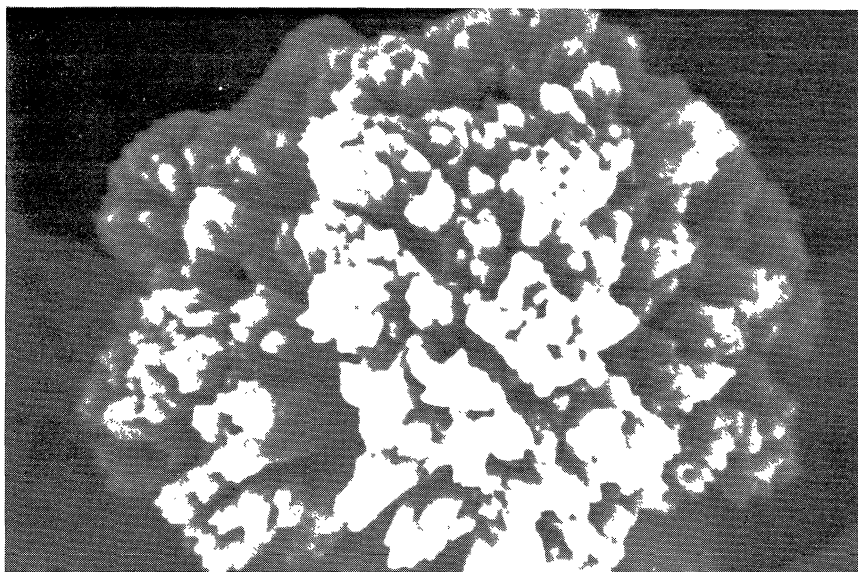
Figure 1.22 Aragonite – spicular form in the inter-mammillary space. Typical of the reptilian eggshell. ×4200.

carbonate, i.e. calcite, aragonite and vaterite. Thus although it is composed primarily of calcite, aragonite is frequently observed within the mammillary layer (Figures 1.21 and 1.22). Both stress and age increase the incidence of aragonite within the mammillary layer (Watt, 1989). The least stable form, vaterite, has been identified on the surface of soft-shelled eggs (Tullett *et al.*, 1976; Chapter 2).

1.3.1 Relating shell structure to function

The eggshell is nature's way of protecting the developing avian embryo outside the hen. Thus in addition to the requirements previously discussed, the shell must be strong enough to withstand the weight of the broody hen, yet be sufficiently weak to allow the chick to break out at the end of incubation. It must be stiff enough to resist distortion but it must also have an inherent elasticity so that it can dissipate and distribute the energy of shock loadings.

Any form of damage or defect in the shell greatly increases the risk of penetration by microorganisms. In a modern intensive battery system, damage to the shell may be caused by the height from which the egg is dropped onto the cage floor at oviposition (Carter, 1970), or when one egg collides with another egg or part of the collecting machinery (Anderson and Carter, 1972). Proper packaging and handling during transportation have also been shown to be of vital importance in ensuring that the egg
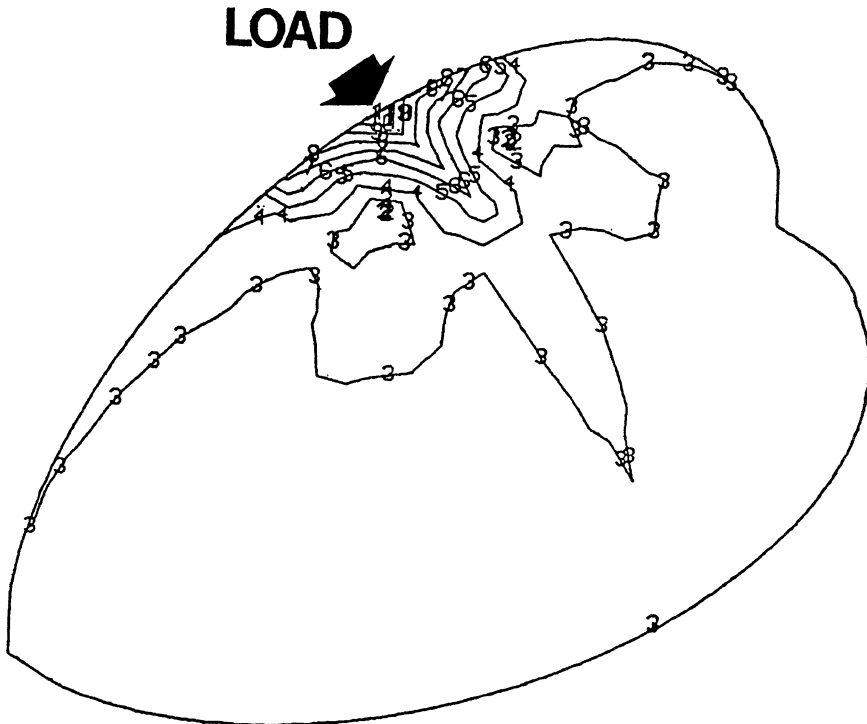
Figure 1.23 According to finite element analysis tensile stresses are induced when a point load is applied to the equatorial region of an egg shell. The numbers on the contour lines represent increasing levels of stress from 1 to 10. The stresses are at a maximum directly beneath the load.

reaches the consumer intact (Nethercote *et al.*, 1974). Ideally, and to satisfy this market, the eggshell might perform better if it were made of a more spongy or elastic material, i.e. if it were tough and flexible rather than hard and brittle. There is now evidence, however, to suggest that an eggshell would be less vulnerable to the above types of damage if it were round rather than elongate, thicker rather than thinner, and more importantly if it possessed ultrastructural characteristics which increased its resistance to crack growth.

1.3.2 Application of modern engineering principles to the case of the eggshell under load

To analyse the reaction of complex multilayered structures to loading, engineers routinely make use of advanced computer modelling techniques such as the finite element method (Ross, 1985). By applying this technology to the case of the eggshell under load (Figure 1.23), Bain (1990) was able to determine the relevant material and structural variables associated with the stiffness and strength characteristics of eggshells.
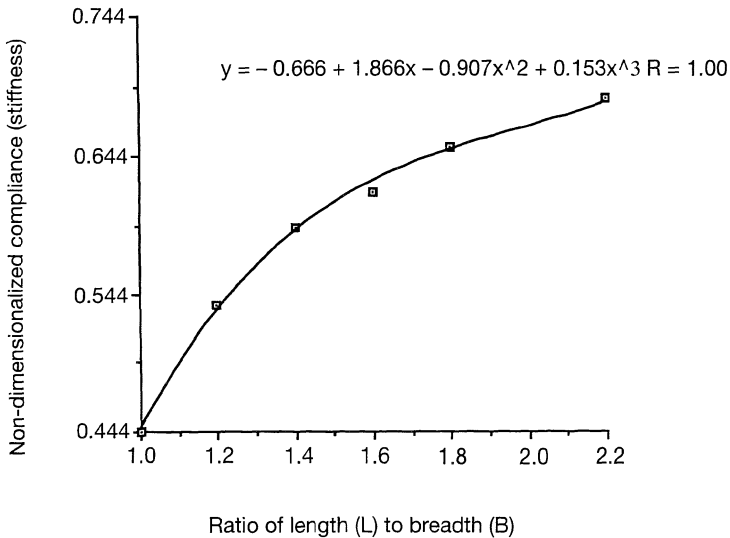
Figure 1.24 The influence of eggshape on shell stiffness characteristics according to finite element analysis (Sphere L:B=1; Eggshape 1<L:B<2.2). (From Bain (1990), with permission.)

1.3.3 Reinterpreting eggshell strength and the mechanism of failure in eggshells:

The stiffness characteristics of the eggshell are determined by the eggshell's
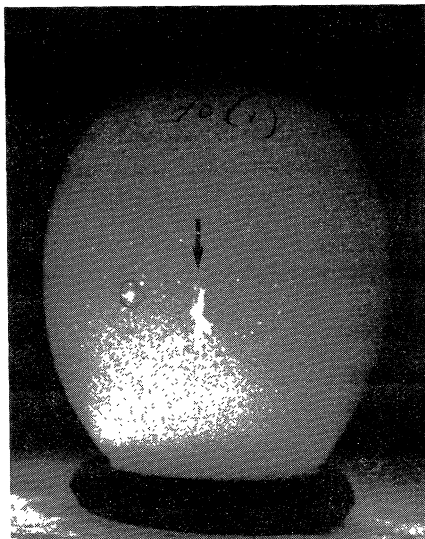
Figure 1.25 This egg was subjected to a non-destructive point load. To the naked eye the shell appeared intact, but candling revealed the presence of a translucent patch directly beneath the point at which the load had been applied (arrow). Upon further investigation microscopic radiating cracks were found within this area. The circled area(s) denote translucent patches which existed prior to testing.
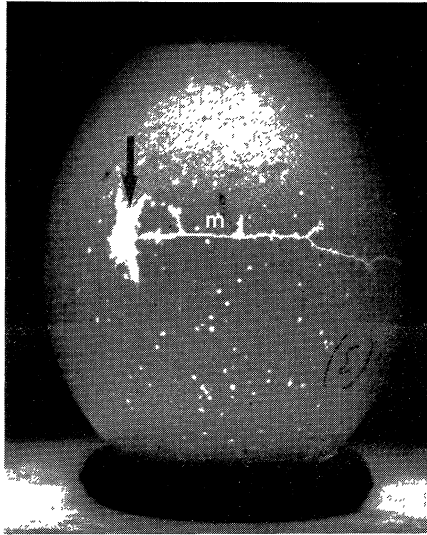
Figure 1.26 Typical type of damage induced to the shell as a result of quasistatic compression tests. A series of radiating cracks were found within the 'crack initiation zone' (arrowed), accompanied by one or more major cracks (m). (From Bain (1990), with permission.)

elastic modulus and the modifying effects on this caused by geometry which includes the combined thickness of the palisade and vertical crystal-line layers (henceforth referred to as the shell's effective thickness) (Carter, 1970; Bain, 1990).

In general the elastic modulus of eggshell is similar to that of other calcified tissues such as bone but on an individual egg basis this material property can vary. Nevertheless strain/age specific differences in stiffness characteristics are best explained in terms of an increase or decrease in the effective thickness of the shell. Egg shape has a significant role to play in determining the eggshell's stiffness characteristics (Figure 1.24).

It is generally accepted that failure is initiated in a structure due to a build up of internal stress. Stress cannot cross empty space and so the character-istic notched appearance of the mammillary layer provides the ideal site for the development of a crack. Experimental observations also indicate that crack initiation precedes the point at which an egg has obviously fractured and failed (Figures 1.25 and 1.26).

The type of localized damage shown in Figure 1.25 is not likely to be detected even by the most experienced candler as it takes several hours for the shell contents to penetrate these traumatized sites. In contrast the broken shell, by virtue of its gross imperfection fails to function in any capacity and in most cases will be identified either at the processing plant or at the retail outlet. In terms of the risk of food spoilage the traumatized eggshell is therefore potentially the more hazardous, and so while attention

should continue to be directed towards reducing the risk of insult experiences by the eggshell in the field, consideration should also be given to improving the fracture toughness of eggshells.

The fracture toughness of an eggshell is a measure of its resistance to the unstable form of crack growth normally associated with absolute shell failure (Figure 1.26). According to Bain (1990) there appears to be a link between the fracture toughness of eggshells and the structural organization of the mammillary layer. Thus where fusion is late, crack propagation through the shell wall, and thereafter outwards from the load point, will occur more rapidly. Similarly the arrangement of mammillae and the presence of depressions and erosions appear to facilitate crack propagation (Figure 1.11) whereas early fusion and a random arrangement of the mammillae resist the growth of cracks. The mammillary layer provides the foundation for subsequent shell formation and a tenuous link between the true shell and the membrane fibres often results in the production of a thinner shell.

1.4 THE MEMBRANES

The close physical bonding observed between the shell membranes and the mammillary caps in the avian egg is not observed in its reptilian counterpart, a feature which permits the latter to remove water from the moist sand substrate into which it is oviposited. In birds, the egg swells *in utero* prior to the process of mineralization. The paired shell membranes are approx.
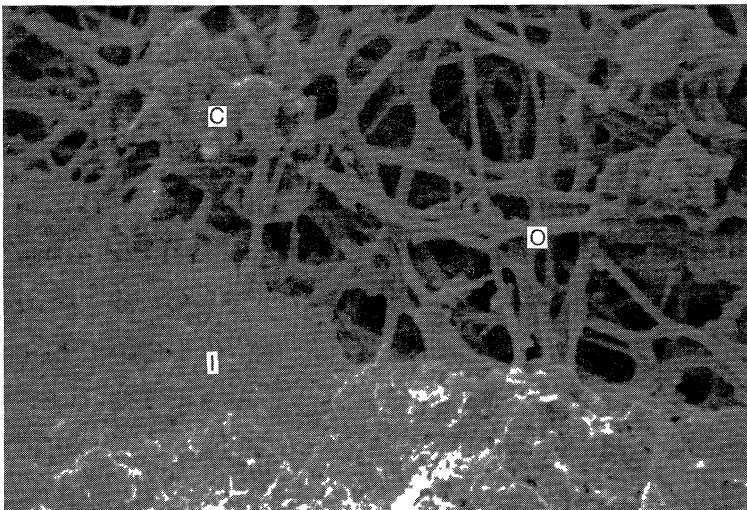
Figure 1.27 The inner (I) and outer (O) shell membranes. Note the calcium deposits (C) associated with the latter. ×965.

70 µm thick (Simons and Wiertz, 1963) and held firmly together, except at the blunt end of the egg, where they separate to enclose the air space. The inner shell membrane (ISM) envelops the albumen, and the outer shell membrane (OSM) is attached to the true shell (Mayes and Takeballi, 1983). Moran and Hale (1936) and Simons and Wiertz (1963) describe three layers of fibres in the OSM whereas the ISM has only two distinct layers (Figure 1.27).

Masshoff and Stolpmann (1961) demonstrated that the egg membranes consist of a network of branched fibres with pores of approx. 1 µm diameter (Wolken, 1951; Bellairs and Boyd, 1969; Fujii and Tamura, 1970; Tung and Richards, 1972). According to Garibaldi and Stokes (1958) and Lifshitz *et al.* (1964), the OSM has interstices larger than bacterial dimensions.

The fibres are on average 0.8–1 µm thick, and each has a core composed mainly of an elastin-like protein surrounded by a less electron-dense mucopolysaccharide mantle of approx. 0.5 µm (Romanoff and Romanoff, 1949). The ISM is reported to be more porous than the OSM (Hays and Sumbardo, 1927 cited in Mayes and Takeballi, 1983), which is surprising in view of the reputation of the former as a more effective barrier to translocation of bacteria (Vadehra and Baker, 1972).

Simons and Wiertz (1963) describe the fibres of the OSM penetrating the inner part of the mammillary layer base and anchoring in its organic matrix. The first contact between membrane and mammillary layer is made by irregular protrusions at the extreme base of the mammilla, penetrating into the meshes of the fibre network. The membrane fibres penetrate each mammilla up to a depth of approx. 20 µm, i. e. about one-fifth of its height and so form the mammillary core (Tyler and Simkiss, 1959). With respect to the contact between ISM and OSM Simons and Wiertz (1963) describe a patchy and tenuous link between the two.

In relation to bacterial penetration, the shell membranes act as a filter (Haines and Moran, 1940; Garibaldi and Stokes, 1958), being more impenetrable to bacteria than the shell. Lifshitz *et al.* (1964) reported that the ISM was the most effective barrier in preventing bacterial penetration of the egg contents, the shell ranked second and the OSM was the least important. Membrane resistance is quickly breached when large bacterial inocula are used (Brooks, 1960; Hartung and Stadelman, 1962; Board, 1964; Board *et al.*, 1968), especially when eggs are held at 37°C (Board and Ayres, 1965), with the microorganisms having been recovered from the inner surface of the ISM within minutes of the challenge (Bean and MacLaury, 1959).

Based on this observation, Board and Fuller (1974) commented that the membranes are capable of imposing only a temporary barrier to the inward movement of bacteria. Once they have passed through the shell membranes, the viscosity of the albumen ensures that they remain in a clump.

Proteolysis has been implicated in bacterial penetration through the

membranes (Mayes and Takeballi, 1983). Brown *et al.* (1965) and Candlish (1972) found zones of hydrolysis surrounding the bacteria located in the membrane, thus supporting the contention that enzymes (mucinase, poly-saccharidase) are actively involved in the penetration process. Hartung and Stadelman (1963) hypothesized that enzymatic digestion by *Pseudomonas fluorescens* could possibly clear the material present between the fibres from the interstices, permitting early and rapid passage. Brown *et al.* (1965) claim the same can happen in a *Ps. aeruginosa* infection. As might be anticipated contrary evidence has also been produced to refute the role of proteolytic enzymes (Wedral, 1971; Vadehra and Baker, 1972).

Some authors claim that the shell membranes possess bactericidal activity. Kortkov (1957) (cited in Board (1966)) and Vadehra *et al.* (1972) detected lysozyme in the shell membranes and hypothesized that it played an important role in the defence of the developing embryo. This role has been categorically denied by Kraft *et al.* (1958) and, *sensu lato*, by Mayes and Takeballi (1983).

Growth of bacteria on the shell membranes *in situ* is restricted unless iron is supplied (Brooks, 1960; Board, 1964), and the antimicrobial properties of the albumen are believed to be primarily responsible for confining initial bacterial contamination to the shell membranes. Multiplication occurs only when the yolk makes contact with the ISM (Board, 1964; Board and Ayres, 1965). It is more prone to happen in eggs held at room temperature (Board, 1964) or in aged eggs (Hartung and Stadelman, 1963). This topic is discussed in Chapter 3.
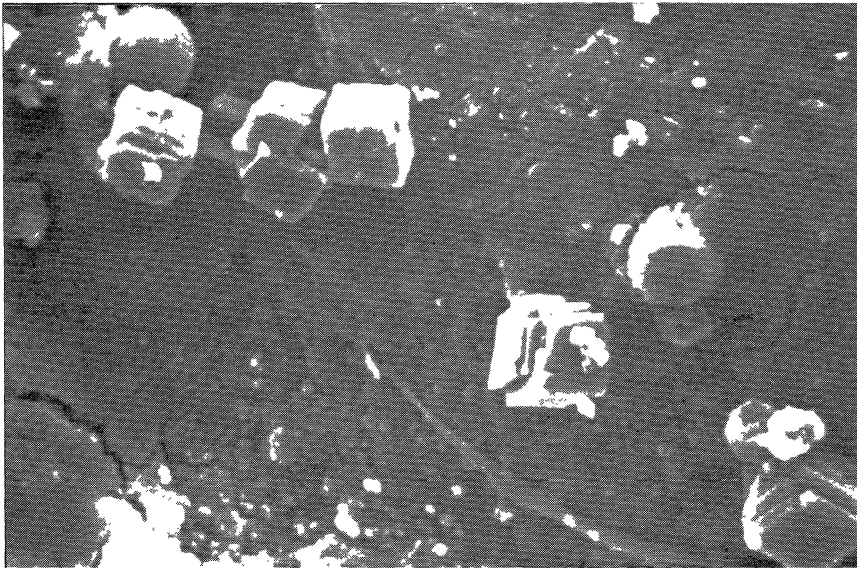
Figure 1.28 Chloride complexes on the surface of the shell after washing, identified using EDAX analysis. ×2100.
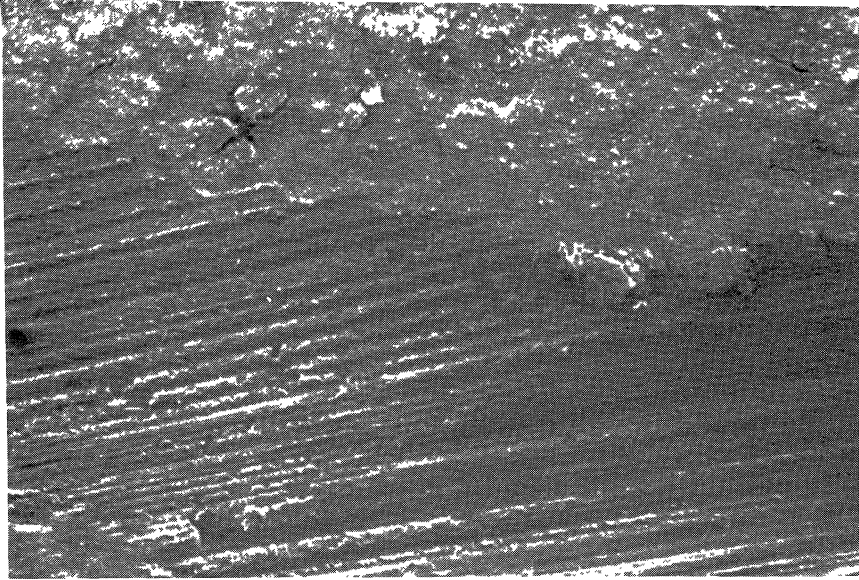
Figure 1.29 The parallel gouges are the result of brush action on the cuticular layer. ×135.

Vadehra *et al.* (1969) showed that cracked eggs were no more liable to infection by *Salmonella* than were normal-shelled eggs, provided they were handled properly. As the membranes are exposed in cracked eggs, the chlorine solutions used to wash the eggs – or even chelating agents such as EDTA or penicillamine (Vadehra and Baker, 1970) – will probably damage the structure of the membranes thus making them more permeable to food poisoning and spoilage bacteria.

Shell quality has been well documented (Bain, 1990) and inter- and intra-clutch differences recorded (unpublished results). Stress effects are manifold; thus slab-sided, body-checked and splashed eggs can be induced by inappropriate husbandry and changes in stock density (Hughes and Black, 1976; Watt, 1989; Solomon and Watt, 1988). Such eggs together with shell-less and soft-shelled eggs are easily detected during routine inspection. These will be downgraded. Many seemingly sound eggs escape the net of inspection only to be broken in transit from the packing station to the consumer.

The subjective process of egg candling, used as it is to highlight cracks and the inclusion of blood and meat spots, also serves to reveal variations in density within the shell (Figure 1.25). These translucent areas which are the result of water accumulation from the breakdown of albumen provide visual evidence of structural imperfection or localized trauma; thus the passage of water is facilitated by late fusion of the palisade columns and the clefts caused by the presence of oviducal debris on the shell membranes, which in turn disrupts the process of mineralization.

Public antipathy towards the cage system has witnessed the development and use of perchery, barn and free range systems, all affording different degrees of freedom, but at what price? Evidence suggests that these systems encourage floor egg laying, feather pecking, cannibalism and in perchery systems an increased incidence of bone breakage.

Floor egg laying carries with it the increased risk of faecal contamination and since 'dirties' do not command a price premium, such eggs will invariably be cleaned to improve their value.

The subject of egg washing is still a matter of debate in this country, although it is practised routinely in the USA, Canada and in certain parts of Australia. Done properly it will achieve its function of rendering the egg clean, while leaving it undamaged, but the egg is porous, it is not a standard product and the improper rinsing of eggs can leave unacceptable residues on the shell surface (Figure 1.28) while uncontrolled wash action can breach the barrier to different depths (Figure 1.29).

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Shell accessory materials: structure and function

N.H.C. SPARKS

2.1 INTRODUCTION

The outer surface of the avian eggshell is frequently covered by a distinct, pigmented layer which may cap or plug the pore canals. This layer occurs in a number of forms (Table 2.1), in contrast to the true shell which is similar in structure and chemical composition for all avian species. As a consequence, various terms have been used to describe the outer layer. Board and Scott (1980) proposed that the term 'shell accessory material' (SAM) be used to describe all layers occurring on the outer surface of the calcitic shell. Board and Scott's terminology has been adopted here, although where the layer is predominately inorganic or organic, the terms 'cover' or 'cuticle' are used respectively.

It is now recognized that SAMs play a crucial role in maintaining the integrity of the shell's gaseous diffusion pathway against obstruction by nest debris, flooding with water and in reducing the probability of bacterial penetration of the shell post-oviposition.

In this chapter, the chemical composition, structure and function of SAMs is discussed, with particular reference to the cuticle on the shell of the domestic chicken (*Gallus gallus domesticus*).

2.2 CHEMICAL COMPOSITION AND STRUCTURE

It has been recognized for many years that SAMs may be either inorganic, organic or a mixture of the two. In the late 1800s, von Nathusius speculated that the covers on eggs from *Phalacrocorax*, *Sula* and *Anhinga* spp. were composed of calcium salts (von Nathusius, 1884, 1887). The organic cuticle on the chicken's egg was described by Baudrimont and St Auge (1847). With the advent of electron and X-ray diffraction techniques, the crystalline

Table 2.1 Chemical composition of shell accessory material

Material	Species	Reference
Inorganic		
Calcium carbonate	Snake bird	Colacino *et al.*
(vaterite)	(*Anhinga anhinga*)	(1985)
	Gannet	Tullett *et al.*
	(*Sula bassanus*)	(1976)
	Cormorant	Tullett *et al.*
	(*Phalacrocorax carbo*)	(1976)
	Blue-Eyed Cormorant	Tullett *et al.*
	(*Phalacrocorax atriceps*)	(1976)
	Shag	Tullett *et al.*
	(*Phalacrocorax aristotelis*)	(1976)
	Brown Pelican	Gould (1972)
	(*Pelecanus occidentalis*)	
	Smooth-billed ani	Board and
	(*Crotophagi ani*)	Perrott (1979a)
(patches of)	Guira cuckoo	Board and
	(*Guira guira*)	Perrott (1979a)
Calcium phosphate	Flamingo	Board (1982)
(amorphous)	(*Phoenicopterus roseus*)	
	Megapodes	Board *et al.*
	(e.g. *Leipoa ocellata*)	(1982)
	Grebe	Board *et al.*
	(*Podiceps cristatus*)	(1984)
Uncharacterized	Rhea	Board *et al.*
	(*Rhea americana*)	(1977)
Organic		
Glycoprotein	Domestic hen	Wedral *et al.*
	(*Gallus gallus domesticus*)	(1974)
Uncharacterized	Kiwi	Board (1982)
	(*Aeptyrex* spp.)	
	Tinamous	Board and
	(e.g. *Eudromia elegans*)	Perrott (1979b)
	Jacana	Board and
	(e.g. *Micropara capensis*)	Perrott (1979b)

nature of many of the inorganic covers has enabled an accurate assessment to be made of the crystal type(s) present. By comparison, organic covers tend to be more complex in their composition. Their amorphous nature precludes analysis by crystal diffraction, and as a consequence, although techniques such as microprobe analysis and wet chemistry can be used to identify the component parts, it is more difficult to determine the overall chemical structure.

2.2.1 Inorganic shell accessory materials

The majority of inorganic SAMs that have been analysed are composed predominantly of vaterite (one of the four polymorphs of calcium carbonate; namely calcite, aragonite, vaterite and amorphous calcium carbonate). It is notable that all the shells examined from sea birds other than gulls
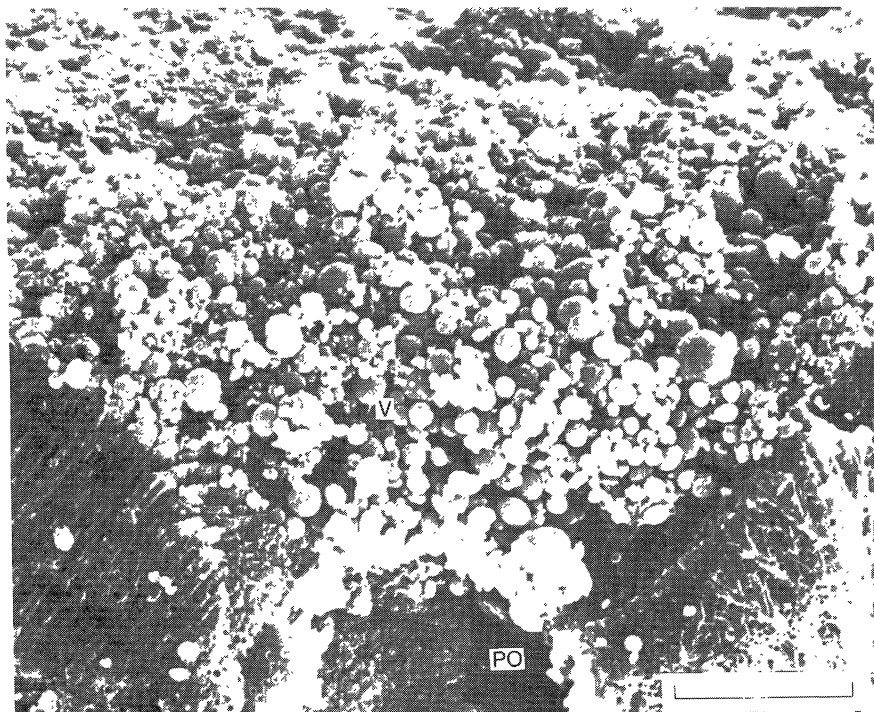
Figure 2.1 Radial section of *Anhinga anhinga* eggshell showing the outer pore orifice (PO) of a pore canal and a layer of spherical vaterite (V). Bar marker 10 μm.

(Board *et al.*, 1977), have a vaterite cover. Tullett *et al.* (1976) reported that the spheres of vaterite on the shell of the gannet (*Sula bassana*) were ca. 0.34–0.35 μm in diameter, comparable in size to the spheres forming the cover of shag (*Phalacrocorax aristotelis*) and cormorant (*Phalocrocorax carbo carbo*) eggs. Spheres of a similar diameter (0.5–3.0 μm) were reported by Colacino *et al.* (1985) on the cover (Figure 2.1) on shells from the snake bird (*Anhinga anhinga*). Although the thickness of the vaterite cover varies according to species, the percentage relative to the total shell thickness is remarkably consistent at ca. 11%. Thus, the cover on the snake bird's egg (Colacino *et al.*, 1985) was ca. 30 μm thick (ca. 10% of shell thickness), on the gannet's egg (Tullett *et al.*, 1976) ca. 60 μm thick (ca. 13% of shell thickness), on the shag's egg (Tyler, 1969) 35 μm thick (ca. 12% of shell thickness) and on the cormorant's egg 31 μm (ca. 10% of shell thickness). These figures are mean values, and it should not be inferred from this that the cover is evenly distributed over the surface of the shell. Indeed, in a study of 25 species (orders Podicipitformes and Pelecaniformes) Tyler (1969) noted 'variations in the cover, even over small areas of shell'. Similarly, the thickness of the cover does not indicate the extent to which the pores may be plugged. The pores in the shell of tinamou (Tinamidae), for example, are plugged (Board

and Perrott, 1979b), the uncharacterized material being confined to the pore orifice. This led Board and Perrott (1979b) to conclude that a substantial proportion of the pore plug and outer layer of the shell were laid down together.

   Given the prevalence of vaterite covers, it is notable that the shells of over 30 species of avians examined by Erben (1970) were predominantly calcite with a trace of aragonite. The mechanism that results in calcium carbonate being expressed as calcite in the shell and vaterite in the cover has yet to be elucidated. The mineralization process can be influenced by the ionic environment. Phosphorus, for example, will 'poison' or modify calcium carbonate mineralization (Simkiss, 1964). Tullett *et al.* (1976) postulated that the switch from calcite to vaterite deposition may be triggered by an increase (from <0.1 to 0.33–0.55 wt%) in the levels of phosphates at the mineralizing face. In a more recent study, Board *et al.* (1984) found that the phosphorus concentration was elevated in the outer part of the shell of the domestic chicken and concluded that factors other than phosphate poisoning of calcite formation were operating in the deposition of vaterite.

   By compartmentalization of biological space, organisms are able to control mineralization by physicochemical and spatial means, the organic macromolecules controlling crystal growth, morphology and aggregation (Mann, 1988). For example, the shell of the mollusc (*Monodonta labio*) is formed from a periodic arrangement of sheets of organic material interspersed with plates of aragonite – the organic phase modelling and determining the mineral phase (Mann, 1988). In contrast, spikey, needle-like crystals result from uncontrolled deposition of aragonite. Similarly, the uncontrolled precipitation of vaterite can give rise to spherical crystals, whereas the introduction of an organic phase can give rise to exquisite florets (Mann, 1988). By controlling the chemical environment and the structure and composition of the organic matrix, the snake bird produces a shell of well-ordered, cubic calcite. It is tempting to speculate that it is the lack of an organic interface and hence control over the mineralization process at the outer surface of the shell that results in the deposition of the thermodynamically more labile vaterite. Indeed, the author has noted the presence of spherical vaterite (0.5–3.0 μm in diameter) on a number of shell samples removed from the uterus of ducks *post mortem*, the vaterite being presumed to form due to the sudden and excessive release of Ca^{2+} into the lumen at the time of stunning (Figure 2.2). The vaterite polymorph may be stabilized by a biological inhibitor (e.g. metal ions, amino acids, phospholipids) blocking sites on the mineralizing face, thereby preventing crystal growth or transformation.

   The SAMs of all avian eggs so far examined are composed of spheres. An extreme example occurs on the grebe (*Podiceps* spp.) egg (Board *et al.* 1984; Sparks, unpublished observations), where a kernel formed from spherules is encapsulated by globular subunits, the resultant spheres being
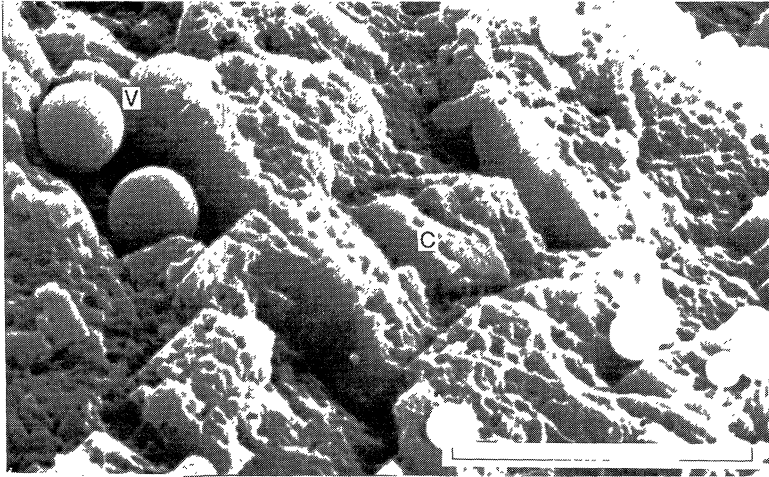
Figure 2.2 Mineralizing face of duck eggshell (approximately 8 h spent *in utero*) showing cubic crystals of calcite (C) overlaid by spherical vaterite (V). Bar marker 10 μm.

less than 2 μm in diameter (Figure 2.3a, b). Spheres, containing amorphous calcium phosphate, form a layer ca. 50 μm thick (17% of total shell thickness), which is capped by a thin fissured layer of amorphous material. Amorphous calcium phosphate is the most labile of calcium carbonate polymorphs. As the solubility of a solid phase increases and the phase becomes thermodynamically more unstable, an organic sheath is required to stabilize the inorganic phase. Board *et al.* (1984) speculated that the high sulphur levels that were detected in the cover resulted from a sulphur-containing organic matrix responsible for the spherules.

2.2.2 Organic shell accessory materials

Baudrimont and St Auge (1847) considered the cuticle on the chicken eggshell to be a structureless epithelial layer. Other studies (Krampitz and Witt, 1979) have indicated that the cuticle is a composite layer. When viewed from above with the electron microscope, the cuticle appears to be uneven, with many star-shaped cracks, fissures and flake-like layers (Parsons, 1982; Sparks, 1985). The larger cracks have a roughly radial orientation, commonly occurring over the outer pore orifice (Figure 2.4a, b). Like the inorganic equivalent, the cuticle is composed of spheres, which on the chicken's egg are <1 μm in diameter, forming an uneven layer 0.5–12.8 μm thick (Simons, 1971). The cuticle is similar in appearance on the eggs of partridge (*Alectoris rufa*), pheasant (*Phasianus colchicus*), domestic turkey (*Meleagris gallopavo*), duck (*Anas platyrhynchus*), and a number of wild ducks, such as the pink-eared duck (*Malacorhynchus membranaceus*) and Cape shelduck (*Tadorna cana*) (Sparks, unpublished observations). It is
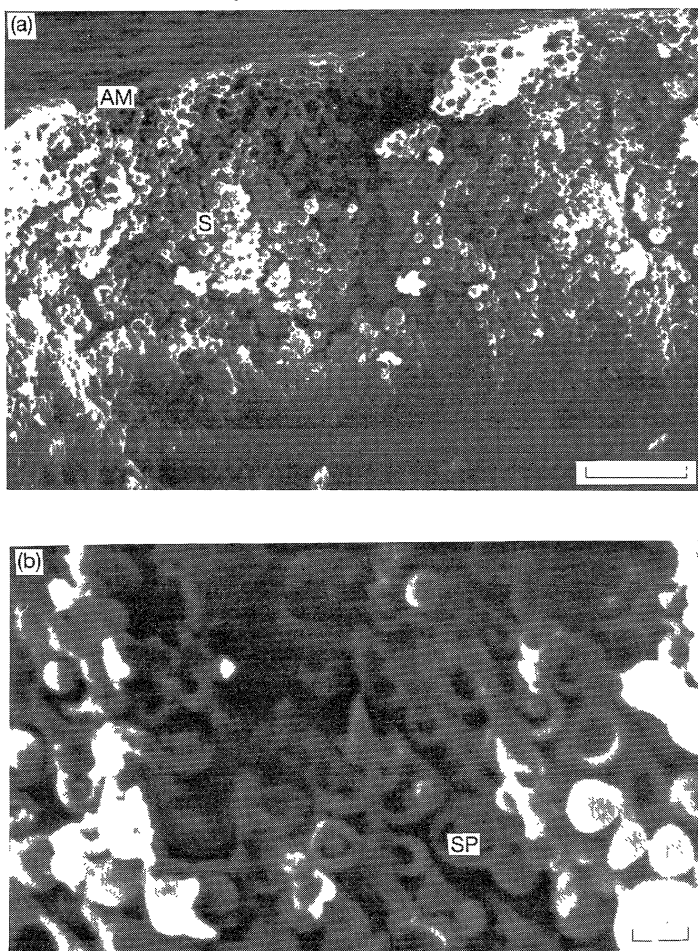
Figure 2.3   (a) Shell accessory material on an eggshell of great crested grebe (*Polideps cristatus*) showing amorphous material (AM) on outer surface of stratum of spheres (S). Bar marker 10 μm. (b) Composite spheres in shell accessory material on great crested grebe eggshell, showing the central aggregate of spherule (SP). Bar marker 1 μm.

notable that, when compared with brown chicken eggs, the cuticle associated with white chicken eggs is less dense in terms of vesicles (Simons and Wiertz, 1963). This is reflected in the more open appearance of the cuticle (Figure 2.5) when viewed using scanning electron microscopy (SEM) (Sparks, 1985).

At oviposition, the cuticle on the chicken's egg has a moist lustre. Within 2–3 min this disappears, subsequent wetting failing to reproduce the characteristic appearance. Sparks and Board (1985) used SEM to observe the changes that take place in the ultrastructure of the cuticle during this 'drying' period. They noted that at oviposition the cuticle had a very open,
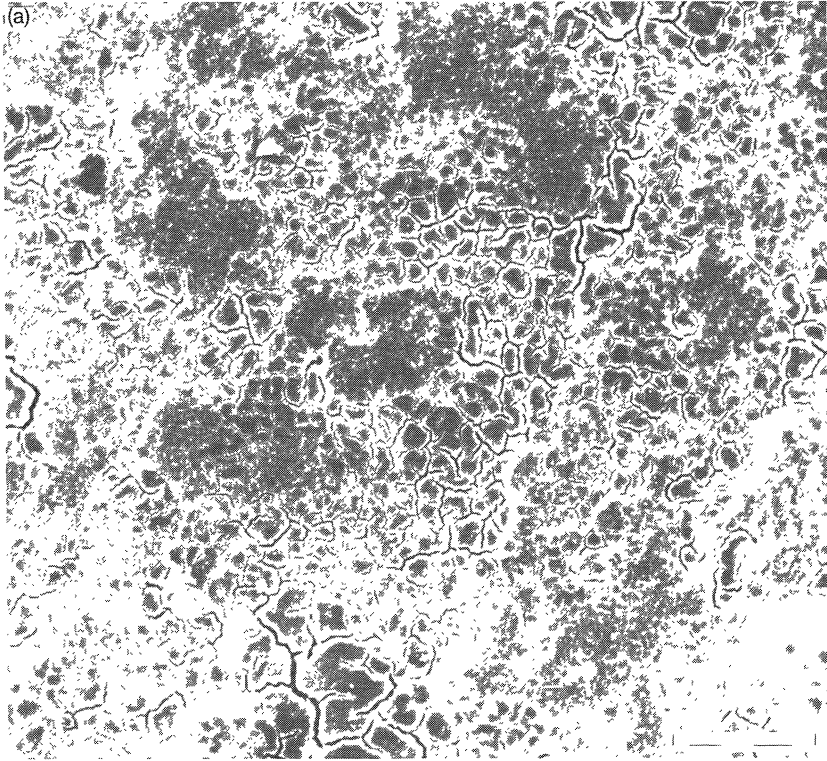
Figure 2.4 (a) Surface view of chicken's brown eggshell showing star cracks over pore orifice. Bar marker 100 µm.

frothy, granular appearance. As the cuticle dried, there was a concomitant change in the structure from open and sponge-like to the fissured, flake-like layers described above. Prolonged storage results in further changes in cuticle structure, the surface of the cuticle becoming flatter and the smaller cracks disappearing (Simons and Wiertz, 1966). The authors proposed that these changes resulted from a shrinking of the vesicles.

A recent study (N.H.C. Sparks, E.M. Drysdale and J. Bruce, unpublished observations) showed that individual chickens would consistently lay eggs which either had a very thin cuticle or were cuticle-less. This supports the conclusions of Ball *et al.* (1975), who suggested that cuticle 'quality' may be heritable. The same study noted that the thickness of the cuticle on one pole (invariably the sharp one) was frequently less than that on the remainder of the egg. Apart from abnormal situations such as 'slab-sided' eggs, which result from two eggs residing in the shell gland at the same time, cuticle thickness showed no variation around any one latitude, but often considerable variation from pole to pole. A similar situation pertains to shell thickness (Tyler and Geake, 1966). Sparks and Board (1984) also noted that
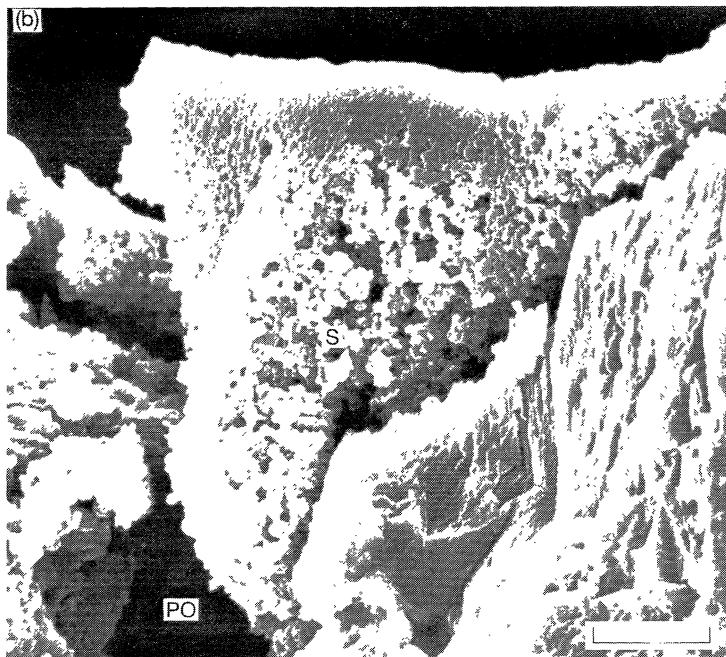
Figure 2.4 (b) Radial section of cuticle on chicken's eggshell, showing the pore orifice (PO) and spheres of glycoprotein (S). Bar marker 10 μm.

there was a significant decrease in cuticle thickness as the age of the flock increased.

The cuticle on the chicken's egg consists of ca. 90% protein (Baker and Balch, 1962), the amino acids having a high glycine content (Krampitz and Witt, 1979). The carbohydrate moiety of the proteoglycan in the cuticle consists of sialic acid, galactosamine, galactose, glucose, mannose and fucose; uronic acids and acidic mucopolysaccharides have not been detected (Balch and Cooke, 1970). The cuticle is manufactured in non-ciliated secretory cells lining the uterus (Solomon, 1991) and is secreted as a granular substance (Cooke and Balch, 1970).

2.2.3 Pigmentation

Highly coloured and patterned SAM results from the presence of shell porphyrins or ooporphyrins, cyclic compounds consisting of four pyrrole rings. Brown and black pigments are associated with protoporphyrin-IX, whereas blue or green shades result from biliverdin-IX or a zinc biliverdin chelate respectively (Kennedy and Vevers, 1975). The concentration of porphyrin is important. For example, the protoporphyrin responsible for the pigmentation of chickens' brown eggs is also present in white eggs, but
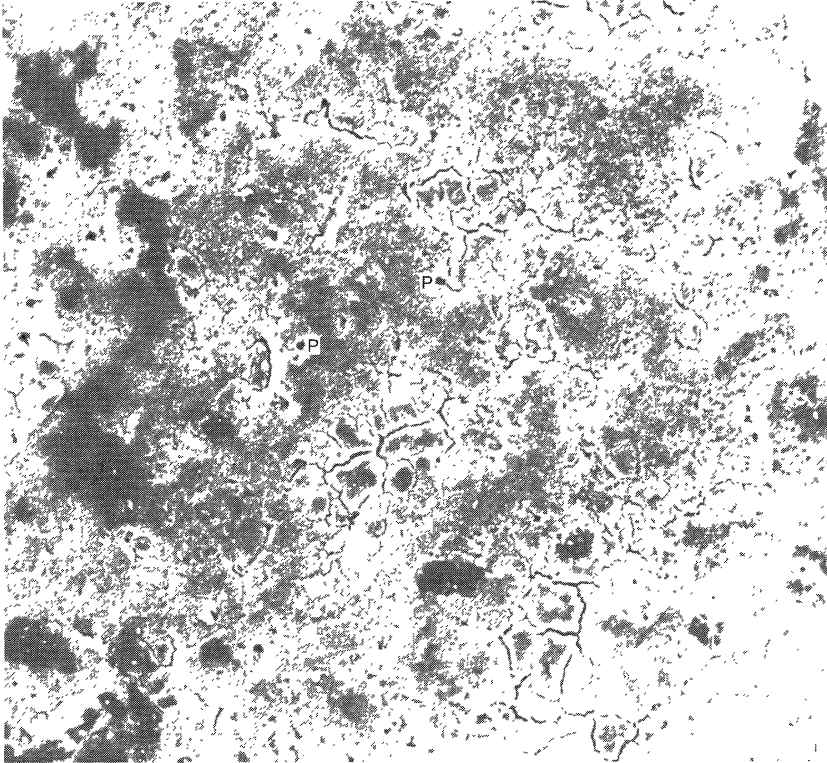
Figure 2.5 Surface view of chicken's white eggshell. Compared with Figure 2.4 (a) the structure is more open, evidenced by a number of uncapped pores (P). Bar marker 100 μm.

at a reduced concentration (Baird *et al.*, 1975). Baird *et al.* (1975) postulated that the porphyrins were synthesized in the blood and transferred via the surface epithelial cells of the shell gland. Other studies have concluded, however, that the pigments are synthesized *de novo* in the shell gland (see Solomon, 1991).

The protoporphyrins responsible for the pigmentation of the chicken's egg occur primarily in the cuticle, yet pigment can be detected in the mineralized shell immediately opposed to the cuticle (Baird *et al.*, 1975; Sparks, 1985). In other orders, such as Gaviiformes (Tyler, 1969), the pigment may be located wholly or partially in the underlying shell.

2.3 FUNCTION OF SHELL ACCESSORY MATERIALS

Arguably, the prime function of SAMs is to act as a physical barrier to liquid water and solids, such as nest debris, and hence microorganisms. Additional functions range from enhancing shell strength to influencing shell

vapour conductance. The structural diversity of SAMs reflects the different emphasis placed on these functions by avians incubating eggs in varied habitats. Thus, the cover on the shell of the pelican's (*Pelecanus* spp.) egg, which is frequently incubated on a rocky ledge, makes a significant contribution to the strength of the shell (Tyler, 1969), compared with the cuticle on the duck (*Anas platyrhynchus*) egg, which makes no significant contribution to shell strength, but does have water repellent characteristics. The functions of SAMs are discussed below.

2.3.1 Pigmentation

A possible function of SAMs may be to carry pigment, which in turn may confer camouflage and temperature control. This is particularly relevant to species that do not indulge in formal nest building or that incubate their eggs in desert environments. In addition, Solomon (1991) suggested that the porphyrin molecule may act as a cushion between the calcite crystals in the true shell, making the shell more resistant to cracking. Alternatively, it is feasible that the lack of structural cohesion seen in the cuticle on white chicken eggs (cf. brown eggs, section 2.2.2) results from the relatively low levels of porphyrin incorporated into the cuticle.

2.3.2 Shell conductance

The porosity or conductance of the majority of shells examined to date is set at oviposition. If the embryo's respiratory requirement is to be satisfied and the correct amount of water is to be lost from the egg during incubation, it is important that the shell's conductance value is not altered (e. g. by obstructing pores or cracking the shell) post-oviposition. By capping or plugging the pore orifice, SAMs play an important role in preventing the occlusion of the pore canals with nest debris. It is notable that pigeons (*Columba* spp.), which nest in a dry environment, lay eggs lacking SAM (Board, 1974). By comparison, the great-crested grebe (*Podiceps cristatus*), lays its eggs on a semi-aquatic platform of soil and vegetation and produces an egg with a shell that is covered with a layer of intricate SAM (section 2.2.1).

  Gaseous exchange between the egg and its environment takes place by diffusion (Wangensteen *et al.*, 1970/71). The water vapour conductance of an egg is determined by the effective total pore area of the shell and is inversely related to the length of the diffusion pathway – normally taken as the pore canal – (Rahn *et al.*, 1977). Therefore, SAMs influence water vapour conductance by altering the length of the diffusion pathway.

  The shell conductance of duck (not Muscovy *Cairina moschata*) (Deeming, 1987), turkey (Christensen and Bagley, 1984) geese *Anser domesticus* (Deeming, 1987) and snake bird (Colacino *et al.*, 1985) eggs is increased by

removing the SAMs. The evidence for chickens' eggs is, however, contradictory. Sparks and Board (1984) found the cuticle's influence on shell conductance to be minimal. This was supported by Deeming (1987) who reported that the conductance of chicken and Muscovy duck eggshells was independent of the cuticle. In contrast, Peebles and Brake (1986) and Peebles *et al.* (1987) found that, under certain circumstances, the shell conductance of broiler breeder eggs could be increased by removing the cuticle. This may reflect the differing selection pressures on broiler breeder birds and egg laying birds. A typical broiler breeder would be expected to lay approximately 180 eggs in a production cycle, compared with >240 eggs from a laying hen over the same time period. The cuticle on a broiler breeder egg may therefore be more substantial than that on an egg produced by a laying bird of similar age. Indeed, in a limited trial, the author (unpublished observations) noted that, although the volume of cuticle deposited on eggs from laying birds decreased as the production cycle progressed, the same was not true for eggs produced by a broiler breeder bird.

The conductance of shells from a small number of species has been shown to change with time. For example, the conductance of egg shells from cliff swallows *Hirundo pyrrhonato* (Sotherland *et al.*, 1980), red-winged blackbirds *Agelaius phoeniceus*, American robins *Turdus migratorius* (Carey, 1979), common canaries *Serinus canarius* (Kern, 1986) and possibly certain Anitidae (see French and Board, 1983), has been shown to increase during the early stages of incubation. Kern concluded that the increase in conductance was not a function of shell abrasion, but noted that it could be accelerated by exposing the egg to incubation temperatures. Given that the geometry of the pore canal is unlikely to change during the initial stages of incubation, it is possible that the change in conductance is the result of a deterioration of the vesicular structure of the SAM, similar to that observed in chicken eggs following prolonged storage (see page 31).

2.3.3 Barrier to bacterial penetration

SAMs can enhance the physical defences of the egg against bacteria in two ways. First, by increasing shell strength and hence reducing the probability of the shell being breached by a crack. Second, by presenting a physical (and in some circumstances, a chemical) barrier to microorganisms, preventing access to the pore canal.

As with water vapour conductance, the SAM would appear to affect shell strength only in as much as it contributes to overall shell thickness. Thus, Tyler (1969) reported that the presence or absence of a cover on eggs from Sphenisciformes, Pelicaniformes and Podicipitiformes '... did not explain any differences in snapping strength'. This author did report, however, that there was limited evidence to suggest that the cover on *Pelicanus* shell enhanced impact strength. The effect of the chicken's egg

cuticle on shell strength is not clear. Tyler and Thomas (1966), for example, noted that shell strength was enhanced by the presence of the cuticle when the shell was snapped inwards. A more recent study (Belyavin and Boorman, 1980) failed to show a significant difference in the strength of chicken's shells with or without cuticle.

The SAM's most important role is as a physical barrier to liquid water and nest debris and hence to microorganisms, in particular, bacteria. Board and Board (1967) showed that bacteria, carbon black particles and water-soluble dyes would pass across the same pores. Board and Halls (1973a, b) used water uptake (induced using a temperature differential dip), as an indication of the ability of the shell on chicken, mallard and guinea fowl (*Numidia meleagris*) eggs to resist bacterial penetration. These authors concluded that the cuticle was the principal barrier to water uptake. A more recent study (Sparks and Board, 1984) supported this conclusion and, in addition, showed that there was no correlation between water uptake and the shell's conductance. Furthermore, there was no correlation between water uptake and cuticle weight, a feature of the uneven distribution of cuticle over the surface of the shell discussed in section 2.2.2. Thus, it is the presence or absence of cuticle on the chicken's eggshell that determines the probability of the shell being penetrated by bacteria, not the shell's conductance.

The vesiculate structure of all SAMs studied to date would appear to be well suited to perform the function of 'pore cap and/or plug'. Furthermore, the chemical nature of some SAMs, such as the oily cuticle on mallard eggs, contributes to the water repellent capacity of these eggs (Board and Halls, 1973a). The importance of an aqueous phase when considering bacterial penetration of the pore canal cannot be over-estimated (Grzimek, 1936). Indeed, it is not certain that bacteria will penetrate the shell in the absence of water (Bruce, personal communication). For example, the once common practice of cleaning the shell of the egg with a damp cloth frequently serves only to transport bacteria from the outer to the inner surface of the shell.

Sparks and Board (1985) contended that it was the presence of an aqueous phase and an immature physical structure that markedly reduced the ability of the cuticle on recently (<30 s) oviposited chickens' eggs to resist bacterial penetration. Only 16% of eggs with a mature cuticle challenged with faeces were penetrated, compared with 100% of eggs with an immature cuticle. The implications of this study for nest box/battery cage hygiene are obvious – an egg laid into a contaminated environment will almost certainly be contaminated internally.

The quality of the table egg at oviposition is determined primarily by the age of the chicken, shell quality for example, deteriorating as the flock ages. Similarly, the quality of the cuticle (in terms of its ability to resist the passage of liquid water) on the shell of the egg produced by laying chickens also deteriorates as the flock ages. This is not the case with the cuticle on eggs

laid by broiler breeder birds (Sparks, unpublished observations – see section 2.3.1 for possible explanation).

Hatching eggs may be fumigated prior to setting, although there is an increasing tendency in the UK to use egg washing/sanitizing regimes (see below). Given that bacterial penetration of the shell is most probable immediately post-oviposition, bacteria that traversed the cuticle during this period would be subsequently protected from the bactericidal action of fumigants and sanitizing agents. Similarly, the practice of 'buffing' (abrading the surface of the shell to remove faecal material) results in contaminated debris being forced down the pore canal. This may cause bacterial contamination of the inner surface of the shell and/or anoxia for the developing embryo through pore occlusion. It is this author's opinion that nest box or cage hygiene is of paramount importance to the producer seeking to minimize bacterial contamination of the egg post-oviposition.

2.4. EGG WASHING/SANITIZING

The washing of table eggs is not permitted within the European Community, although it is a feature of the egg production industry in most other countries in the world. Hatching eggs, however, may be washed. An increasing number of breeding companies are turning to egg washing as an alternative to fumigation with formaldehyde, use of which is seen as unacceptable in the UK under the current Control of Substances Hazardous to Health (COSHH) Regulations.

Egg washing has had a chequered history (Moats, 1978). In the 1950s, a number of studies highlighted the problems associated with washed eggs, and in particular, the increased probability of such eggs rotting in store (Alford *et al.*, 1950; Brooks, 1951; Brooks *et al.*, 1952). Further studies were undertaken and from these came an understanding of the causes of egg contamination through washing. To wash an egg successfully, the following principles should be applied: (1) the temperature of the wash-water must exceed the temperature of the egg; (2) the bacterial load of the wash-water must be minimal; (3) sanitizer efficacy must be maintained; (4) the shell should not be damaged as a result of the washing procedure and (5) the egg should be dried quickly post-washing.

2.4.1 Egg washing technology

Improved technology has allowed machines to be constructed that meet the principles outlined above. The earlier practice of submerging eggs in a tank or 'bucket' of wash-water has been superseded by the so-called
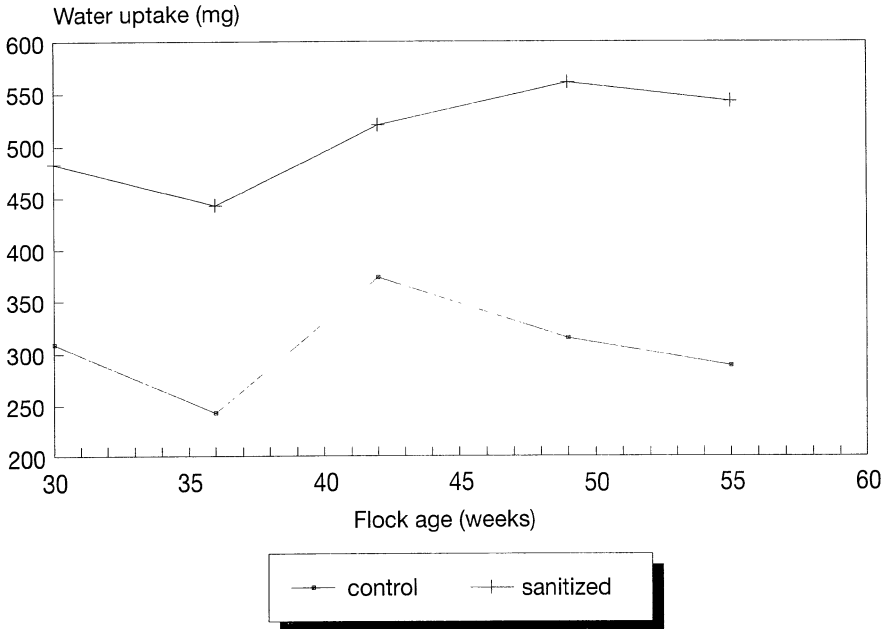
Figure 2.6 Effect of shell sanitizing on the ability of the cuticle to resist water movement across the shell. (After Sparks, 1992.)

continuous egg washers. A typical machine will consist of three chambers. In the first, the tray of eggs is sprayed with warm (43°C) water containing a detergent, the high pressure jets removing faecal material, etc. The eggs are then rinsed in a second chamber with a sanitizing agent (e. g. quaternary
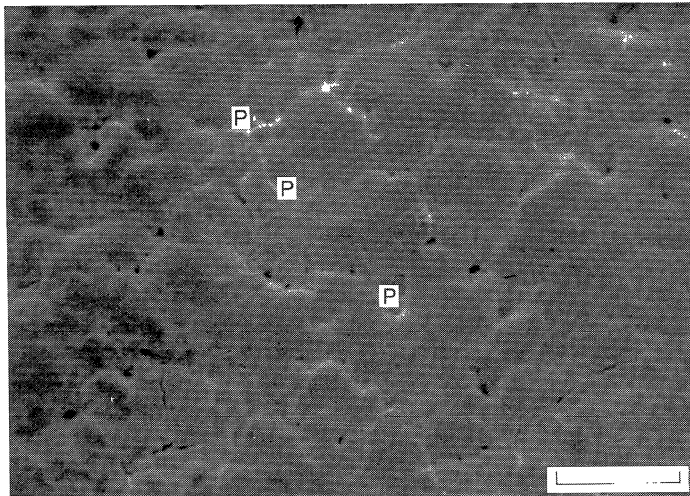
Figure 2.7 Cuticle on brown chicken's eggshell, post washing. Compared with Figure 2.4 (a), there is a significant increase in the number of exposed pores (P). Bar marker 100 μm.

ammonium, chlorine based agents). Finally, in the third chamber, the eggs are dried, by jets of warm air and/or heat from infrared lamps.

2.4.2 Effect on the cuticle of egg washing and implications for bacterial contamination

Egg washing needs to be considered in the context of the end user. Providing table eggs are not stored for long periods post-washing, there is no evidence that eggs correctly washed and stored present a greater potential health hazard to the consumer than unwashed eggs (Bryant and Sharp, 1934; Moats, 1978). By comparison, Sparks (unpublished observations) has repeatedly noted a significant increase in the incidence of bacterial rots when fertile hatching eggs were washed prior to incubation. Board *et al.* (1986), citing unpublished work by R.G. Board and F. Sykes, contended that incubation of fertile eggs brings about changes in the egg such that contaminants are restricted to the shell membrane. Thus, egg washing may, through the provision of water and/or trace elements such as iron (Chapter 3), negate the hatching egg's chemical defence against bacteria.

Some studies (Kuhl, 1987; Overfield, 1989) have reported that continuous egg washers of the pressure spray type (cf. those reliant on rotating brushes) do not damage the cuticle under normal operating conditions. In contrast, a study by Sparks (1992) showed (Figure 2.6) that such washers caused a significant decrease in the ability of the cuticle to resist water uptake by the egg and a concomitant deterioration in the structure of the cuticle (Figure 2.7). Although damage to the cuticle *per se* is not cause for concern, a compromised cuticle and wash-water (possibly with a high bacterial load) is.

One of the stated (Kuhl, 1987) advantages of egg washing over fumigation is that the latter does not remove faecal material from the shell. There is, however, the possibility, if not certainty, that this feature of egg-washing machines will be abused, heavily soiled eggs (table or hatching) being repeatedly passed through the machine until they appear clean. Three problems are inherent in this practice. First, there is a high probability that heavily soiled or stained eggs are already contaminated with bacteria internally. When 'cleaned', these eggs will appear to be no different from nest clean eggs and will be treated as such by the hatchery, thereby substantially increasing the risk of cross-contamination at hatching. Second, repeated passes through the egg-washing machine prolongs the contact time between the wash-water and the shell, increasing the probability of water translocation across the shell and third, more cuticle is removed with each successive pass. Once the cuticle has been removed from the pore orifice, water is likely to pass rapidly along the pore canal, the canal offering little resistance to the movement of water (Board, 1974). The probability of uptake is increased due to the addition of a detergent,

which will significantly reduce the surface tension of the water (Board and Halls, 1973b; Sparks, 1985). Thus, the repeated washing of heavily soiled eggs should be actively discouraged.

When faced with a new problem such as egg washing, there is a tendency for the poultry industry to adopt an empirical approach. In part, this is due to the lack of fundamental knowledge concerning the basic biology of the egg. The ability to manipulate the egg to its full potential, in production terms, will only be possible if this is rectified.

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The egg: a compartmentalized, aseptically packaged food

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Of the many contributions of the female to the overall breeding success of her species, two are of particular relevance in the context of this chapter. First, she endows an egg with a large, dense (50% solids and 50% water) store of nutrients in the yolk and a reservoir of water, mainly in the albumen. Both are sufficient in amount to support embryogenesis and to sustain the chick for a short while after hatching. Second, she incubates the eggs and influences the colonization of the gut of a newly hatched chick with an appropriate flora.

3.1 COMPARTMENTALIZATION

Figure 3.1 emphasizes that there is a marked difference in the time scale for the laying down of the yolk and that for the deposition of water in the albumen. The former takes place over many days. The incremental addition of material is evident in a cross section of a mature yolk (Figure 3.2). The yolk is enveloped in albumen and the shell membranes and the egg is 'plumped' with water within hours. The difference between the two time scales is obviously the initial cause of the compartmentalization of an egg. The maintenance of compartments calls for physical protection of such mechanically weak entities as the yolk and white and mechanisms that hinder the exchange of materials between the two. In the former case, the shell plays an important role in protecting the yolk, especially the vitelline membrane (Figure 3.3), from gross damage. Some have also claimed that, through acting as a 'shock absorber', the albuminous sac contributes to the protection of the vitelline membrane (Palmer and Guillette, 1991).
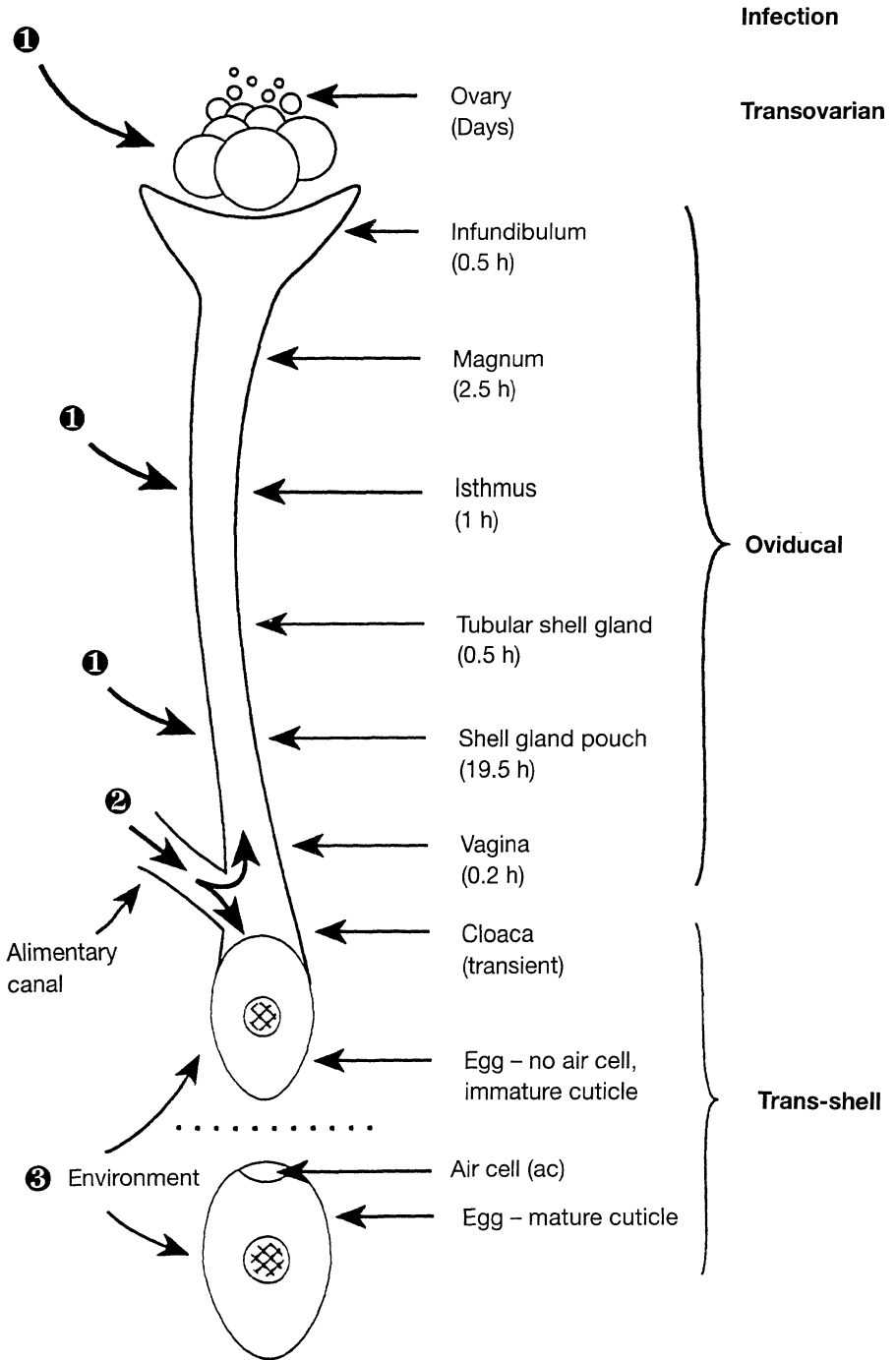
Figure 3.1 Schematic diagram of oviduct showing the times (hours) of various events in egg formation and sites (numbers on a black background) at which contamination may occur.
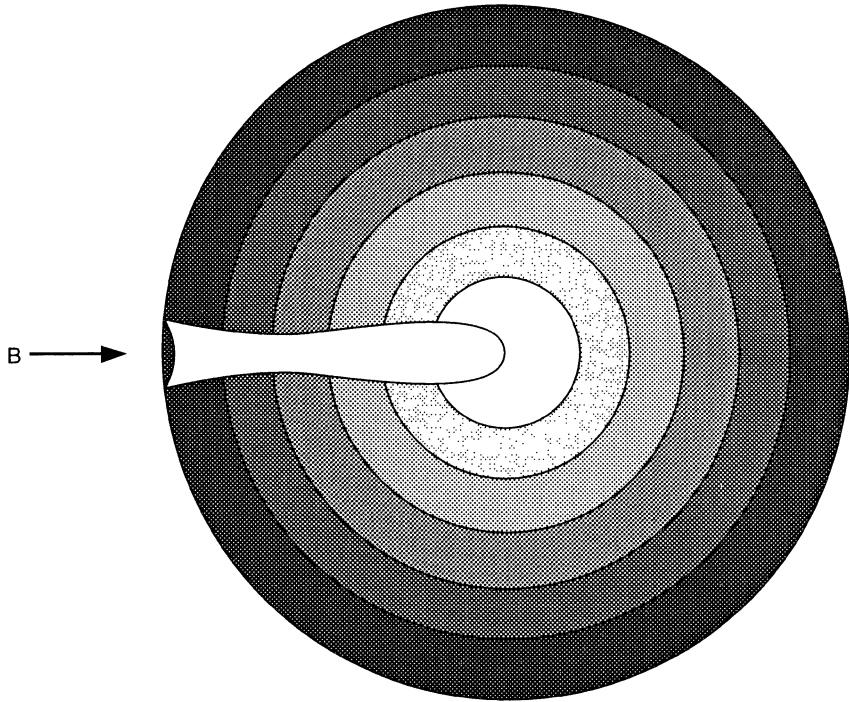
Figure 3.2 A cross section of the mature yolk of a hen's egg, showing concentric layers of yolk. B, blastodisc.

With the egg produced for human consumption, the two compartments – the yolk and the white – retain their identity throughout protracted storage. However, their spatial arrangement within the egg and physical characteristics do change appreciably. This is due in large part to the decay of the albuminous sac (Table 3.1) together with changes in the chalazae (Figure 3.3) both of which result in the yolk moving from a central to a peripheral location within an egg. A change in location is aided also by the diffusive loss of water from the albumen to the atmosphere around an egg

Table 3.1 Factors influencing the decay of the albuminous sac

Initial Albumen Quantity	-Breed and age of hen (Proudfoot, 1962; Jeffrey, 1941) -Health of hen (Gilbert, 1971) -Degree of ovomucin (α and β) and lysozyme interaction (Cotterill and Winter, 1955; Burley and Vadehra, 1989)
Storage Conditions	-Temperature (Hinton, 1968) -Humidity (Hinton, 1968) -CO_2 concentration (Sharp, 1929; Fletcher *et al.*, 1959)
Treatment of Eggs	-e.g. Oiling (Romanoff and Romanoff, 1949)
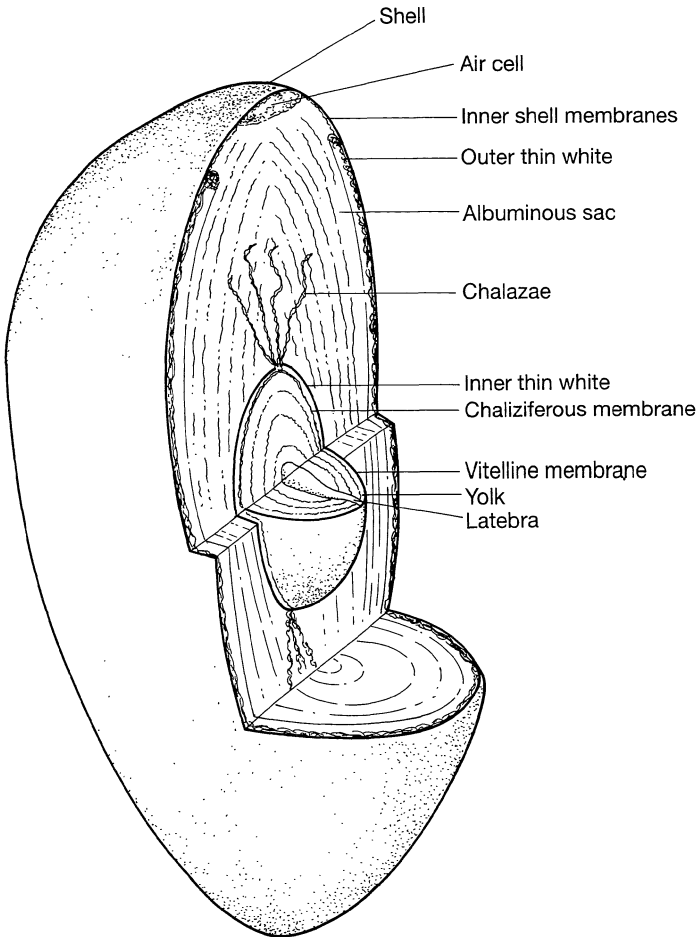
Figure 3.3 An artist's impression of the hen's egg as shown by cut-away sections.

(Ar, 1991) as well as the migration of water from the white to the yolk.

   The acellular vitelline membrane enveloping the yolk behaves as a semipermeable membrane *in vitro* (Burley and Vadehra, 1989). In view of the potential for osmotic forces (ca. 1.8 atm) to mediate such movement, it would not be expected to hinder appreciably the movement of water from the albumen (freezing point, 0.42 °C) to the yolk (freezing point, 0.57 °C). In practice, the emulsion state of the yolk (Burley and Vadehra, 1989) prevents the unfettered flow of water. Although the rate of water movement is slow, it does lead in time to a diminution in yolk viscosity and density as well as a stretching and consequent weakening of the vitelline membrane (Oosterwoud, 1987). Diffusive loss and water movement causes an increase in the density of the albumen so that the yolk which settled at oviposition

tends to float. Yolk movement becomes less constrained as the albuminous sac decays (Table 3.1). The distance over which the yolk moves in a table egg stored broad pole uppermost is dictated in part by the size of the air cell. This becomes larger with time as a consequence of the diffusive loss of water from the albumen. In practice the rather flabby yolk of an old egg comes to rest against the air cell and the side of an egg (Figure 3.4). In a fertile egg there is a very pronounced movement of water from the albumen to form the subgerminal fluid on which the embryo sits for the first few days of incubation. In this instance, water movement is an active process associated with epiblast cells (Stern, 1991) rather than with diffusion across the acellular vitelline membrane (Burley and Vadehra, 1989).

It needs to be stressed that the changes in the table egg discussed to date are of major importance in terms of egg quality. Indeed every effort is made by those wishing to sell high quality eggs to retard the rate of decay of the albuminous sac (Oosterwoud, 1987) and to minimize diffusive water loss. Various strategies have been advocated over the years in order to minimize quality loss in table eggs during passage from the producer to the consumer. Some of these are listed in Table 3.2.

So far in this discussion of the compartmentalization of an egg, there has been no compelling need to consider the movement of materials from the yolk to the albumen. In practice such movement can lead to gross changes in the appearance of the white and yolk. This has been an occasional problem with hens which have access to mallow seeds or certain other feeds, for example, improperly prepared cotton seed meal (Burley and Vadehra, 1989). Both contain cyclopropene fatty acids, sterculic and malvalic, which affect the yolk but not the vitelline membrane so that iron migrates into the albumen and forms a salmon-pink complex with ovotransferrin. Movement of water in the opposite direction results in the rapid formation of a large, flaccid yolk. Uncontrolled exposure to temperatures below 0°C may also disrupt the emulsion state of the yolk. This results in the salmon-pink white discoloration noted above and a gross enlargement of the yolk (Hale, 1950). At one time it was considered that microorganisms may have been the cause of these changes and the term, yellow rot, was applied to such eggs. However, Mckenzie *et al.* (1955) failed to substantiate this view.

Table 3.2 Strategies for the preservation of hens' eggs

Hygienic production and handling
Immersion in lime water or water glass
Oiling/waxing of the shells
Temperature control
Humidity control
Carbon dioxide (high and low concentrations)
addition to the atmosphere
Modified Atmosphere Packaging – eggs in packs
wrapped in film with high resistance to
gaseous diffusion

Egg at Oviposition

Air cell - small

Outer thin white

Chalaza

Yolk - denser than albumen

Albuminous sac - well formed
and holds yolk centrally

Albumen pH - ca 7.2

After storage

Air cell - large due to diffusive loss
of water from albumen

Yolk - enlarged as a result of uptake
of water from albumen

Chalaza
Albuminous sac - decayed (see Table 3.1)

Thin albumen - dense due to loss
of water to atmosphere and yolk

Albumen pH - ca 9.5

Figure 3.4   A summary of the changes occurring in an egg during storage.

As the quality of eggs laid by hens receiving an appropriate diet is not compromised by an out-flow of small molecules from the yolk, the literature contains very little information on this topic. Ducay *et al.* (1960) found a gradual but small increase in the concentration of amino acids in the albumen of eggs stored for 83 days. We too have found little if any change in the amount of free amino acids in albumen of eggs stored for 34 days at 20°C. Much more work has been done on the movement of substances of small molecular weight from the yolk to the white of fertile eggs during the first day of incubation. Changes in the amino acid and glucose content of the albumen and a gradient in both substances from a high point in the albumen immediately adjacent to the vitelline membrane have been demonstrated (Garcia *et al.*, 1983; Pons *et al.*, 1985). The induction of embryogenesis may be responsible for such changes rather than passive diffusion across the vitelline membrane.

3.2 AN ASEPTICALLY PACKAGED FOOD

The oviduct is an open-ended tube (Figure 3.1), one end of which is located in the cloaca and the other in the peritoneum adjacent to the ovary. There is a direct link between the cloacal contents and the sterile cavity of the female's peritoneum. It is remarkable that the mechanisms preventing the movement of bacteria from the former to the latter have received little attention. Early workers (Horowitz, 1903; Lamson, 1909), introduced bacteria into the oviduct and noted that these were eliminated about a day later. There is a general consensus that in some way the secretion of albumen proteins having antimicrobial properties plays an important role in ridding the oviduct of chance contaminants. Thus Harry (1963) had to use enrichment methods in order to isolate bacteria (*Pasteurella haemolytica*, *Lactobacillus* spp. and *Micrococcus* spp.) from the ovaries. It was notable that the incidence of contamination of the ovaries of out-of-lay hens was greater than that of those of laying ones.

In the past few years several surveys of ovaries taken from hens at slaughter in commercial poultry-processing plants have revealed an apparently high incidence of ovarian contamination with a range of *Salmonella* serotypes (Jones *et al.*, 1991; Poppe *et al.*, 1991). This information should be interpreted with certain qualifications. The conditions obtaining before and at the moment of slaughter in a commercial plant can be nothing other than stressful to the hen. Could this cause salmonellas to pass from other parts of the hen to the ovaries? Moreover, is there violent antiperistalsis in the oviduct during the agonal period such that contaminants from the cloaca are transported to the peritoneum?

If direct studies of the oviduct and ovaries is an uncertain means of

assessing their microbiological status, and hence of the contents of eggs produced therein, then direct examination of egg contents may provide an indirect means. In the early years of this century several attempts (e.g. Pennington, 1909) were made to answer the question: what is the incidence of contamination of the contents of hens' eggs? These studies recorded contamination but again caution must be exercised in interpreting the results. As the work was done before the introduction of laminar flow cabinets and other aids to the aseptic technique, one must recognize the problems of applying this technique to such viscous materials as egg albumen and yolk in a general laboratory environment. Indeed it was considerations such as these that led Brooks and Taylor (1955) to the generalization that 'roughly 90% of newly laid eggs are free from micro-organisms and the true values may be even higher'. It should be noted that these workers were concerned mainly with rotting of table eggs.

The current pandemic due to *Salmonella enteritidis* (Rodrigue *et al.*, 1990) has led once again to studies of egg contents. The available evidence on the incidence of this organism in contents is discussed in Chapter 5. For present purposes it is sufficient to note that the incidence of contamination is relatively low and that the contaminants would appear to be derived from the oviduct (oviducal infection) more commonly than from the ovary (transovarian infection).

It is evident from the above discussion that it is not possible to give an unequivocal answer to the question: what is the incidence of contamination of egg contents at oviposition? *A priori* reasoning might well lead one to the answer that selection pressures have presumably assured a very low incidence of contamination with pathogens or microorganisms capable of causing breakdown of the egg contents, particularly the main food reserve in the yolk. Alternatively, the incidence may be larger than one would imagine and that the large investment made by the female to the breeding biology of her species is protected by antimicrobial defence systems operating in an egg pre- and post the onset of incubation (Board and Fuller, 1974). In the case of pathogens, defence may well be based on antibodies secreted into the yolk (Rose *et al.*, 1974). With saprophytic microorganisms, a non-specific defence system would be needed. The chemical defence system (Table 3.3) in the albumen of the hen's egg supports the latter supposition. It is essential to recognize that, in nature, the non-specific defence system would need to be effective for a relatively short period only. If one takes the domestic hen as an example, the first egg in a clutch of 12 would require protection for about 12 days, in other words the interval between the laying of the first egg and the onset of incubation. It is noteworthy also that, within a short while of incubation, new compartments have formed and certain resources essential to embryogenesis redistributed in a fertile egg. Thus within 9 days the yolk is enveloped by the cellular yolk sac membrane (Burley and Vadehra, 1989) and by 20% of

Table 3.3 Properties of the main proteins of hen albumen

Protein	Amount in albumen(%)	M_r	Characteristics
Ovotransferrin	12	80 000	Chelation of metal ions particularly iron
Ovomucoid	11	28 000	Inhibition of trypsin
Lysozyme	3.4	14 000	Hydrolysis of β-1, 4-glycosidic bond in peptidoglycans. Electrostatic interaction with ovomucin
Ovomucin	3.5	ND[*]	-
Ovoinhibitor	1.4	44 000–49 000	Inhibition of several proteases
Ovomacroglobulin	0.5	760 000–900 000	-
Ovoglycoprotein	1.0	24 400	-
Ovoflavoprotein	0.8	32 000	Chelation of riboflavin
Avidin	0.05	70 000	Chelation of biotin

*Value not determined or reported
For further details, see Tranter and Board (1982).

the time into incubation (Ar, 1991) a large amount of the water reserve in the albumen has been transferred to a part of the yolk to form the subgerminal fluid (Deeming, 1991). The albumen, which is highly concentrated by day 18 (Ar, 1991), is eventually transferred to the embryo's alimentary canal (Deeming, 1991). The information on the antimicrobial defence systems of the incubating egg was reviewed by Board and Fuller (1974).

3.3 ANTIMICROBIAL DEFENCE

Since the pioneering study of egg microbiology by Gayon (1873), an associate of Louis Pasteur, a great many investigations have shown that the hen's egg is endowed with chemical and physical defences against microorganisms (Table 3.3). The physical defence systems are discussed in Chapter 1 and 2. The purpose here is to consider the contribution of these systems to the well-being of the table egg. Much of the information stems from studies that were undertaken at a time when laying flocks were small, egg-packing plants plentiful, long-term egg storage was the norm, and addled eggs relatively common (Board, 1965). Additionally the majority of studies were probably concerned with eggs infected post oviposition (trans-shell infection in the terminology of Duguid and North, 1991).

Infection of the outer surface of an eggshell is patently the first step in

trans-shell infection. Many studies have shown that the shell acquires a broad range of contaminants through contact with nest material, egg boxes etc. It is notable that Gram-positive bacteria are commonly dominant (Chapter 4, Table 3). Contamination of the shell must be followed by translocation of microorganisms across the shell. In terms of the role of microorganisms, translocation is active (micro-fungi) or passive (bacteria). When eggs are stored in humid conditions, micro-fungi grow on, and may even digest, the cuticle enveloping the shell. Their hyphae penetrate the pore canals and the shell membranes and their mycelia ramify throughout the albumen (Board *et al.*, 1964). Mould growth on the surface of the shell was referred to as 'whiskers' in the trade. *Cladosporium herbarum* appears to have been one of the most common micro-fungi of eggs stored under unfavourable conditions (Weston and Halnan, 1927). The factors that influence the transfer of bacteria across the shell have been discussed in Chapters 1 and 2. The shell may be considered as a series of resistances (Figure 3.5) and, as noted in Chapters 1 and 2, work needs to be done to overcome their water repellency and, more importantly, water resistance. There are no reasons to believe that translocation would preferentially select particular organisms from the heterogeneous flora on the surface of the shell. It is therefore of note that the contents of addled eggs contain mainly Gram-negative bacteria (Chapter 4, Table 5) although Gram-positive bacteria dominate the flora on the shell's surface. Two studies (Seviour and Board, 1972; Dolman and Board, 1992) have shown that selection of the former at the expense of the latter occurs in the shell membranes.
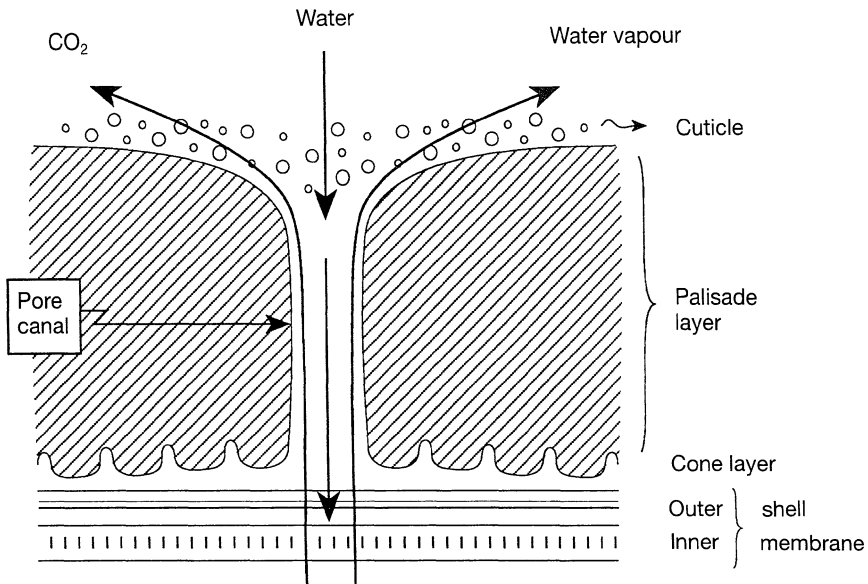
Figure 3.5 A radial section of the hen's eggshell showing the resistance to water movement.

There is one common feature in the vast majority of studies on the course of infection of table eggs with rot-producing bacteria at ambient temperature (Table 3.4). There is a lag of 10–20 days between infection of the shell membranes with bacteria suspended in water containing <1–2 ppm Fe(III) and overt signs of infection of the egg contents. This lag has also been noted in eggs infected with salmonellas. In this case, however, the presence of large populations in the albumen (Clay and Board, 1991) rather than changes in the appearance of the yolk and white was used as an indicator of the termination of the lag period. Gillespie and Scott (1950), who were probably the first to emphasize this lag period, concluded that it reflected the time taken for bacteria to penetrate the shell membranes. In other words, gross infection of and microscopic changes to the albumen did not occur until organisms had 'grown' through the shell membranes. However, Brooks (1960) suggested that the shell membranes were initially an unfavourable niche for microbial growth but some undefined changes in their structures around day 13 of storage led to an improvement and the onset of microbial growth within the membranes and in the underlying albumen. Board (1965) concluded that, with eggs infected deliberately and stored at ambient temperatures, the lag period was terminated when the yolk moved upwards and made contact with an infected shell membrane. In other words the loss of the highly organized (compartmentalized) structure of the egg contents negated the antimicrobial defence of the albumen. This hypothesis has subsequently been modified. Board and Ayers (1965), who studied the infection process in eggs infected deliberately with fluorescent pseudomonads and incubated at 10 °C, concluded that a generalized infection of the egg contents occurs as a result of contaminants in the albumen colliding with the yolk before the latter makes contact with the shell membrane. This interpretation was put forward (Clay and Board, 1991; Dolman and Board, 1992) to account for the onset of the generalized infection of the contents of eggs the shell membranes of which had been infected deliberately with *S. enteritidis*. In subsequent studies *in vitro*, Lock (unpublished observations) demonstrated that salmonellas fail to grow in albumen seeded with a piece of shell membrane containing *Salmonella*. The organisms did grow, however, when a piece of infected shell membrane was put into the albumen of whole eggs that had been broken out into sterile containers. These observations demonstrate that the yolk plays an important role in the infection process. Loss of the closely ordered compartmentalization of the newly laid egg results in a situation in which the yolk short-circuits the chemical defences of the albumen.

Yet another interpretation has been put forward to account for the sudden onset of a generalized infection of eggs with salmonellas. Humphrey *et al.* (1991) and Humphrey (1991) contend that quiescent cells in the albumen are induced to multiply by materials diffusing out from the yolk.

As was noted previously, there is a paucity of information on the

Table 3.4 Time taken from the penetration of the shells of fresh eggs and the occurrence of gross contamination or macroscopic changes in the albumen

Test organisms	Infection method	Temp$_*$(°C) (Days*)	Reference
Bacillus			
prodigious	Immersion†	15–18	Zagaevsky and
pyocyaneus	Immersion	(15–20)	Lutikova (1944)
Pseudomonas	Immersion	20	Gillespie and
		(14)	Scott (1950)
Salmonella			
bareilly	Rolled in	22	Bigland and
oranienberg	faeces	(6–16)	Papas (1953)
kentucky	containing		
typhimurium	test organism		
Rot producers	Natural and	7–37	Miller and
	artificial	(14)	Crawford (1953)
	(not specified)		
Ps. ovalis	Immersion	15	Elliot (1954)
		(7–11)	
Salmonella			
oranienberg	Immersion	29	Stokes *et al.*
typhimurium		(15)	(1956)
montevideo			
pullorum			
gallinarum			
Pseudomonas	Immersion	20	Orel (1959)
		(21)	
Ps. aeruginosa	Immersion	26	Fromm and Monroe
		(10–15)	(1960)
Pseudomonas			
ovalis	Immersion	15	Garibaldi and
fluorescens		(25)	Bayne (1960)
Pseudomonas	Air cell‡	20	Brooks (1960)
		(13)	
Ps. fluorescens	Air cell	27	Board (1964)
		(12–30)	
Serratia	Air cell	30	Board and Ayres
marcesens		(10–15)	(1965)
Salmonella			
lexington	Immersion	22–25	Rizk *et al.*
anatum		(14)	(1966)
Ps. aeruginosa	Immersion	21–23	Vadhera *et*
		(15)	*al.* (1970)
Salmonella			
enteritidis	Air cell	25	Clay and Board
		(12–34)	(1991)
Salmonella			
enteritidis	Air cell	20	Dolman and
		(15)	Board (1992)

*The number of days before the occurrence of gross contamination or macroscopic changes in the albumen.
†The eggs were immersed in a suspension of bacteria.
‡The air cell was located by candling, a hole drilled in this area and the air cell membrane inoculated with the test organism.

diffusion of substances from the yolk to the albumen. We have failed to demonstrate an appreciable increase in the amount of amino acids in albumen. Nevertheless it is feasible that diffusion may play a role in egg infection; not by supplying growth-promoting quantities of nutrients but rather by establishing a gradient (Garcia *et al.*, 1983: Pons *et al.*, 1985) that induces a chemotactic response in microorganisms.

In the work discussed so far, destructive sampling techniques were used to study egg infection. It is notable that a lag of about 15 days between infection of the shell membranes with a pseudomonad and the appearance of fluorescent pigment in the albumen was a feature of eggs candled repeatedly throughout storage with ultraviolet light (Vadehra *et al.*, 1970). It was stressed above that the studies (Table 3.4) selected for discussion of the process of bacterial infection of an egg's contents were those in which the Fe(III) content of the medium used to suspend an inoculum was < 2 ppm. Garibaldi and Bayne (1962) associated rapid addling of washed eggs with the use of water containing *ca* 4.8 ppm Fe(III). Subsequent studies (Board *et al.*, 1968) showed that contamination of a bacterial suspension with this level of iron led to extensive growth of bacteria in the shell membranes and a progressive build up in the level of contamination of the albumen. The amounts of iron used in these studies were far less than that which would be required to quench the chelating potential of ovotransferrin in the albumen of infected eggs. Tranter *et al.* (1983) give an interpretation of this apparent paradox. They contaminated the shell membrane with a solution of an iron salt such that approximately 96 μm of Fe(III) was introduced on to the membrane. Histochemical techniques demonstrated that the iron penetrated the mantles of the fibres of the shell membrane but that there was a very slow migration of this element from the mantles to the albumen. An iron-contaminated portion of the shell membrane was providing a nidus for bacterial growth. This caused a build up in the albumen of contaminants, some of which eventually multiplied, thereby producing gross contamination of an egg's content.

3.4   CHEMOTAXIS AND EGG INFECTION

Recent studies (Lock *et al.*, 1992) have provided a new perspective on the infection process of eggs and one which may help resolve some of the conflicting opinions discussed above. The experimental protocol used in these studies is given in the legend to Figure 3.6. In practice, the results led them to conclude that the following stages resulted in a generalized infection of an egg's contents (and hence addling when rot-producing bacteria are present).
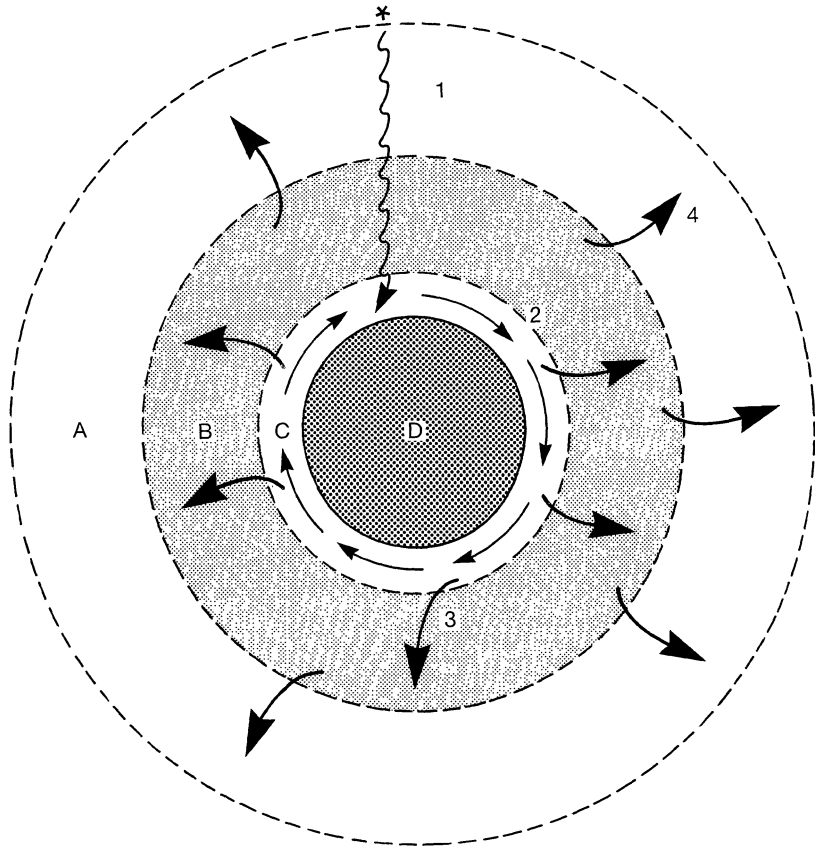
Figure 3.6 Sequence of events leading to generalized infection of egg contents with *Pseudomonas putida*. Contents of a freshly laid egg were poured into a square (10 × 10 cm) Petri dish and inoculated in the outer thin albumen (asterisk) with pseudomonads in a plug of water agar. The numbered arrows refer to steps in the process (see text for details). A, outer thin white; B, albuminous sac; C, inner thin white; D, yolk.

1. Organisms from the site of infection invade the thin albumen.
2. Some of these then pass through the albuminous sac and gain access to the inner thin white.
3. Some of the initial invaders of the inner thin white begin to grow probably as a consequence of obtaining essential nutrients from the yolk.
4. Within a short time, the whole of the inner thin white is heavily contaminated.
5. Gross contamination passes outwards into the albuminous sac where it appears to be temporarily constrained before finally moving out into the outer thin white.

Pseudomonas putida was used in these studies. Viable counts, direct

microscopical examination as well as the development of the organism's fluorescent pigment were all used to monitor the progress of infection. The results, summarized in Figure 3.6 highlight the role of the yolk in the process leading to gross bacterial infection of eggs. Additionally, the possible role of chemotaxis in this process was indicated. Other and as yet unresolved questions are raised by these recent observations. For example, it has been postulated (Garibaldi, 1970) that contaminants of the albumen may overcome the bacteriostatic action of ovotransferrin by the production of siderophores. Tranter and Board (1984) failed to obtain evidence to support this contention but it needs to be stressed that they studied only albumen. With the protocol given in Figure 3.6, it is conceivable that the organisms that made contact with the yolk obtained sufficient iron to initiate growth as well as the synthesis of siderophores. Thus, once growth had been triggered by the yolk, subsequent growth was sustained by the organisms overcoming iron deprivation through siderophore production. The coincidence of growth with the production of fluorescent pigment in the albumen – the synthesis of which is known to be accentuated by iron deprivation – supports this notion.

3.5 COLONIZATION OF THE GUT

As is evident in other Chapters of this book, many means of minimizing the passage of salmonellas along the chain animal feed/environment → laying hen → eggs → humans have been proposed and/or implemented. This implies that one method of control is probably an unattainable solution and safeguarding of human health can only be achieved by imposing a series of hurdles as discussed in other contexts by Leistner (1985) and Mossel and Struijk (1992). One such hurdle can be provided by administration of probiotic supplements (Fuller, 1990) exemplified by the competitive exclusion concept (Nurmi and Rantala, 1973). With this, the newly hatched chick is exposed to microorganisms that are components of the indigenous gut microflora of healthy mature hens. The rationale behind this approach is that such organisms block colonization of the caeca of newly hatched chicks by salmonellas and hence impede or eliminate their spread among the chicks and eventually hens kept under intensive management systems. Of course the success of this approach will be determined by many factors such as the freedom of a chick at hatching from contamination with salmonellas or other microorganisms, the effective dosing of every chick, the selection of appropriate organisms to dose the chick.

It was noted above that there is still a great deal of uncertainty about the extent of microbial contamination of an egg at and after oviposition. Indeed it was suggested above that one reason for the evolution of complex

antimicrobial defence systems in eggs is that asepsis during egg formation is not assured and that the breeding success of birds depends on this defence. It is remarkable that discussions of this topic rarely, if ever, consider the experiences of those who study gnotobiotic chicks. The evidence from such work suggests that a germ-free chick can be produced if the shell is effectively sterilized before the onset of incubation (Coates *et al.*, 1963).

In nature the chick is first exposed to the infection at pipping, a process reviewed by Bond *et al.* (1988). In the modern poultry industry, the chick at the pipping stage is in a totally alien environment and one in which every effort is taken to ensure high standards of hygiene (Baxter-Jones, 1991). This is in marked contrast to the pipping chick in the wild where it will be exposed to the microflora that has built up in the nest during incubation. Although this flora has not been studied, it is pertinent to speculate on the possible contribution of the broody hen, particularly in the context of organisms derived from the alimentary canal. It is well known that non-brooding birds pass two types of stools, a large dropping capped with crystals of uric acid and a small one having the appearance and consistency of yeast extract. The latter is caecal in origin and of infrequent occurrence (1 or 2 per day). The broody hen, on the other hand, produces a large dropping on the rare occasions when she leaves the nest. Its appearance is markedly different from that of either of the two noted above. It is tempting to speculate that the third form of dropping is:

1. The product of fermentative digestion occurring in the distal part of the gut because of the restricted food intake of a broody hen;
2. The source of organisms that colonize the gut, especially the caeca, of the 'pipping' chick. In practice this is the second major contribution (see introductory paragraph) of the female to the success of the egg in the breeding biology of birds.

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Trans-shell transmission

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4.1 INTRODUCTION

Microbial contamination of eggs is a well-established phenomenon and has important economic implications to the poultry industry. In the case of table eggs spoilage may occur and if the organism is of public health significance the affected eggs may be the cause of spread of disease. Contamination of hatching eggs may reduce hatchability, be responsible for transmission of poultry pathogens and impair the quality of chicks produced.

There are two ways in which eggs can become contaminated, namely, by the transovarian and trans-shell routes. In transovarian contamination the egg becomes contaminated prior to oviposition, with the source of contamination originating in the egg-laying apparatus of the bird. In the case of trans-shell contamination organisms gain access to the egg after oviposition by penetration of the shell.

4.2 ROUTES OF TRANSMISSION

4.2.1 Transovarian transmission

Although this chapter is directed at trans-shell transmission a brief discussion of transovarian contamination is included to elucidate the differences and to indicate the relative importance of the two routes.

It is generally accepted that microorganisms are absent from the vast majority of eggs while in the oviduct of healthy hens (Brooks and Taylor, 1955; Board, 1968). The microbiology of the hen's oviduct has been studied by several authors (Harry, 1963a; Jacobs *et al.*, 1978; Blankenship *et al.*, 1982; Bruce and Drysdale, 1991). In these studies the bacterial flora recovered from the oviducts was found to differ markedly from that found in the eggs indicating that contamination occurs most frequently after oviposition. It can be seen therefore that in a healthy bird the risk of transovarian infection

is regarded as being quite low. However, it has to be emphasized that there are several poultry and human diseases of bacterial and viral origin which can be disseminated via the egg. *Mycoplasma* spp. including *M. synoviae*, *M. gallisepticum* and *M. meliagridis* are examples of microorganisms capable of undergoing this vertical mode of transmission. Other organisms such as *Staphylococcus aureus*, *Salmonella* spp. and *Pasteurella* spp. are also known to produce infection (Mayes and Takeballi, 1983). The recent outbreaks of *Salmonella enteritidis* PT4 infection of eggs occurred as a result of this organism's ability to infect the reproductive tract. These so-called invasive strains can infect the ovary or oviduct and in certain instances can contaminate the contents of the egg before it is laid.

4.2.2 Trans-shell transmission

In practice problems associated with microbial penetration of the shell are normally manifested in some observable way, e.g. spoilage or reduced hatchability. However, it is important to appreciate that the egg presents to the invading organism a complex series of defensive barriers (Board, 1966, 1980; Board and Fuller, 1974) and although microbes may successfully penetrate the shell of the egg, further development of the organism may be arrested or delayed. Furthermore, it is well established that different microorganisms differ in their ability to overcome the defence mechanisms of the egg and grow in the egg contents (Board, 1969; Bruce and Drysdale, 1991). Consequently bacterial penetration of the shell may occur without any ensuing growth of the organism taking place or any damage to the egg. In this connection it is noteworthy that in the case of hatching eggs, even the recovery of bacteria from the egg contents of incubated eggs which have failed to hatch does not necessarily implicate the organism in the arrestment of embryonic development, as the embryo may have died for other reasons prior to the invasion or multiplication of the contaminating organism (Bruce and Johnson, 1978; Baxter-Jones, 1991).

Several workers have demonstrated trans-shell transmission of microorganisms in a variety of ways. The technique most frequently adopted follows the method used by Haines and Moran (1940) in which warm eggs are immersed in a relatively cool bacterial suspension so that the contraction of the egg contents draws the bacteria through the pores (MacLaury and Moran, 1959; Williams *et al.*, 1968; Vadehra *et al.*, 1970 a, b). Other workers incorporated dyes into the bacterial suspensions and were thus able to demonstrate points of penetration by examination of the inner shell membrane (Alls *et al.*, 1964). Board and Halls (1973) used suspensions containing carbon black particles and were able to demonstrate that these particles were drawn through the shell. Board and Board (1967) developed a method to demonstrate where bacterial penetration of eggs had occurred, in which the sharp end of eggs to be examined was sterilized and a hole

(1.5 cm diameter) was cut in the shell. The contents were aseptically removed and any albumen adhering to the membranes was removed by washing with sterile Ringer's solution. The egg was then filled with a molten agar medium containing 0.1% 2,3,5-triphenyltetrazolium chloride and the hole was sealed with molten wax. After incubation at 37°C for 24 h the egg was cut longitudinally and the agar block removed. Where bacterial penetration of the shell had occurred organisms grew and reduced the tetrazolium compound to formazan which is red in colour. In this way points of bacterial penetration are clearly visible. The method has subsequently been adopted by a number of workers (Baxter-Jones, 1983; Smeltzer *et al.*, 1979; Sparks and Board, 1985). A disadvantage of this method is that, as not all bacteria reduce tetrazolium compounds, it may not detect penetration by all types of bacteria.

4.3 FACTORS AFFECTING TRANS-SHELL INFECTION OF EGGS

4.3.1 Temperature differential

Due to the porous nature of eggshells, eggs are particularly vulnerable to trans-shell contamination at oviposition because from the point of lay, as the warm egg cools, a negative pressure is created down the pores which may result in contaminated material being drawn across the pores and into the egg contents. This phenomenon was first demonstrated by Haines and Moran (1940) and is widely accepted as one of the main factors governing microbial contamination of eggs.

4.3.2 Moisture

Water, either as a liquid or in the vapour state, appears to be essential for microbial penetration of the pores of the calcitic shell (Board *et al.*, 1979). Flooding of the pores with contaminated water is sometimes regarded as the initial stage in the infection of the egg contents (Board and Fuller, 1974). 'Sweating' is a term given to the condensation of water droplets on the surface of an egg which can form when the egg is removed from refrigerated storage and placed at room temperature. The occurrence of 'sweating' is widely believed to induce bacterial penetration (Forsythe *et al.*, 1953; Fromm and Margoff, 1958) and in certain circumstances can allow mould growth to occur (Sharp and Stewart, 1936). However, it is well established that penetration will be greatly enhanced in circumstances where in addition to moisture the egg is exposed to a temperature change which causes the contents to contract and draw any water present through the open pores (Haines and Moran, 1940; MacLaury and Moran, 1959; Williams *et al.*, 1968; Vadhera *et al.*, 1970b; Board and Halls, 1973). In addition to water in the

liquid state acting as a vehicle to penetration it has been shown (Graves and MacLaury, 1962) that there is a significant positive correlation between the amount of water vapour present in the atmosphere at the time of laying and the incidence of contamination in eggs.

4.3.3 Presence of contamination

Clearly the presence of microbial contamination is an essential prerequisite for successful penetration of the egg to take place. As most eggs contain no viable organisms prior to oviposition the first major opportunity for contamination to take place is at the point of lay.

Several reports exist in which workers have recorded the level of contamination on the shells of eggs produced under different conditions (Haines, 1938; Harry, 1963b; Board *et al.*, 1964; Board, 1969; Quarles *et al.*, 1970; Metwally *et al.*, 1984). The level of contamination ranges from 10^3–10^5 c.f.u. per egg in clean conditions to 10^7–10^8 c.f.u. under dirty conditions (Baxter-Jones, 1991) and generally confirms that contamination on eggs is related to the environment in which the egg is laid. However, external contamination levels need not relate directly to incidence of spoilage or hatchability levels as other factors determine whether the organisms will actually penetrate the shell, grow and cause problems.

If it is accepted that the egg is most susceptible to penetration at the point of lay it follows that the microbiological status of the environment into which the newly laid eggs are deposited must have a major influence in determining the incidence of contamination in eggs. There is ample evidence that eggs laid into a heavily contaminated environment suffer more bacterial spoilage than those laid into a clean environment (Harry, 1963b; Smeltzer *et al.*, 1979).

Comparisons made between eggs laid on the floor of henhouses and those laid in clean nests have shown that floor eggs have a higher incidence of bacterial penetration (15.3%) compared with nest eggs (10.5%) (Smeltzer *et al.*, 1979). Joyce and Chaplin (1978) investigating a problem of poor hatchability in duck eggs demonstrated a higher level of bangers and bacteriologically spoiled eggs in floor eggs than in nest eggs and Baxter-Jones (1991) found turkey eggs laid on the floor had a higher incidence of bacterial contamination than corresponding nest eggs. It is noteworthy that Tullet (1990) reported that in broiler breeders hatchability of floor eggs is consistently 10–15% lower than those from clean nest eggs. Also comparisons of contamination levels in eggs laid in roll away cages indicated that they are usually more free from contamination than eggs laid in nests (Harry, 1963b; Carter *et al.*, 1973; Bruce and Drysdale, 1991).

Bruce and Johnson (1978) examined the contents of intact incubated broiler breeder eggs which had failed to hatch in the belief that the incidence of contamination would be a useful index of the level of hygiene employed
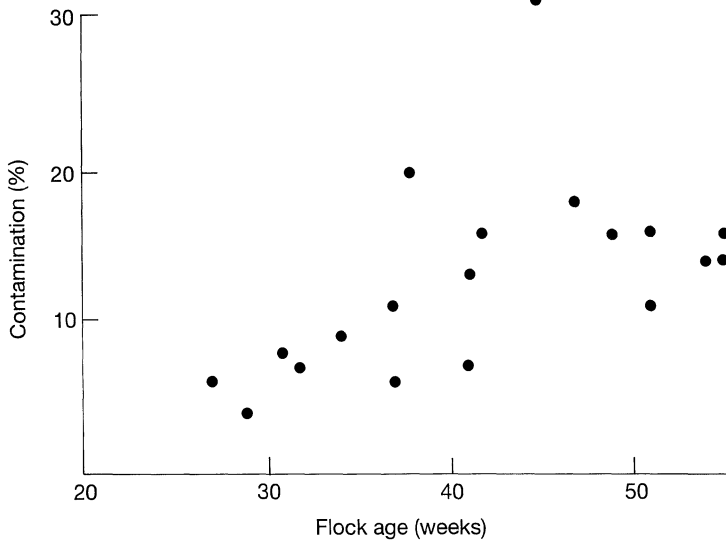
Figure 4.1 The percentage contamination of eggs failing to hatch at different flock ages.

in their production and the bacterial challenge the eggs had met between laying and hatching. This method of assessing bacterial contamination in hatching eggs was also used by Baxter-Jones (1983, 1991) and by Bruce and Drysdale (1983) to examine turkey eggs. Bruce and Johnson (1978) observed that eggs produced from grandparent flocks had a lower incidence of contamination than eggs from parent flocks (Table 4.1) which was thought to reflect the higher levels of hygiene generally applied in the production and hatching of grandparent eggs. They also demonstrated that as flocks became older the incidence of contamination in eggs failing to hatch increased (Figure 4.1). Three possible reasons were postulated to account for this observation: (a) the defence mechanisms of the egg deteriorate with flock age; (b) the environment becomes more contaminated; (c) the egg-laying apparatus of the hen becomes more contaminated with age and produces more contaminated eggs. The last proposition was investigated by Bruce and Drysdale (1991) and found to be an unlikely cause. Drysdale (1985) investigated the effect of flock age on the effectiveness of some of the physical barriers to bacterial penetration and showed that cuticle deposition deteriorated with flock age. In addition it is considered that a general

Table 4.1 Incidence of contamination in eggs produced from parent and grandparent flocks

Type of eggs	Number of eggs failing to hatch examined	Contamination of eggs failing to hatch (%)
Ex parent flocks	2002	14.8
Ex grandparent flocks	540	5.9
Total	2542	12.7

decline in the hygienic status of the environment during the life of the flock and in particular contamination of nest boxes is likely to play an important role.

Contamination of the nest-box litter is mainly derived from material tracked into the nest by the birds although air-borne contamination may also play a part in some circumstances (Quarles *et al.*, 1970; Metwally *et al.*, 1984). In this connection Quarles *et al.* (1970) and Carter *et al.* (1973) have shown that husbandry conditions influence the level of contamination found in the air and on eggshells. They observed that in general wire-floored systems of housing resulted in lower levels of contamination than litter or litter and slat systems.

The above observations have implications for the current trends which involve a return to more traditional methods of egg and poultry production in which the emphasis is placed on animal welfare and in particular the degree of freedom allowed to the laying hen. This desire to move from intensive to extensive husbandry systems has resulted in a range of housing systems coming into use of which 'Free Range' and 'Barn' systems are among the most common. In all the systems the birds have access to the outside environment, are free to move around inside the housing, lay in nests and have areas of litter for scratching etc. The consequence is that the nest material is prone to becoming contaminated and the eggs are likely to be more contaminated than cage-laid eggs. In addition as the birds have access to the outside world they are exposed to cross-infection from wild birds many of which harbour *Salmonella* and *Campylobacter*.

Investigations by workers who have tried to study bacterial penetration of eggs by deliberately contaminating the nesting material with organisms known to induce spoilage or reduce hatchability have produced some noteworthy results. Haines and Moran (1940) who dropped newly laid eggs into straw which had been sprayed with a strain of *Pseudomonas* known to cause spoilage found that none of the 45 eggs so treated showed any spoilage after three weeks' incubation at 25°C. Drysdale (1985) also failed to induce spoilage in a similar experiments in which eggs were naturally laid into nests containing wood shavings sprayed with a strain of *Bacillus cereus* known to cause spoilage of hatching eggs. Drysdale (1985) subsequently subjected eggs to a much more severe challenge by incorporating into the nest litter a mixture of fresh poultry faeces, soiled deep litter and shavings sprayed with a suspension of *Proteus vulgaris* and *Proteus mirabilis*, both from poultry sources. A fluorescent brightener (Calcafluor) was incorporated in the spray which allowed the distribution of the contaminated shavings to be observed using ultra-violet light.

This highly contaminated mixture resulted in a bacterial challenge of more than 10^9 c.f.u./g of nest-box litter. Control nests were employed in which the litter was changed on a daily basis resulting in a contamination level in the control boxes of less than 10^6 c.f.u./g. Approximately 100 eggs

Table 4.2 Incidence of *Proteus* contamination in eggs laid in highly contaminated nest-boxes compared with clean nest-boxes

Treatment	Number of eggs contaminated with *Proteus* spp.	
	Treatment pen	Control pen
1. *Proteus* spp.+ faeces	8	0
2. *Proteus* spp.	1	0
3. *Proteus* spp.+ high moisture	2	0

were collected from both the control and the treated nests. After being incubated, the eggs failing to hatch were opened aseptically and the contents examined for the presence of *Proteus* spp. This procedure resulted in eight eggs from the treated nests being contaminated with *Proteus* spp., and none of the eggs from the control nests. When the experiment was repeated with shavings sprayed with the *Proteus* cultures but without faeces or used deep litter only one egg examined from the treated nests was found to be contaminated with *Proteus*. It was thought that the higher moisture level which occurred in the nest litter containing faeces and deep litter may have been responsible for the differences in contamination levels observed. Consequently a third trial was undertaken in which shavings in the treated nests were sprayed with the *Proteus* cultures and the moisture level adjusted to that recorded in the nest litter containing faeces and deep litter. In this case only two of the eggs from the treated nests contained *Proteus* spp. These results (Table 4.2) indicate that surprisingly few eggs become contaminated even under conditions which would have been expected to induce serious contamination problems. Nevertheless the presence of faecal material and deep litter waste appears to increase contamination which cannot be attributed solely to increased moisture levels. Graves and MacLaury (1962) also used deep litter for nest material and sprayed it with a mixture of *P. vulgaris, Ps. aeruginosa* and *S. faecalis*. Over a series of trials they found that in incubated eggs which failed to hatch, 53.2% contained the test bacteria with a range of contamination levels of 1.8–87.4%. The results of Graves and MacLaury (1962) complement those of Drysdale (1985) and lend support to the view that heavily soiled nest material will increase the frequency of contamination in eggs. There are a number of possible explanations for this occurrence. Faecal or other soiling material may contain substances which reduce the surface tension of any moisture present and this has been shown to increase the rate of bacterial penetration (Board and Halls, 1973). Alternatively, the faeces or other soiling may contribute some chemical, e.g. iron, which interferes with the natural defence mechanisms of the egg thereby allowing bacteria to establish more easily in the egg once penetration has taken place. This suggestion is supported by the observation (Board, 1964) that when bacteria were injected into eggs as a suspension in an infusion of deep litter material,

Table 4.3 Comparison of the microflora on the surface of the egg and within spoiled eggs (Mayes and Takeballi, 1983)

Type of organism	Frequency of occurrence[*]	
	On the shell	In rotten eggs
Micrococcus	+++	+
Achromobacter	++	+
Aerobacter	++	-
Alcaligenes	++	+++
Arthrobacter	++	+
Bacillus	++	+
Cytophaga	++	+
Escherichia	++	+++
Flavobacterium	++	+
Pseudomonas	++	+++
Staphylococcus	++	-
Aeromonas	+	++
Proteus	+	+++
Sarcina	+	-
Serratia	+	-
Streptococcus	+	+

*The more plus signs, the more frequent the occurrence.

growth of the injected bacteria was enhanced compared with the same organism suspended in distilled water. In this connection it is noteworthy that MacLaury and Moran (1959) observed that suspensions of bacteria in mud applied to the surfaces of eggs resulted in higher levels of egg contamination than when bacterial suspensions were used alone.

4.3.4 Type of contaminating flora

The importance of the type of microbial flora which challenges the egg has to be appreciated as not all organisms are capable of penetrating the outer structures of the egg and surviving the adverse conditions in the albumen. A number of workers have reported on the flora present on eggshells. These observations have been summarized and compared with the types of bacteria isolated from spoiled eggs (Table 4.3) (Board, 1966; Mayes and Takeballi, 1983). In this connection Mayes and Takeballi (1983) have noted that although the flora found on the eggshell varies quantitatively and qualitatively in different geographical areas the spoilage flora of eggs tends to be similar irrespective of geographical area or husbandry methods, indicating that the intrinsic defence mechanisms of the egg influence the selection of spoilage types.

Seviour and Board (1972) compared the bacterial flora present in eggs derived from two domestic duck hatcheries, waterfowl of various types and chickens. 'Dead-in-shell' duck and waterfowl eggs were not examined and in the case of chickens only incubator clears were examined. The results (Table 4.4) showed that in wildfowl and duck eggs Enterobacteriaceae

Table 4.4 Microbial flora of eggs from different bird types

	% of Isolates					
	Duck A[*]	Duck B[*]	Waterfowl[*]	Hen[*]	Hen[†]	Turkey[‡]
Enterobacteriaceae	65.4	40	66	11.8	31.5	71.4
Staphylococcus spp.	2.5	4	11.4	23	9.2	7.7
Micrococcus spp.	1.2	0	21.3	63.8	34.6	0
Streptococcus spp.	0	0	0	1.2	15.3	8.5
Pseudomonas spp.	16	56	0	0	2.5	1.5
Acinetobacter spp.	6.2	0	0	0	0	0
Bacillus spp.	8.6	0	0.9	0	1.2	3.9
Moulds	0	0	0	0	0.2	1.6
Unidentified	0	0	0	0	5.5	5.4

*Seviour et al. (1972)
†Bruce and Johnston (1978)
‡Bruce and Drysdale (1983)

(mainly *E. coli*) constituted the main part of the flora, whereas in chicken eggs micrococci were the predominant group. Other major differences are that the duck eggs had a high incidence of *Pseudomonas* spp. while the other waterfowl and chicken eggs did not have pseudomonads present. Although it is difficult to account for all the observed differences in flora, Seviour and Board (1972) noted that the shells of the waterfowl and duck eggs were stained, indicating poor nest hygiene which could have provided an opportunity for contamination with faecal organisms to take place. They also proposed that under the conditions employed for incubating the eggs, coliform organisms would be preferentially selected in favour of pseudomonads.

In another examination of the flora of incubated hatching chicken eggs, Bruce and Johnson (1978) found a similar range of bacteria to Seviour and Board (1972) (Table 4.4) but there were differences in the relative proportions of the various groups. In this connection, however, it should be noted that Seviour and Board only examined incubator clears whereas the majority of eggs examined by Bruce and Johnson contained dead embryos. Bruce and Johnson (1978) found the incidence of contamination with *Pseudomonas* spp. and Enterobacteriaceae increased significantly with flock age ($P < 0.01$), which was thought to reflect a declining level of environmental hygiene; furthermore the presence of *Pseudomonas* spp. showed a significant ($P < 0.01$) correlation with decreased hatchability. It was also observed that of all eggs examined (2542), 2.4% were found to be contaminated with more than one organism with the combination of Enterobacteriaceae and *Streptococcus* spp. occurring more frequently than would be expected by chance. This can be explained by assuming that the organisms originate from a common source, namely faeces. Harry (1957) showed, under *in vitro* conditions, that *S. faecalis* and *B. cereus* formed a synergistic relationship which enhanced their individual spoilage potential of egg yolk. If such a synergistic relationship exists for *Streptococcus* spp. and Enterobacteriaceae it may account for the

reduced hatchability which correlated with their joint presence. Baxter-Jones (1991) reported recovering a similar flora from turkey eggs. However, Bruce and Drysdale (1983) found some major differences between the flora of turkey eggs and that reported for chicken eggs (Table 4.4). In particular the absence of micrococci from the turkey eggs and the relatively high incidence of Enterobacteriaceae, predominantly *E. coli*, are worthy of note. The turkey eggs were laid into roll-away nests which was reflected in the low incidence of contaminated eggs (4%) and it may have been expected that this nesting system would have reduced contamination with *E. coli*. However, the birds were fertilized by artificial insemination and it is thought this procedure may have introduced *E. coli* as Gale and Brown (1966) reported that 53.5% of the turkey semen samples they examined were contaminated with coliforms.

Invasion and growth of bacteria in eggs have been studied by inoculating bacteria into the air-sac between the shell membranes. This is a useful technique as it allows the direct introduction of bacteria into the egg and eliminates variability introduced by the use of penetration techniques such as dipping. MacLaury and Moran (1959) found it was the most successful method of inducing bacterial contamination. Using this method Board (1964) studied the growth of a range of Gram-negative bacteria in eggs. The results indicated that once penetration has taken place with such Gram-negative organisms there is a localized and restricted phase of growth in the area of the membranes where penetration has occurred. The degree of growth at this stage is dependent, among other factors, on the presence of iron which counteracts the effect of conalbumin, a chelating agent present in the albumen. A second and more extensive phase of growth was found to occur when the yolk made contact with the membrane. Board (1964) indicates that this phenomenon could account for the lag of 10–20 days reported by other workers, which occurs between bacterial penetration taking place and the subsequent recovery of bacteria from or detection of changes in the egg contents. Board also noted that different bacteria produced different and characteristic rots which were related to the metabolic activity of the bacteria.

Inoculation studies in hatching eggs have also shown that some bacteria are more effective in reducing hatchability than others (Bruce and Drysdale, 1991). Figure 4.2 shows that *Proteus mirabilis, Staphylococcus aureus* and Group D *Streptococcus* spp. reduced hatchability to zero whereas other organisms such as *Micrococcus* spp. had a less-dramatic effect on hatchability. In this connection it is noteworthy that both *Pr. mirabilis* and *Staph. aureus* are lecithinase positive. In addition to hatching and spoilage problems, the egg can also act as a vector in the transmission of food-poisoning organisms. *Campylobacter* infection in hens is widespread and the transfer of this pathogen to humans is often associated with the consumption of chicken, particularly raw or undercooked chicken. Despite the prevalence
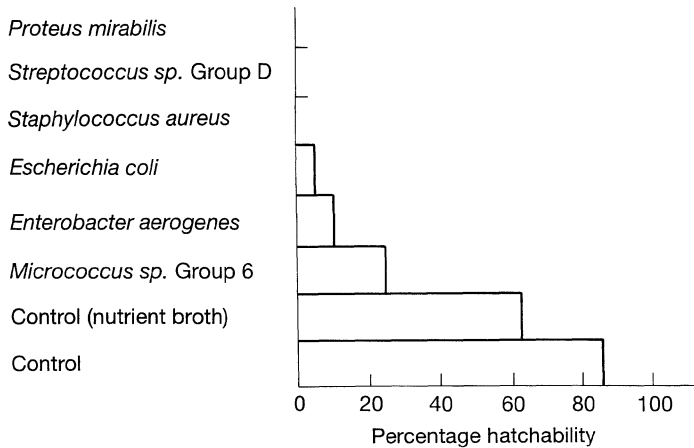
Figure 4.2 The percentage hatchability of eggs inoculated with various bacterial cultures.

of *C. jejuni* in hens it is unclear whether eggs have the ability to transmit the organism. Finch and Blake (1985) reported a case in which 26 of 81 people contracted campylobacteriosis after consuming undercooked eggs. Work carried out by Doyle (1984), Shane *et al.* (1986) and Shanker *et al.* (1986) showed that eggs are not internally infected with *C. jejuni* when laid by hens known to carry and excrete the organism, suggesting that vertical transmission may not occur naturally in flocks. Several workers have since undertaken penetration studies in both table and fertile eggs to determine whether *C. jejuni* is capable of penetrating intact eggs and subsequently surviving within the egg contents (Clark and Bueschkens, 1985; Doyle, 1984; Shane *et al.*, 1986; Jones *et al.*, 1991). Their findings in fertile eggs showed that penetration can take place as long as a pressure or temperature differential is applied (Clark and Bueschkens, 1985) and the presence of iron was again found to enhance the infection rate. Contamination of commercial table eggs with faecal suspensions containing *C. jejuni* resulted in a low incidence of penetration. Viability on the shell surface was retained for only 16 h, this was attributed to the desiccation of the faecal suspension. The susceptibility of *Campylobacter* spp. to desiccation may play an important role in the inability of the organism to survive long enough for transmission to take place. A field survey of three commercial laying farms demonstrated that hens shown to be faecal shedders of *C. jejuni* (12–62%) did not produce infected eggs (Shane *et al.*, 1986). The occurrence and survival of the organism on the shell surface is not only temperature dependent but also dependent on the relative humidity (Kollowa and Kollowa, 1989). Storage at lower temperatures but with higher humidity permitted longer survival times. Inoculation studies also showed better survival within the lower temperature range (4–7°C). Studies carried out in broiler breeder eggs support the view that transmission of this organism

via eggs is highly improbable. Although penetration of the eggs was achieved under artificial conditions, survival of the organism was not detected on the shell or the membranes of fertile incubated eggs after 6 h. This may in part be attributable to its sensitivity to atmospheric oxygen (Neill *et al.*, 1985).

Amin and Draughon (1990) evaluated the ability of *Yersinia enterocolitica* to penetrate and infect shell eggs. The eggs were immersed in various suspensions containing *Y. enterocolitica* (10^6/ml), stored at 10°C and then examined microbiologically. The organism was not recovered from the eggs immediately after inoculation. However, after 7 days it was present in 14% of the eggs and in 100% of the eggs after 14 days. This lag period primarily reflects the antimicrobial properties of the egg white. The supplementation of iron substantially increased both the rate and extent of infection as has been found with other pathogenic organisms and common egg spoilage bacteria. Due care should be exercised in washing, storage and handling of eggs to prevent contamination by this organism since its alkalotolerance, its ability to survive at the relatively low pH of 4.6 in addition to its ability to grow at refrigeration temperatures makes it a psychotrophic food-borne pathogen of public concern.

In the UK, the level of food poisoning cases arising from *Salmonella* infection has increased dramatically. This increase has also been noted in the USA and Europe. Not only is poultry meat a major vehicle of human salmonellosis, but there is evidence that eggs are also an important source of human *Salmonella* infection. Recent infections have been caused by *Salmonella typhimurium* and *Salmonella enteritidis* due to their invasive characteristics. Observations from infected hens have shown that contaminated eggs are laid intermittently and that these infected eggs may only contain a few salmonellae. The subsequent storage conditions of these eggs are of importance as at room temperature the salmonellae are known to be able to multiply and reach high levels within the eggs very quickly (Board *et al.*, 1989). However, it should be noted that in certain outbreaks the infective dose has been relatively small (Cowden, 1990), showing that large inocula are not prerequisites for the induction of human illness. In particular *S. enteritidis* PT4 has been responsible for the major increase in food poisoning associated with eggs in recent years. The ultimate eradication of *S. enteritidis* from both poultry layer and broiler flocks is largely dependent on the efforts of the veterinarian and the poultry industry.

4.3.5 Porosity of the eggshell

Pores are essential to the development of embryos in hatching eggs as they allow the exchange of respiratory gases and water vapour conductance (Burton and Tullett, 1983). Since the only route available for bacteria to penetrate the intact shell of the avian egg is via the pores, the structure of

the pores may have a bearing on how readily penetration takes place. In the hen eggshell the pores consist of broad funnel-like openings forming single unbranched channels penetrating the crystal layers and terminating in clefts formed between adjacent mammillary knobs (Parsons, 1982). This pore structure is found in the commercially important birds, e. g. hen, turkey, guinea fowl, duck and goose, however branched pores are found in other breeds of bird, e. g. swans, ostrich, and the rhea (Board, 1980).

The number of pores per egg ranges from 6000–10 000 in hen eggs (Marshall and Cruickshank, 1938; Romanoff and Romanoff, 1949; Tyler, 1953; Board, 1969; Tullett, 1976). Several workers have shown that the pores are unevenly distributed over the surface of the eggshell (Tyler, 1953, 1955). and that the broad pole is generally more porous than the narrow pole (Romanoff and Romanoff, 1949; Tyler, 1958; Alls *et al.*, 1964). Tullett (1976) found that chicken eggs had a greater number of pores per egg compared with turkey eggs but the pore diameters were larger with an average of 18 µm compared with 11.9 µm in chicken eggs. With increased flock age the number of pores increases but their dimensions remain fairly constant (Rahn *et al.*, 1981). The above observations of the physical dimensions of pores in eggshells indicate that bacteria would pass through freely and indeed yeasts which are considerably larger than bacteria have been shown to be capable of passing through the pores of hens' eggs (Haines and Moran, 1940).

It has been demonstrated that water vapour conductance can be used to assess the overall porosity of hatching eggs (Ar *et al.*, 1974; Tullett, 1981). Drysdale (1985) used this technique to study the relationship between porosity and incidence of contamination with a view to establishing whether eggs with a high porosity are more susceptible to bacterial penetration under commercial conditions. In the course of this study the porosity of >3000 hatching broiler breeder eggs was measured and the incidence of bacterial contamination in the eggs failing to hatch was determined. The results indicated that there was no direct relationship between porosity, as measured by water vapour conductance, and susceptibility to bacterial contamination. These findings may be accounted for by the presence of the cuticle layer which is capable of covering many of the pores thus preventing penetration by bacteria but still allowing gaseous exchange to take place.

4.3.6 Cuticle on the eggshell

It can be seen therefore that in studies of bacterial penetration through the pores the presence of the cuticle has to be taken into account. The cuticle of the hen's egg is the outermost layer and is glycoprotein in nature; it often extends into the pores to form cuticular plugs which are effective in preventing microbial penetration. Although the cuticle *per se* is permeable

to gases either through cracks in the cuticle layer or between the individual spherules (Marshall and Cruickshank, 1938; Board and Halls, 1973; Board, 1980; Sparks and Board, 1984) the presence of a mature cuticle confers on the domestic hen eggshell a defence barrier to water uptake (Board, 1982; Sparks, 1985). Sparks and Board (1984) showed that eggs naturally lacking cuticle took up more water than those having cuticle. Scanning electron micrographs have endorsed the fact that the cuticle forms an outer covering on the egg, often plugging up the outer orifices of the pore canals, thus protecting them from flooding (Tullett *et al.*, 1975).

The cuticle has been shown to vary in thickness across the surface of the egg. In the domestic fowl this thickness ranges from 0.5–12.8 μm with an average of 10 μm (Simkiss, 1961; Simons, 1971; Parsons, 1982). Simons (1971) expressed the cuticle weight as being 0.2% of the egg weight. The cuticle of brown eggs has been shown to be thicker than that of white eggs (Simons, 1971; Board and Halls, 1973). Cooke and Balch (1970) measured the mean cuticle weight of brown eggs as 18 mg/shell (250 μg/cm^2) and as the flock age increases the cuticle is reported to get thinner (Simons, 1971).

Sparks and Board (1985) showed that the physical state of the cuticle alters immediately after the egg has been laid and can have an important bearing on the egg's susceptibility to microbial infection at this particular stage. When the egg is freshly laid the cuticle appears 'wet' but then takes on a 'dry' appearance after approximately 3 min. Bacterial penetration studies were carried out on areas of shell which were 'wet' and 'dry' and a higher incidence of contamination was found across the shell which still had 'wet' cuticle. Electron microscopy studies of the 'wet' shell revealed a frothy, open, granular appearance to the cuticle whereas the 'dry' cuticle took on the sphere structure of mature cuticle thus resulting in less penetration through the pores. This study showed that in addition to the temperature differential across the eggshell acting as a driving force, microbial penetration is closely associated with the physical state of the cuticle at oviposition. Sparks (1985) also concluded that the mature cuticle especially in brown eggs was the major barrier to the movement of water while in the liquid but not the vapour state. It would be reasonable to assume, therefore, that microbial contamination would be less likely to take place once the cuticle has set.

Board and Halls (1973) developed a simple staining technique to assess cuticle deposition using the food dye Edicol Pea Green which is a mixture of Tartrazine and Green S (72:28). In this procedure eggs are immersed in a 1% aqueous solution of the dye for a period of 5 min then rinsed in fresh water to remove excess dye prior to drying. The depth and distribution of staining is taken as an index of cuticle deposition. Drysdale (1985) used a reflectometer fitted with a red filter to measure the amount of Green S taken up by the cuticle which showed an acceptable degree of correlation with cuticular dry weight measurements. This application of the reflectometer
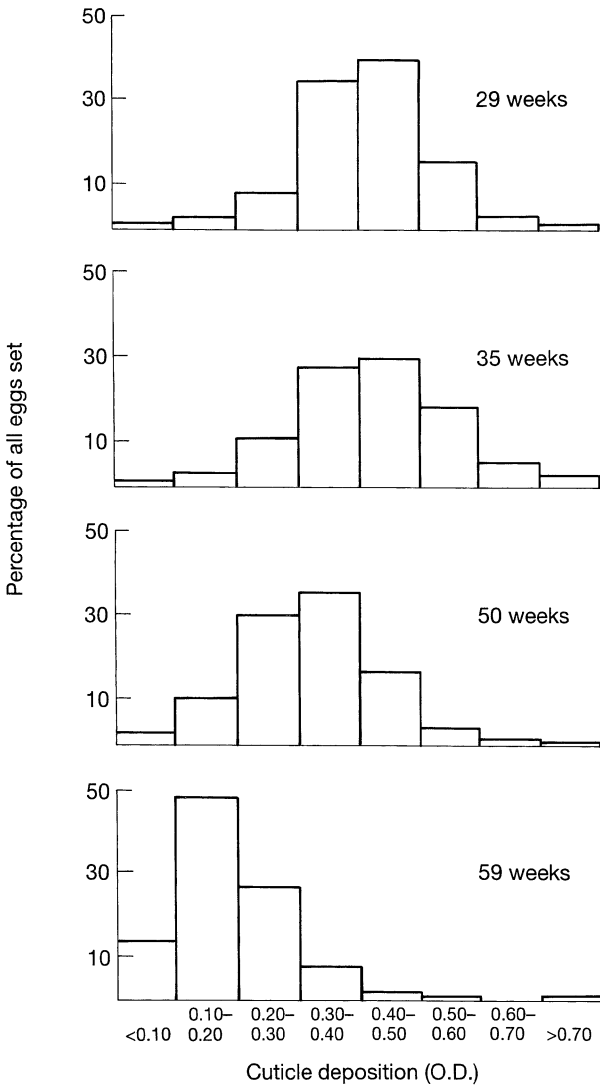
Figure 4.3 The effect of flock age on cuticle deposition.

allowed cuticle deposition on a large number of eggs to be assessed and numerical values allocated to observed degrees of cuticle deposition. In this particular study cuticle deposition was measured on >3000 hatching eggs and bacteriological analysis was carried out on all the eggs failing to hatch. The incidence of bacteriological contamination within the eggs was found to be significantly higher in eggs which had a poor cuticle (40%) compared with eggs with a medium or good quality cuticle (26%) confirming that the cuticle layer is of primary importance in the prevention of microbial trans-

mission across the integument of the egg. It should be noted, however, that cuticle deposition over the entire surface of the egg is seldom uniform and often large areas of the porous shell are devoid of cuticular cover. Staining techniques have allowed the distribution of the cuticle over the surface of the egg to be assessed. In a survey on a commercial flock of brown egg layers it was found that approximately 3.5% of eggs were laid totally devoid of cuticle and approximately 8% lacked cuticle at either the broad or narrow pole (Board and Halls, 1973). Scanning electron microscopy revealed that cuticleless surfaces of brown layer eggs showed open pores and the surface was irregularly contoured and in many instances deeply fissured (Board, 1975).

The observation that cuticle deposition declines as the flock ages was confirmed in a broiler breeder flock (Figure 4.3) and may be a factor in explaining why eggs produced from older flocks are more prone to bacterial contamination (Bruce and Johnson, 1978). In addition, Ball *et al.* (1975) found that a great deal of variation in cuticle deposition existed in eggs from different hens and different breeds. They also observed that several other factors had deleterious effects on cuticle quality, e. g. the influence of egg fumigation, washing and storage conditions. Egg storage, especially at higher temperatures had an adverse effect, fumigation was found to reduce cuticle staining intensity, possibly due to the action of formic acid. Similarly brush marks and lighter staining were apparent in washed eggs. The uric acid present in poultry manure also affects the cuticle. In addition, it has been established that certain bacteria such as pseudomonads are capable of digesting the cuticle layer (Board *et al.*, 1979), however, this activity only occurred when humidity levels approached 100%. Other organisms found to be capable of cuticle digestion include *Alcaligenes bookeri* and *Streptomyces* spp. (Lifshitz *et al.*, 1965; Board and Halls, 1973). All these factors are bound to play a role in enhancing trans-shell transmission of microorganisms by breaking down the primary barrier to infection.

The crucial factor in determining whether an egg is likely to suffer bacterial penetration of the shell is not the number of pores but the number of pores not plugged with cuticle, through which bacteria can pass under suitable conditions. Generally the proportion of open pores is low (Board, 1966) and pores are largest and most frequently found on the blunt end of the egg (Mayes and Takeballi, 1983). Allied to this observation Vadehra *et al.* (1970a) studying contamination routes in table eggs demonstrated that penetration with *Ps. aeruginosa* occurred most frequently at the poles of the egg and that the blunt end was more prone to penetration than the pointed end. Also it has been observed that cuticle deposition is not always complete at the poles (Board and Halls, 1973; Drysdale, 1985). It is interesting to speculate why the blunt end should have more open pores than the other areas of the shell. Given that the concept of the egg contents contracting on cooling and creating a vacuum within the egg is correct and accepted, it

seems probable that where the two shell membranes separate at the air sac the inner membrane will act like a diaphragm and will move with the contracting contents. This will have the effect of transferring most of the vacuum effect onto the area of the shell covering the air sac. Air will try to move into the egg through the pores in this area and the cuticle layer will be stressed and may rupture. The larger the diameter of the pore the more likely the cuticle will be to rupture and the thinner the cuticle layer the more likely it will be to rupture. Furthermore, when an open pore is formed it will allow free passage of air and reduce the stress on the cuticle covering other pores. This theory may account for the low number of open pores found in eggs and their higher incidence at the blunt end of eggs.

The importance of shell thickness in its own right is not completely clear as some studies found that it did not significantly affect the transmission of bacteria across the shell (Williams *et al.*, 1968; Vadhera *et al.*, 1970; Smeltzer *et al.*, 1979; Drysdale, 1985), but Sauter and Peterson (1974) found that shell thickness affected the ability of bacteria to penetrate the egg. Irrespective of the importance of shell thickness any fracture of the shell will enhance the likelihood of penetration. Miller and Crawford (1953) reported an increased incidence of penetration and egg spoilage in cracked eggs. Brown *et al.* (1966) also found heavy contamination in cracked eggs especially if the eggs were wet. Wetting a cracked egg in the presence of bacteria apparently sweeps the bacteria into the shell crack and onto the membranes.

4.3.7 Shell membranes

Underlying the shell matrix are the shell membranes. In the domestic hen the membranes consist of three distinct layers: the inner and outer membranes which consist of a network of randomly orientated fibres and a homogeneous third layer of electron-dense material called the limiting membrane (Tranter *et al.*, 1983). Apart from the physical role of protecting the albumen from microbial infection (Bean and MacLaury, 1959; Williams and Whittemore, 1967) the membranes are involved in the diffusion of respiratory gases to and from the chorioallantois (Tullett and Board, 1976) and the movement of Ca^{2+} from the shell (Coleman and Terepka, 1972).

Due to the osmotic forces acting across the shell membranes it might be expected that flooding of pores and formation of a thread of water along the pore canal would result in continuous flow and thus translocate bacteria across the integument of the egg. It has not been possible to demonstrate such a phenomenon in intact eggs, presumably because the drag imposed by the pore canal is greater than the osmotic forces.

Kraft *et al.* (1958) found that the membranes are important barriers to bacterial transmission. Penetration studies (Lifshitz *et al.*, 1964) to establish the relative importance of the shell and the outer and inner membranes in

affecting this transmission process showed that the inner membrane was found to be the most important sole barrier. It should be noted that the effect of the cuticle was not taken into account in these studies. Although little is known about the mode of penetration through the inner shell membrane, when whole eggs are challenged externally with large numbers of bacteria, the organisms are recovered from the inner surface of the inner membrane within minutes (Bean and MacLaury, 1959). Thus the shell membranes pose only a temporary barrier to the inward movement of bacteria. Tracer studies by Walden *et al.* (1956) indicated that the membranes could provide mechanical restraint for a period of up to 15–20 h. *In vitro* studies showed that shell membranes from eggs of different porosities did not vary in their capacity to resist penetration by *Ps. fluorescens* (Hartung and Stadelman, 1963). However, it was found that membranes derived from older eggs underwent a greater degree of bacterial penetration compared with fresh eggs. As the pH increases rapidly in egg white with age this may result in alkaline hydrolysis of proteins in the egg white located in the interstices of the membranes thus permitting a more rapid entry by bacteria.

Electron microscopy has shown that membranes become more permeable after bacterial penetration has taken place which tends to suggest that enzymatic activity may also be involved in the penetration process (Brown *et al.*, 1965). Board (1965) has shown that commonly occurring contaminants of rotten eggs can multiply in a buffered solution of mineral salts containing intact shell membranes and Lifshitz *et al.* (1965) demonstrated that the exterior structures of the egg contained sufficient nutrients for bacteria such as *S. paratyphi*, *Ps. fluorescens* and *Alcaligenes bookeri* to grow while penetrating the eggshell.

The permeability of the inner membrane and the transmission of bacteria is known to be affected by charge and chelating agents (Maesso *et al.*, 1970). The iron-binding properties of the membranes also play a major role in the transmission of bacteria into the egg contents. Garibaldi and Bayne (1962) were the first to notice the influence of iron on the incidence and rate of rotting of eggs washed under farm conditions. Studies by Board (1968) showed that iron contamination of the shell membranes enhanced the growth of bacteria trapped in the membranes and resulted in heavy contamination of the underlying albumen. More recent inoculation studies with enteropathogenic organisms have shown that water, especially in the presence of iron, greatly enhanced the growth of bacteria (Becirevic *et al.*, 1988). The iron appears to bind onto the medulla of the shell membranes and is capable of persisting for several weeks thus ensuring a constant supply for microorganisms trapped on or between the fibres (Tranter *et al.*, 1983).

4.3.8 Chemical defence mechanisms

Once microorganisms penetrate the integument of the egg the viscosity of

the albumen ensures that they remain localized and in fresh eggs the combined workings of the chalazae and albuminous sac preclude contact of the contaminants with the yolk.

The presence of ovotransferrin (conalbumin) in the albumen chelates iron and is one of the major defence mechanisms against bacterial growth. Studies of microorganisms grown in a nutrient medium containing ovotransferrin showed that the consequent iron deprivation resulted in a switch from respiration to glycolysis (Theodore and Schade, 1965). Garibaldi and Bayne (1960) showed that bacterial growth does not occur unless this iron binding agent is quenched with Fe^{3+} ions. Therefore the ability of the membranes to harbour excess iron can pose problems by inactivating the ovotransferrin and thus aiding the penetration process.

The albumen has a relatively high pH of 9.5 which can itself have a deleterious effect on bacterial growth. Other substances found in the albumen include avidin, which combines with biotin, and a protein which combines with riboflavin, thereby making these chemicals unavailable to the microorganism (Board and Fuller, 1974). Lysozyme present in albumen hydrolyses the peptidoglycan in the bacterial cell wall by breaking the β1,4 linkage between muramic acid and glucosamine. Gram negative organisms are generally regarded as being resistant to the action of lysozyme due to the presence of the lipoprotein layer in their cell walls (Chander *et al.*, 1984) and this may account for the preponderance of Gram negative organisms found in spoiled eggs (Board, 1966).

4.4   CONTROL OF TRANS-SHELL TRANSMISSION

4.4.1 Hygienic status of environment

As microbial transmission can often occur at the point of lay, methods which improve the hygienic status of the environment are regarded as being of paramount importance (Harry, 1963b; Smeltzer *et al.*, 1979). The use of roll-away cages in layer flocks reduces the risk of contamination (Quarles *et al.*, 1970; Bruce and Drysdale, 1991). In order to facilitate the rapid transfer of eggs, the slope of the floor is crucial. Otherwise the eggs would remain in the cages and be subjected to faecal soiling. The choice of nest box material has been shown to influence the general cleanliness of the eggs and a variety of nest box materials have been investigated in an attempt to minimize soiling of eggs in the nest (Dawson and Watts, 1952; Baker and Balch, 1962). Baker and Balch (1962) demonstrated that fine perlite and calcined clay were better nesting material than the most commonly employed material, woodshavings. However, for economic and other practical reasons woodshavings are currently the most commonly employed nesting material and are likely to continue to be so into the foreseeable future.

Table 4.5 Level of bacterial contamination in untreated nest-box woodshavings and chemically disinfected woodshavings (log c.f.u. g^{-1}).

	Day 1	Day 2	Day 3	Day 4
Untreated woodshavings	8.90	8.80	8.81	9.04
Treated woodshavings	4.35	5.24	6.33	6.71

Regular changing of nest-box material is also considered important (Vest, 1978), as moisture and faecal material can accumulate and act as sources of contamination. The use of floor eggs for hatching purposes should be avoided as they have a tendency to explode in the incubator thereby contaminating surrounding eggs (Vest, 1978).

Some workers have studied the potential benefits of trying to improve the microbiological quality of the nest box litter by including antimicrobial agents to control microbial contamination which inevitably takes place. Hodgetts and Dance (1974) showed that including formaldehyde flakes in the nest box litter reduced the level of contamination on eggshells and in the nest litter. Their results indicated that eggs from treated nests tended to have a higher hatchability but this observation could not be substantiated by statistical analysis. Drysdale (1985) also investigated the possibility of controlling nest box hygiene. In this case the woodshavings used for nest box litter were treated by spraying with a commercially available disinfectant mixture of chlorxylenol, sulphonic acid and liquid paraffin (10:10:80). In the trial a Ross 1 Broiler Breeder flock, aged 48 weeks, was housed in ten deep litter pens with approximately 150 birds per pen. Each pen had two rows of nest boxes. In one row the woodshavings were replaced with treated shavings while the shavings in the other row were left untreated as a control. Samples of shavings were collected over the period of the trial and eggs were collected three times a day. After incubation all eggs failing to hatch were opened aseptically and the contents examined for bacterial contamination. The nest box litter in chemically treated nests showed a lower level of contamination over the four-day period of the trial (Table 4.5) compared with the litter in the untreated nests and the incidence of contamination in the eggs examined from the treated nest boxes was 3.7% compared with 6.8% in eggs from untreated boxes. However, there was a higher incidence of embryo mortality in the eggs from the treated nests which occurred during the later stages of incubation and was thought to be related to the oil blocking pores and inhibiting respiration of the embryo. It appears therefore that reduction in nest box contamination is possible and this can be reflected in reduced contamination of eggs, however the choice of chemical is of paramount importance and is a matter which merits further research.

4.4.2. Egg collection and storage

Egg collection, especially in the case of hatching eggs should be frequent (three or four times daily) in order to minimize the risk of soiling and to prevent breakages occurring (North, 1984). It has been shown that eggs handled with gloved hands have a lower level of surface contamination compared with eggs handled normally (Rosser, 1942).

In addition to the length of storage, the temperature of storage is of importance. If infected eggs are kept at room temperature, microorganisms such as salmonellas can multiply rapidly and reach high levels within the egg, whereas cold storage has been shown to completely inhibit the growth of salmonellas in both the yolk and the albumen (Board *et al.*, 1989) . It is also important to store eggs under suitable moisture conditions. Correct humidity conditions are essential to prevent excessive evaporation from the egg contents. Care should also be taken to prevent 'sweating' of eggs, i. e. condensation of water when cool eggs are moved into warm conditions.

4.4.3. Egg washing

The majority of reports on the effect of egg washing have established that various egg washing practices can result in an increase in the number of infected or spoiled eggs (Lorenz and Starr, 1952; Miller and Crawford, 1953; March, 1969; Moats, 1978, 1981). There are several factors which are regarded as being important in influencing this penetration process. The bacteriological quality of the wash water is of prime importance and in general spray systems which employ fresh water are better in this respect than immersion systems in which the water tends to become contaminated. It is also important to ensure that the washwater temperature is higher than that of the eggs. However, care should be taken to ensure that the temperature difference is not too great or the shells may crack and allow contamination to penetrate readily. The type of contaminating flora present on the surface of the egg or in the washwater also plays an important role in determining whether infection takes place as some bacteria are more prone to cause problems than others. Even if correct temperature differentials are adhered to Brant and Starr (1962) found that high bacterial loads in the washwater could still lead to contamination. Moats (1981) found that ineffective cleaning of the washing equipment was an important source of contamination of washwater and recommended that washing of any description should be avoided. At present the washing of table eggs is not recommended in the UK but this does not apply to eggs intended for hatching purposes. It has been shown that the presence of iron (4.8 mg/l) in the washwater can result in the iron as well as microorganisms being deposited on the shell membranes (Garibaldi and Bayne, 1962) and this is known to accelerate the rotting process. Generally, alkaline products are

added to the washwater in order to increase the pH thus making microorganisms such as *Salmonella* spp. more susceptible to the heat (Humphrey *et al.*, 1981; Kinner and Moats, 1981). The eggshell is more sensitive to acidic conditions, therefore the pH of egg washwater is usually adjusted upwards. Holley and Proulx (1986) emphasized the importance of monitoring the conditions of egg wash water. In their studies they found that salmonellas would not survive very long in egg washwater with a pH >10 and with temperatures in excess of 38°C. It was found that washwater at pH 10 and 42°C had a greater lethal effect than washwater at pH 7 and 60°C. It is recommended that egg washers should have a minimum temperature of 32°C or 11.1°C warmer than the eggs being washed (IAMFES, 1976). A combination of high temperature and high pH would probably help to eliminate salmonellas from egg washwater. However, care should be taken as corrosion of equipment may arise as a result of using too high a pH (Holley and Proulx, 1986). In addition it is known that certain detergents can have a deleterious effect on the cuticle thus enhancing bacterial transmission across the shell. Simons and Wiertz (1966) examined the ultrastructure of the surface of the eggshell using an electron microscope to determine the effect of different washing techniques. They found that water >40°C had an effect on the cuticle layer and the addition of detergents brought about even greater changes. Early studies (Haines, 1938) showed that soaking clean fresh eggs in a suspension of *Pseudomonas* (5×10^4 c.f.u./ml) produced no spoilage whereas similar eggs which had been previously washed and dried, had a 40% incidence of spoilage within 14 days' storage at 20°C. These studies highlight the potential problem posed by the washing process on egg spoilage.

Yersinia enterocolitica is known to survive and grow in egg washwater (Southam *et al.*, 1987), and *Listeria monocytogenes* has also been shown to survive in egg washwater under normal operating conditions (pH 10.5/42°C) for up to 4 h. These washwater conditions had been selected by Holley and Proulx (1986) to minimize the survival of salmonellas (Laird *et al.*, 1991). The fact that both *Y. enterocolitica* and *L. monocytogenes* are alkali tolerant makes them better suited to survive the alkaline conditions established for egg washwater. Although *Listeria* spp. are susceptible to lysozyme they have been cited as being capable of survival and growth in liquid egg (Laird *et al.*, 1991). These findings emphasize the possibility that egg washing conditions may serve as a source of contamination of shell eggs. It is therefore understandable that egg washing is not recommended for table eggs in the UK.

4.4.4. Egg fumigation

Fumigation of eggs has widely been used as a means of disinfecting the surface of the shell, as efficient fumigation has been shown to reduce the

shell-borne bacterial population by 99.8% (Williams, 1970). Some organisms such as cocci and spore formers are resistant to the fumigation process. The destruction of bacteria was found to be greater on brown eggs compared with white eggs even though the bacterial load on unfumigated brown eggs was consistently higher. This is believed to be because brown eggs have a thicker cuticle than white eggs (Simons, 1971; Board and Halls, 1973), and are therefore able to absorb the fumigant to a greater degree. The gas acts primarily on the surface of the egg and does not penetrate to any great extent under the shell or into the albumen and does not provide any residual bactericidal effect.

Formaldehyde has several advantages: it is a relatively efficient disinfectant; its gas phase confers more penetration power down the pores of the shell; it is low cost; it has a non-corrosive nature; and it does not damage the eggs or the developing embryos. However, the practice of egg fumigation is becoming less popular due to the health risks associated with long-term exposure. Formaldehyde is now regarded as an occupational carcinogen. It is toxic by inhalation or ingestion, it is irritating to the eye, respiratory system and skin. Therefore, it is of paramount importance to ensure the fumigation procedure is carried out correctly and that formaldehyde exposure is monitored in order to minimize the dangers (Attar, 1990).

4.4.5 Other forms of egg sanitation

Studies by Arhienbuwa *et al.* (1980) have indicated that sanitized eggs have significantly lower bacterial counts than fumigated eggs. Chemicals such as chlorine dioxide and quaternary ammonium compounds are known to be effective sanitizers for eggs although they tend to lose their killing power in the presence of organic material (North, 1984).

Ozone is an effective biocide and has been evaluated and compared with formaldehyde fumigation of eggs. It was found to be as effective as formaldehyde in reducing microbial counts but there was a concomitant reduction in hatchability. This adverse effect on embryo development precludes its use in the sanitation of hatching eggs (Whistler and Sheldon, 1989).

UV irradiation of eggs as an alternative to formaldehyde fumigation has also been examined both for hatching purposes (Latala and Wakula-Radzik, 1990) and for human consumption (Latala and Dobrzanski, 1989). Although the bacterial load on the shell can be reduced, it was found that excessive UV dosage reduced hatchability.

4.5 SUMMARY

Trans-shell contamination of the avian egg is a well-established phenomenon and is known to affect the shelf-life of table eggs and the hatchability of incubated eggs. The major factors identified as being important to the transmission of bacteria through the shell are: temperature differential, presence of moisture, quality of cuticle deposition, presence of open pores and the presence of contamination. Once through the shell growth of the microorganisms and development of subsequent spoilage defects depend on the type of microorganisms present, temperature, time and integrity of the internal defence systems of the egg.

Trans-shell contamination can be minimized by controlling the hygienic status of the environment, particularly nest box hygiene. Husbandry and management systems will also affect contamination levels and sanitizing procedures have been shown to reduce contamination on eggs.

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Contamination of eggs with potential human pathogens

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5.1 INTRODUCTION

As with any food of animal origin, eggs may be contaminated with organisms which are potentially pathogenic for humans. The most important of these are salmonellas and most of this chapter will be devoted to these organisms and to *Salmonella enteritidis* in particular. Poultry can be infected or colonized with other potential human pathogens. These may contaminate eggs. Problems caused by this will also be discussed.

5.2 HISTORICAL BACKGROUND

There has been a long history of association of human salmonellosis and eggs. Initially the association was mainly with duck eggs (Scott, 1930; Anon, 1944). During and after the Second World War, a number of outbreaks caused by a variety of *Salmonella* serotypes occurred in the United Kingdom as a result of contamination of spray dried and bulk liquid egg. This led to the introduction of the Liquid Egg (Pasteurisation) Regulations, 1963 requiring all liquid egg to be heated to a temperature of not less than 64.4°C for 2.5 min. Heated products also had to satisfy the α-amylase test. These regulations were some of the most effective pieces of Public Health legislation and it is significant that, in the two years following their introduction, the reported number of cases of human salmonellosis in England and Wales fell by 25%.

In the United Kingdom, egg-associated cases remained at a low level until, in the middle to late 1980s, in common with a number of other countries, it became apparent that eggs were being implicated more frequently as vehicles of infection in salmonella outbreaks, especially in those where *S. enteritidis* was the causative organism (St Louis *et al.*, 1988; Anon,

1989). There are over 30 phage types (PTs) of *S. enteritidis*. PT13a and PT8 are currently important in North America and there has been a recent increase in human cases associated with *S. enteritidis* PT1 in Eastern Europe.

It is generally recognized, however, that *S. enteritidis* PT4 is the most important strain. Cases caused by this organism have shown a recent, marked increase in many countries including some in Western Europe. *S. enteritidis* PT4 is closely associated with poultry (Anon, 1992) and, as with the North American phage types, human illness is believed to be associated with contaminated poultry meat (Rampling *et al.*, 1989) and eggs (Anon, 1989).

5.3   *SALMONELLA* CONTAMINATION OF EGGS

Salmonellas can be isolated from either the shells or contents of egg.

5.3.1 Shell contamination

Eggshells can become contaminated with salmonellas either as a result of infection of the oviduct or faecal carriage. With salmonellas other than *S. enteritidis*, the latter is probably the more important route. Colonization of the intestinal tract commonly occurs after the consumption of contaminated feed (Williams, 1981) and chickens can acquire a wide range of salmonella serotypes by this route. In a recent survey in Canada (Poppe *et al.*, 1991) 35 different serotypes were isolated from environmental samples (including eggshells) from 295 flocks. Investigation of 42 laying flocks examined at slaughter (Barnhart *et al.*, 1991) found 32 (76.2%) to be salmonella-positive. *S. heidelberg* was the most frequent isolate (56.5% of those detected) but 14 other serotypes were also present.

The evidence for the incidence of eggshell contamination by *S. enteritidis* is very variable. In Spain, Perales and Audicana (1989) examined 372 eggs from flocks associated with human cases of salmonellosis. Four (1.1%) were shell-positive for *S. enteritidis* PT4. With eggs from flocks not implicated in cases or outbreaks, the incidence of shell contamination was lower with five (0.5%) of 998 eggs being PT4-positive. In the United Kingdom, Mawer *et al.* (1989) reported that none of 360 eggs from a small free-range flock implicated in a school-associated outbreak of salmonellosis was shell-positive for PT4 even though the organism was isolated from egg contents. In later studies with the same birds (Humphrey *et al.*, 1989a), *S. enteritidis* PT4 was isolated from the shell of five (7.4%) of 68 eggs. In investigations with other case-associated commercial laying flocks (Humphrey *et al.*, 1989b) *S. enteritidis* PT4 was recovered from the shells of 10 (5.2%) of 194 intact eggs. Prevalence varied, but in a sample of eggs from a battery flock of 25 000 birds, six (19%) of 32 eggs were shell-positive.

The contamination of spray-dried and bulk liquid egg referred to earlier also prompted investigations of likely sources of the salmonellas. Solowey *et al.* (1946) reported that, with eggs taken from egg-processing plants, salmonellas were isolated from the shell surfaces of 3% of clean, intact eggs and 5% of 'dirty' eggs both before and after washing. Cantor and McFarlane (1948) isolated either *S. montevideo* or *S. anatum* from the shells of 13 (0.6%) of 2132 eggs sampled at processing plants. All isolations were from 'dirty' eggs. Carter *et al.* (1950) found salmonellas in 3.2% of eggs purchased from grading stations. The techniques in this investigation in which shells and contents were incubated together did not allow the site of contamination to be identified. Earlier investigations, quoted by Cantor and McFarlane (1948), demonstrated that a wide range of serotypes, including *S. thompson, S. typhimurium, S. bareilly, S. oranienburg, S. montevideo, S. tennessee, S. derby, S. essen* and *S. worthington,* could be isolated from eggshells. As the majority of these strains are not thought to be invasive in chickens, faecal contamination is their most likely source.

Faecal carriage can also lead to eggshells becoming contaminated with *S. enteritidis* and Gast and Beard (1990a) reported that there was an apparent relationship between faeces positivity and eggshell contamination in hens artificially infected with *S. enteritidis* PT13a. With *S. enteritidis* PT4 in particular, infection of reproductive tissue also appears to be important. Humphrey *et al.* (1991a) using Specific Pathogen Free (SPF) hens artificially infected with *S. enteritidis* PT4, found that eggshells were contaminated in the absence of faecal carriage. Eleven of the infected birds laid eggs with salmonella-positive shells for up to 46 days after intestinal carriage had ceased. Five of these birds were never shown to be faeces-positive even though faecal samples were examined, using standard techniques, each day for 60 days after infection. This raises the possibility that the shell gland or another part of the oviduct may have been a site of infection.

Little information is available on the numbers of salmonellas on eggshells. In one study (Baker *et al.*, 1985), 'dirty' duck eggs were found to be carrying 5×10^5 salmonellas per egg, compared to less than 1×10^2/egg with 'clean' eggs.

Salmonellas on eggshells can die rapidly (Baker, 1990) but survival is enhanced by high relative humidities (Lancaster and Crabb, 1953) and low temperature (Lancaster and Crabb, 1953; Rizk *et al.*, 1966; Baker, 1990) during storage.

5.3.2 *Salmonella* contamination of egg contents

Egg contents can become contaminated with salmonellas as a result of either infection in reproductive tissue or the passage, through the shell, of organisms derived from either the intestinal tract or the environment. With salmonellas, other than *S. enteritidis*, the latter route is probably more

important and can be facilitated by the presence of moisture on the shell surface (Scott, 1930; Brown *et al.*, 1940) storage at ambient rather than at refrigeration temperatures (Wolk *et al.*, 1950; Stokes *et al.*, 1956; Rizk *et al.*, 1966) and damage to the shell (Vadehra *et al.*, 1969; Humphrey *et al.*, 1989a). Contact with contaminating organisms before the shell cuticle has dried (Sparks and Board, 1985; Padron, 1990) may also facilitate penetration of the shell by salmonellas (Chapter 4).

When eggs are broken, salmonellas present on egg shells may contaminate the contents. These contaminants may grow rapidly in broken out egg if storage is at ambient temperature. This route of contamination was believed to have contributed to two large outbreaks of human salmonellosis in North America in 1962 and 1963 (Thatcher and Montford, 1962; Sanders *et al.*, 1963; Ager *et al.*, 1967).

Evidence is accumulating which supports the view that the contamination of egg contents with *S. enteritidis* is the result of infection of the reproductive tissue rather than passage through the shell after lay. Investigations of eggs from naturally infected hens (Mawer *et al.*, 1989; Humphrey *et al.*, 1989b, 1991b) revealed that there is no association between shell contamination and the presence of *S. enteritidis* in egg contents. Studies with artificially infected birds have also shown that there is no relationship between faecal carriage of *S. enteritidis* and contamination of egg contents (Gast and Beard, 1990a; Humphrey *et al.*, 1991a). *S. enteritidis* PT4 can be isolated from the reproductive tissue of naturally infected hens in the absence of intestinal colonization (Lister, 1988; Bygrave and Gallagher, 1989). A similar pattern has been observed with hens infected artificially (Timoney *et al.*, 1989).

In a recent study in which commercially reared hens were given doses of *S. enteritidis* ranging from 10^3–10^8 per bird by the direct introduction into the crop, the only bird to lay eggs containing salmonella in the contents was from the group receiving 10^3 cells. She was also faeces-negative throughout the study and produced only negligible levels of antibody (Humphrey *et al.*, 1991c).

In 55 of 56 naturally contaminated eggs with contents positive for *S. enteritidis* (Table 5.1), the organism was present in pure culture. This may also provide further evidence of the involvement of reproductive tissue in the transmission of salmonellas. There is, as yet, no indication that *S. enteritidis* can move through eggshells and the underlying membranes more effectively than other competing faecal organisms. Indeed, recent work (Dolman and Board, 1992) has demonstrated that the bacterium does not compete effectively with other potential contaminants in the shell membranes or in the albumen in eggs stored at room temperature. Further evidence of the role of reproductive tissue in the contamination of egg contents with *S. enteritidis* is provided by the observations that, although a range of serotypes have been isolated from egg shells (De Louvois, personal

Table 5.1 Prevalence of egg contents naturally contaminated with *Salmonella enteritidis* PT4

Flock type	No. of flocks	No. of eggs examined	No. contents positive	%
Commercial layer–Free Range	9	2412	24	1.0
Commercial layer–Battery	8	2489	10	0.4
Layer–breeder	1	1120	1	0.1
Broiler–breeder	2	1558	10	0.6
Experimental*	2	1119	11	1.0
Total	22	8698	56	0.6

*Two small flocks of naturally infected laying hens were caged individually with each egg laid by each hen examined over a three month period.

communication; Humphrey *et al.*, 1991b), only *S. enteritidis* has been isolated, in recent surveys, from the contents of intact eggs.

5.3.3 The prevalence of eggs with *Salmonella*-positive contents

Results of surveys can only be compared if reference is made to the techniques used. This is particularly relevant in the examination of egg contents for the presence of salmonellas. There are a number of factors, including the size of sample, timing of sampling and the site(s) within the egg that are tested, which have an influence on the observed prevalence of eggs with *Salmonella*-positive contents. The importance of technique, however, should not be underestimated. Independent investigations with eggs from artificially (Gast and Beard, 1990a) and naturally infected hens (Humphrey and Whitehead, 1992) have shown that, when eggs are bulked for culture, the isolation rate can be increased significantly by extending the culture time from 24 to 48 h. Surveys which did not take account of this (Chapman *et al.*, 1988) may have underestimated the prevalence of egg contamination. The principal site of contamination in eggs (see later) would seem to be either the albumen or the outside of the vitelline membrane (Gast and Beard, 1990a; Humphrey *et al.*, 1991b). Thus techniques where yolks only are examined (Nicholas and Andrews, 1991) may also either fail to detect *Salmonella*-positive eggs or underestimate their prevalence. Consideration of the results of the various surveys outlined below should, therefore, take account of the factors described above.

Prior to the recent problems with *S. enteritidis* and eggs, there had been few surveys on the prevalence of hens' eggs with *Salmonella*-positive contents. Philbrook *et al.* (1960) isolated *S. typhimurium* from the contents of three eggs (0.3%) from a sample of 1137. More recently, Chapman *et al.* (1988) were unable to detect *S. typhimurium* in the contents of 1000 eggs taken from infected flocks.

Rather more work has been done on eggs from flocks known or thought to be infected with *S. enteritidis*. The observed prevalence of eggs with *Salmonella*-positive contents has been very variable. A survey of eggs from

22 naturally infected flocks, undertaken by the Exeter Public Health Laboratory between 1988 and 1991, revealed an overall prevalence of 0.6% (Table 5.1). Results from individual flocks were in the range 0.1–10%. Perales and Audicana (1989) examined eggs from Spanish flocks implicated in *S. enteritidis* PT4 outbreaks and found 0.1% to be contents-positive. In the United Kingdom, Paul and Batchelor (1988) tested 10 eggs from a small free-range flock and isolated *S. enteritidis* PT4 from the contents of five.

Although differences in technique could account for some of the observed variations, it has also become apparent that there can be clustering of eggs with *Salmonella*-positive contents. Thus, at or around the same time, a number of different birds in the same flock lay contaminated eggs. This was first observed during an outbreak investigation (Humphrey *et al.*, 1989b) and again during studies with naturally infected hens caged individually (Humphrey *et al.* 1989a). The latter investigation used 35 hens from two small flocks. In a study lasting over three months, every egg laid by each hen was examined for the presence of salmonellas in the contents. Eleven (1%) of 1119 eggs were positive for *S. enteritidis*; they were produced by ten birds. Over one two-day period, three hens laid contaminated eggs. During a similar period, six weeks later, five different hens did so. On one day, four of 18 eggs (22%) were *Salmonella*-positive. The explanation for this phenomenon is not known but it has also been observed with infected broiler–breeder flocks (Lanning, personal communication).

5.3.4 Numbers of salmonellas in egg contents

Almost all currently available evidence on the numbers of *S. enteritidis* in the contents of clean, intact eggs from either naturally (Humphrey *et al.*, 1989a,b; 1991b; Mawer *et al.*, 1989) or artificially infected hens (Timoney *et al.*, 1989; Gast and Beard, 1990a) indicates that fresh eggs contain few salmonellas. Low levels of contamination were also recovered from egg contents following the inoculation of *S. pullorum* onto ovaries (Forsythe *et al.*, 1967). At present, there is only one exception to the above findings (Salvat *et al.*, 1991). During outbreak investigations, these workers isolated *S. enteritidis* at a level exceeding 10^7/g, from the contents of a clean, intact egg thought to be five days old.

Recent work (Humphrey *et al.*, 1991b) has demonstrated a highly significant association between the age of an egg and the numbers of *S. enteritidis* in its contents (Table 5.2). Thus, in this study, all eggs examined within three weeks of lay contained less than 20 cells, whereas over 50% of positive eggs that were three or more weeks old contained more than 100 cells of *S. enteritidis*. Three eggs were heavily contaminated (Table 5.2). The apparent delay before *S. enteritidis* grows in an egg's contents may be caused by a number of factors. Investigations (Bigland and Papas 1953; Stokes *et al.*, 1956) using methods of contamination designed to facilitate the passage of

Table 5.2 The influence of storage at room temperature (20 °C) on the numbers of *Salmonella enteritidis* in the contents of naturally contaminated, intact hens' eggs[*]

Length of storage at room temperature (days)	No. of eggs containing salmonellas at the following level per egg	
	<20	>100
<21	13	0
>21	7	6[†]

[*]Only clean, dry intact eggs examined.
[†]Three eggs contained 7.2×10^3, 1.5×10^4 and 1.2×10^5 cells of *S. enteritidis* respectively.

salmonellas through eggshells found that egg contents did not become heavily contaminated until two weeks after inoculation. They concluded that this was governed by the time taken for salmonellas to cross the shell membrane. The viscosity of the albumen may also limit the movement of salmonellas (Gillespie and Scott, 1950). Clay and Board (1991) found that significant growth of *S. enteritidis*, inoculated on to the inner membrane of the air cell, did not take place until the yolk had made contact with the air cell. Humphrey *et al.* (1991b) showed that storage-related changes, possibly either to the vitelline membrane or the albumen, were important in allowing *S. enteritidis* to grow in albumen (section 5.3.5).

5.3.5 The growth of salmonellas in egg contents

The demonstration that some naturally contaminated eggs can carry a large number of salmonellas implies that *S. enteritidis* is able to multiply in egg contents.

Many experiments have been carried out on the growth of salmonellas and other potential human pathogens in egg contents contaminated artificially. This approach has been adopted by investigators because of the low incidence of naturally contaminated eggs (Table 5.1). These experiments have provided valuable data but results may not necessarily reflect what happens with natural contamination. Either the removal of egg contents or their inoculation with microorganisms might disturb the inherent antimicrobial properties of albumen. This may facilitate the growth of bacteria. With this in mind, it would not be unreasonable to assume that where growth does not take place in artificially contaminated eggs, it is unlikely to do so in those that are contaminated naturally.

When inoculated into the yolk of 'intact' eggs, *S. enteritidis* is able to grow from a low inoculum (<5 cells per egg), in eggs of any age, whether they come from either infected or uninfected hens and in either the presence or absence of oxygen (Bradshaw *et al.*, 1990; Humphrey *et al.*, 1991b). These results are essentially the same as those from earlier studies with other salmonellas (Ayres and Taylor, 1956) and could be taken to suggest that, if

yolk contents were the principal site of contamination, the majority of salmonella-positive eggs would be heavily contaminated. There is also the possibility that the profuse growth of salmonellas following yolk inoculation results from disturbance of the yolk emulsion and disruption of possible inherent control properties which prevent the growth of micro-organisms. This is clearly difficult to disprove.

Studies with egg contents removed, intact, at lay using aseptic techniques have revealed that once albumen has reached pH 9.0, the growth of *S. enteritidis* in egg contents held at 20°C is governed by the size of the initial population of bacterial cells, the position of inoculum in relation to the yolk and the age of an egg. Thus, provided an inoculum of <10^3 cells per egg is used, significant growth will only take place, in the majority of eggs, if the salmonellas are next to the vitelline membrane in eggs that have been stored at 20°C for three weeks or more (Humphrey *et al.*, 1991b). This observation provides a possible explanation for the relationship between egg age and levels of contamination. An important controlling factor on the growth of salmonellas in albumen is iron limitation (Tranter and Board, 1982) and it is possible that during storage, changes to the vitelline membrane (Romanoff and Romanoff, 1949) either allow iron or some other material to pass from the yolk into the albumen or permit easier access to yolk contents. There is also the possibility that the inherent inhibitory properties of egg albumen lessen with egg age, allowing organisms to utilize nutrients in yolk contents.

5.3.6 Site of contamination in egg contents

S. enteritidis PT4 can be isolated from the ovaries and oviduct of broiler–breeder hens (Lister, 1988) and commercial layers (Hopper and Mawer, 1988). This, as discussed earlier, will result in the contamination of contents before eggs are laid.

The isolation of *S. enteritidis* from ovaries and ovules may have, understandably, given rise to the belief that the principal site of contamination in eggs is yolk contents. This does not seem to be the case and there is increasing direct and indirect evidence that the albumen, or possibly the outside of the vitelline membrane is more likely to be contaminated than the yolk.

(a) Direct evidence on the sites of contamination

During studies on a flock of naturally infected free-range hens Humphrey *et al.* (1989a) reported that in six eggs, where the site of contamination was identified, four were positive in the whole yolk (contents plus membrane) and two in the albumen only. In later investigations with eggs from a number of flocks (Humphrey *et al.*, 1991b), the site of contamination was

identified in 15 eggs. Twelve (80%) eggs contained *S. enteritidis* in the albumen only, two in the whole yolks and, one egg, in both the whole yolk and the albumen. Both the whole yolk and the albumen were also positive in two eggs from another small free-range flock (Paul and Batchelor, 1988).

Studies with hens infected artificially gave similar results. Gast and Beard (1990a), using *S. enteritidis* PT13a, and a high infecting dose, isolated salmonellas from a large number of eggs. None of the yolk contents was positive even though *S. enteritidis* was recovered with high frequency, from either albumen or whole yolks. Timoney *et al.* (1989), using *S. enteritidis* PT4, recovered the organism from a low percentage of yolk contents although they too found some eggs were only positive in the albumen. Shivaprasad *et al.* (1990) repeated the above work but used *S. enteritidis* PT8, and isolated the organism with low frequency from yolk contents, but albumen was, again, the principal site of contamination.

The factors which may determine the site of contamination in egg contents have not yet been elucidated. Recent work has, however, revealed some most interesting information on the route of infection and the site of contamination in egg contents. There would also appear to be strain-to-strain differences between isolates of the same phage type of *S. enteritidis*. Thus Shivaprasad *et al.* (1990) observed that when *S. enteritidis* PT8 strain Y-8P2 was given orally to chickens, albumen was positive but not yolk. When the organism was given either intravenously or via the cloaca, some yolks were positive but the bulk of the isolations were still from albumen. In contrast, when strain 27A was used to infect birds by the oral route, more eggs were positive in the yolk than in the albumen. Gast and Beard (1990a) observed differences in the frequency of albumen contamination between birds infected by the direct administration of *S. enteritidis* PT13a into the crop and those infected by contact with inoculated birds. Infection via the cloaca also appears to lead to contamination of the albumen only (Bale, personal communication).

It is possible that flocks of laying hens or individual birds may vary in their susceptibility to salmonella infection and that different routes of infection may also be involved. Thus, a survey involving flocks of different age and breed and reared under different conditions (Humphrey *et al.*, 1991b) may be expected to produce different results from investigations of individual flocks (Paul and Batchelor, 1988; Humphrey *et al.*, 1989a).

It is also becoming clearer, that an important site of contamination in egg contents may be the area of albumen immediately surrounding the yolk (Humphrey *et al.*, 1991b). Thus technical differences in, for example, the amount of albumen left adhering to the vitelline membrane may influence the results of the various investigations.

(b) Indirect evidence

Indirect evidence on the close relationship between egg age and levels of contamination (Humphrey *et al.*, 1991b) and the involvement of dishes such as meringue in outbreaks of *S. enteritidis* PT4 infection (Anon, 1992) also suggest that albumen may be an important site of contamination. Mayonnaise is a major vehicle of infection (Anon, 1992), and this could be taken to indicate contamination of yolk contents. It is very difficult to separate completely egg yolk and albumen and it is likely that some albumen will still be present on separated yolks. It should also be borne in mind, however, that in some recipes egg albumen is included in mayonnaise.

5.4   EGGS AND EGG DISHES AS VEHICLES FOR HUMAN SALMONELLOSIS

Each year since 1988 in England and Wales, eggs or dishes containing egg have been implicated as the suspect food in between 40–60 outbreaks of *S. enteritidis* PT4 infection (Anon, 1992). Mayonnaise was implicated most frequently, followed by mousse and meringue. Cowden *et al.* (1989a) have reported on two outbreaks; in the first, in which 18 people were infected, ice-cream containing raw egg was implicated. In the second, where 96 people became ill, almond parfait made with lightly cooked egg yolk and raw egg white was associated with infection. Stevens *et al.* (1989) described a large outbreak of *S. enteritidis* infection following a wedding reception: 173 people met the case definition of illness and lightly cooked egg-based sauces were strongly associated with infection. An unusual feature of the outbreak was that some people reported incubation periods of less than three hours.

In addition to outbreak investigations, case-control studies have also been undertaken into sporadic cases of *S. enteritidis* PT4 infection. These investigations were important as sporadic cases account for the great majority of human infections. In the first study (Coyle *et al.*, 1988), 17 of 19 cases but only 10 of 19 controls reported eating eggs or egg products in the three days before illness. The proportion of cases eating soft boiled egg in an average week was also greater than that of the controls although the odds ratio of 3:1 was not significant.

The second study (Cowden *et al.*, 1989b) was on a much larger scale: 232 cases were identified and data were obtained on 160 patients. Up to three matched controls were asked the same questions as the cases, in the same calendar period. Illness due to *S. enteritidis* PT4 was found to be significantly associated with the consumption of products containing raw egg (mayonnaise, home made ice cream and milk drinks containing eggs) and shop-bought sandwiches containing either mayonnaise or eggs although

the association was stronger with the former. Illness was also linked to consumption of precooked hot chicken and lightly cooked, but not soft-boiled, eggs.

As with many other enteric infections, those most at risk are the very young, the infirm or the elderly. It was these groups that the United Kingdom Chief Medical Officer sought to protect when he issued advice on the preparation and consumption of eggs (Anon, 1988a). The higher risk groups were advised to eat eggs which had been cooked until the yolk was solid. Other people were cautioned against the use of raw eggs.

5.4.1 Salmonellas and mayonnaise

The International Commission on Microbiological Specifications for Foods (ICMSF) has defined the lower pH limit allowing initiation of growth of salmonellas as pH 4.0–4.5 (Anon, 1980). Ferreira and Lund (1987) found that some *Salmonella* serotypes grew at pH 3.8 but that this was temperature dependent. Thus, at 30°C, growth occurred at minimum pH values of 3.8–4.0 within one to three days. At 20°C, growth was slower, the lag phase was extended to between three and five days. At 10°C, growth did not take place below pH 4.4.

The pH of mayonnaise is clearly a critical factor in controlling the growth of salmonellas. The type of acid used to reduce the pH of the egg/oil mix is also important. Collins (1985) examined the survival of *S. muenster* in mayonnaise at pH values between 4.2 and 5.2 with acetic or citric acid as the acidulant. Marked differences in survival were recorded and in mayonnaise made with citric acid, viability was little affected at pH values exceeding 4.8 over a ten-day period at 25°C. In contrast, in mayonnaise made with acetic acid and at pH 5.0, the bacterial population declined by over 90% within two days.

Perales and Garcia (1990) examined the behaviour of *S. enteritidis* PT4 in home-made mayonnaise; their results are similar to those of Collins (1985). When vinegar was the acidulant, growth did not occur, even at pH 5.0. At pH values below 4.5, death of *S. enteritidis* was rapid. When lemon juice was used, viability was unaffected at either pH 3.6 or pH 4.0 although multiplication did not take place. Growth occurred rapidly, however, above pH 4.5. Perales and Garcia (1990) also found that death rates were temperature-related and, irrespective of the acid used, occurred more rapidly at 35°C than at 24°C. Miller and Martin (1990) examined the survival of *S. enteritidis* and *S. typhimurium* in an Italian salad dressing at pH 3.49–3.67. Death was rapid and viable cells were not recovered after 5 min exposure.

In addition to the type of acidulant, pH and storage temperature, Radford *et al.* (1991) demonstrated that the oil used in the preparation of mayonnaise had an impact on the survival of *S. enteritidis*. Death rates were

always faster in products made with extra virgin olive oil than in mayon-
naise prepared from either blended olive oils or sunflower oil. The authors
concluded that this may be linked to the greater acidity of the extra virgin
oil.

5.4.2 *Salmonella enteritidis* and its heat resistance

Cooked egg dishes have been implicated in both cases and outbreaks of *S.
enteritidis* infection. This prompted an investigation of the heat resistance
of the bacterium and its ability to survive certain kinds of heat treatment.

S. *enteritidis* PT4 appears to be more heat resistant than some other
poultry-associated salmonellas (Humphrey *et al.*, 1990). For example, it was
recovered from artificially contaminated eggs which had been scrambled
whereas *S. typhimurium* PT141 was not (Humphrey *et al.*, 1989c). Death rates
at 64°C (Humphrey *et al.*, 1990), however, indicate that *S. enteritidis* should
not survive pasteurization. To date, in England and Wales, no outbreaks
have been associated with products made from pasteurized egg.

Earlier investigations with *S. pullorum* demonstrated that the bacterium
could be recovered from the contents of whole eggs boiled for four minutes
(Rettger *et al.*, 1916; Stafseth *et al.*, 1952). The destruction of *S. typhimurium*
inoculated into the yolks of 'intact' eggs was dependent upon the method
of boiling used. Thus, with a 'jumbo' egg placed directly into boiling water,
a nine log reduction in the number of salmonellas was achieved within 7–8
min (Licciardello et al., 1965). When eggs were placed into water at ambient
temperature and brought to the boil, the D value increased to 18.8 min.
Baker *et al.* (1983) also examined the survival of an inoculum of approxi-
mately 1×10^8 cells of *S. typhimurium* in cooked eggs. Boiling for 7 min was
sufficient to kill the inoculum. With fried eggs, destruction was achieved
within 4 min at 70°C when the eggs were covered and by 3 min on each
side for turned-over eggs cooked at 64°C. Humphrey *et al.* (1989c) found
essentially similar results.

The realization that albumen may be an important site of contamination
in egg contents led to investigations into the impact of albumen-like alka-
line conditions on heat resistance. Exposure to broth at pH 9.2 (Humphrey
et al., 1991d) significantly increased subsequent heat resistance, particularly
when cells were heated at pH 7 rather than at pH 9.

It has also become apparent that within the various phage types of *S.
enteritidis* there can be considerable variation in heat resistance. The reasons
for this have yet to be elucidated but Shah *et al.* (1991) found that D 60°C
values with strains of *S. enteritidis* PT8 in liquid whole egg varied from 11.8
to 31.3 s. Similar results have been obtained with *S. enteritidis* PT4 (Line,
personal communication).

Low temperature storage maintains the quality of egg contents (Wil-
liams, 1992) and can also prevent the growth of salmonellas (Bradshaw *et*

al., 1990; Humphrey, 1990a). In addition, exposure to either 4 or 8°C significantly increases the heat sensitivity of *S. enteritidis* PT4 (Humphrey, 1990b). There are thus distinct public health advantages in the storage of eggs at refrigeration temperatures, particularly in the home (Anon, 1988a).

5.5 *SALMONELLA* INFECTION IN LAYING HENS

Poultry-associated salmonellas fall into two groups. There are serotypes, principally *S. pullorum* and *S. gallinarum*, which are host adapted. The remainder are primarily non-host specific and can infect or colonize a range of animal species. *S. pullorum* is an internationally important poultry pathogen. Although it has been eradicated in the United Kingdom, infections caused by this organism represent a considerable economic loss to the poultry industry in other parts of the world. The bacterium has a predilection for the reproductive tissue and in a study with hens infected artificially (Forsythe *et al.*, 1967), 10 (26%) of 38 eggs were contents-positive. In contrast, hens infected with large doses of either *S. anatum* (Forsythe *et al.*, 1967) or *S. typhimurium* (Baker *et al.*, 1980) did not lay eggs with *Salmonella*-positive contents.

The behaviour of *S. enteritidis* in chickens shows some similarities to *S. pullorum*, to which it is antigenically related. In broiler chickens, *S. enteritidis* can cause a variety of clinical conditions, including pericarditis (Rampling *et al.*, 1989) and economic losses can be high in some commercial flocks (Anon, 1988b). With hens used for commercial egg production, the response to infection with *S. enteritidis* is different. In some birds at *post-mortem*, infected ovaries (Lister, 1988) and oviducts (Cooper *et al.*, 1989) have been observed. These findings may not be typical and it is of interest to note that abnormalities were not observed in any of the 56 contents-positive eggs referred to earlier (Table 5.1). Diarrhoea has been recorded in birds infected artificially (Timoney *et al.*, 1989; Shivaprasad *et al.*, 1990; Humphrey *et al.*, 1991a,c). This appears to be associated with high infecting doses and birds which received fewer organisms (10^3 per bird) remained clinically well throughout the trial (Humphrey *et al.*, 1991c). Some hens have also shown a decrease in egg production after artificial infection with *S. enteritidis* (Gast and Beard, 1990a; Shivaprasad *et al.*, 1990; Barrow and Lovell, 1991) but, again, this was probably in response to high infecting doses.

The majority of naturally infected hens do not show the above responses. During the investigation of some cases or outbreaks of human infection, the Exeter Public Health Laboratory identified 20 commercial flocks that were infected with *S. enteritidis* PT4. They ranged in size from a small, free-range flock of 20 birds to battery units which exceeded 300 000 birds.

Table 5.3 Routes of infection used in the study of *Salmonella enteritidis* infection in laying hens

Contaminated food
Crop inoculation
Contaminated aerosols
Via the conjunctiva
Spread to contact birds
Cloacal inoculation
Vertical transmission

There was no common factor in either management regimens, feed or breed of bird. None of the flocks had shown any increase in mortality or any change in behaviour, feeding patterns or egg laying. When some birds were examined at slaughter, at approximately 74 weeks of age, few signs of abnormality were observed in either ovaries or oviduct even though the organism was cultured from these sites.

Infected birds produce antibodies against *S. enteritidis* (Cooper *et al.*, 1989; Chart *et al.*, 1990). Serum samples have been shown to contain both IgG and IgM but the immune response and the levels of the two antibodies are governed by the infecting dose (Humphrey *et al.*, 1991c) and the route of infection (Chart *et al.*, 1992).

The economic importance of *S. enteritidis* to the poultry industry and its prominence in human infection have meant that much work has been undertaken on the infection of chickens. Laying hens can be infected easily by a variety of routes (Table 5.3). It is also possible to produce systemic infection with as few as 100 cells of *S. enteritidis* PT4 (Humphrey *et al.*, 1989d) although in studies of artificial infection, higher doses are generally used.

Irrespective of either the route of infection, phage type or the dose used systemic infection commonly results. A variety of tissues are involved (Table 5.4) and there can be a relatively short-lived septicaemic phase. *Salmonella enteritidis* must be regarded as being highly invasive in chickens. It can spread easily from bird to bird (Gast and Beard, 1990 a,b,c) and can be isolated from tissues, including reproductive tissue in the absence of faecal carriage in naturally (Bygrave and Gallagher, 1989) and artificially (Gast and Beard, 1990b,c; Shivaprasad *et al.*, 1990) infected hens.

5.5.1 Control measures

Measures, which might limit the risk to public health from the infection of laying hens with *S. enteritidis* and the associated contamination of eggs, can be applied at a number of points in the chain from farm to home.

(a) On-farm measures

In the United Kingdom, bovine tuberculosis and brucellosis were controlled

Table 5.4 The isolation of *Salmonella enteritidis* PT4 from the tissues of laying hens following artificial infection*

Tissue	No. of specimens examined	No. Salmonella-positive	%
Blood	16	3	19
Liver	51	19	37
Spleen	51	13	26
Kidney	23	8	35
Ovary/Ovules	51	15	29
Oviduct	51	11	22
Lung†	15	11	73

*Birds were infected by contaminated aerosols, direct introduction of *S. enteritidis* into the crop or via the conjunctiva.
†Aerosol-infected birds only.

by the regular testing of cattle herds and the slaughter of reactor animals. A similar approach can be adopted for the control of the infection of laying flocks with invasive salmonellas. From 1989 to early 1993, such a system has formed an important part of the United Kingdom government's anti-salmonella measures. Under the Poultry Laying Flocks (Testing and Registration) Order 1989, either composite samples of fresh faeces or cloacal swabs were examined, at 12-week intervals, for the presence of salmonellas. A different regulation, the Poultry Breeding Flocks and Hatcheries (Registration and Testing) Order 1989, is applied to breeding flocks. In addition to faecal sampling, the salmonella status of these birds is monitored by examination of chick box liners and either culled or dead chicks.

Between 1989 and 1991, following the introduction of the above regulations, and the amended Zoonoses Order (1989), 233 poultry flocks were slaughtered because of infection with *S. enteritidis*. These comprised 58 broiler–breeder flocks, 10 layer–breeders and 165 commercial layers (Anon, 1992).

Properly applied, a slaughter policy can have a marked impact on the presence of diseased animals; the virtual eradication of bovine tuberculosis and brucellosis from the United Kingdom is evidence of this. Efficacy is dependent, however, on a number of factors:

1. Sampling frequency and the number of animals tested should be high enough to detect low levels of infection.
2. Samples taken should reflect the behaviour and ecology of the target pathogen.
3. Techniques applied to the samples should be those which maximize the detection of the organism being sought.

Control measures may be subject to economic constraints and it may be necessary to amend testing protocols to take account of this. Thus, rather

than slaughter hens at each sampling time, the monitoring of laying flocks in the United Kingdom largely relies upon the examination of faecal material. This may lead to an underestimate of the prevalence of infected birds (Bygrave and Gallagher, 1989). Detection levels have been set at 5% with a maximum of 60 samples being examined irrespective of flock size. Serological tests are being developed for the detection of *S. enteritidis* infection (Cooper *et al.*, 1989; Gast and Beard, 1990c; Chart *et al.*, 1990; Nicholas and Cullen, 1991). These are likely to be less expensive and easier to perform than microbiological tests. This will enable a greater number of birds to be tested, giving increased sensitivity, and will largely overcome the difficulties caused by the fact that *S. enteritidis* can be isolated from viscera in the absence of faecal carriage. Serological tests have a potential disadvantage in that they may detect past infection where a bird is anti-body-positive but is no longer infected. For this reason, it may be necessary for both the regulatory authorities and the poultry industry to use serology as an initial screen with confirmation of positive results by the microbiological examination of culled birds.

The success of a slaughter policy is also dependent upon replacement stock being free from infection and an absence of environmental contamination. The latter poses particular difficulties in the poultry industry because of the large scale and intensive nature of much of the production. Duguid and North (1991) have reported that re-infection has occurred in replacement flocks on some farms where previous flocks were slaughtered because of infection with *S. enteritidis*. It is clear that further studies are needed on the survival of salmonellas in the poultry-house environment.

There are other control measures which could be used either to minimize or prevent the infection of laying hens with salmonellas. Although *S. enteritidis* has only rarely been isolated from poultry feed, contaminated feedstuffs are commonly the most important source of infection or colonization of poultry with other *Salmonella* serotypes. Salmonellas in feed are largely associated with the protein component especially that of animal origin (meat-and-bone meal, fish meal, etc.), although vegetable protein and other ingredients may also be contaminated. Heat-processing in feed production should be sufficient to destroy salmonellas or reduce their incidence below the level required to infect poultry, but may fail to do so, especially when the conditions of treatment are inadequate or opportunities exist for recontamination of the finished product.

Sampling of domestic protein products in England and Wales from 1982 to 1985 led to salmonella isolations at 55 of the 102 registered premises examined (excluding those ensiling poultry manure). Contamination rates for different products varied from 0.7% for white fish meal to 25.7% for feather meal (Matthews, 1986). In total, 29 *Salmonella* serotypes were isolated from 318 positive samples and they corresponded closely with those found in food animals. The sampling of imported animal protein over the

same period gave a similar picture, with 16.4% of 2020 samples being salmonella-positive by comparison with 9.5% for domestic production. In the United Kingdom, the recent Zoonoses and Protein Processing orders (1989) should do much to reduce the prevalence of salmonella-contaminated poultry feed.

Heat-processing is the most widely used means of reducing salmonella contamination of animal feed but other types of treatment are available (Williams, 1981). One approach is to incorporate an antimicrobial compound such as formic or propionic acid or a mixture of the two. This treatment does not require any withdrawal period. Commercial utilization has so far been limited, probably because of cost, but Humphrey and Lanning (1988) found that treatment of feed given to broiler–breeder birds reduced significantly the contamination of hatchery waste and papers from chick boxes with a range of *Salmonella* serotypes. In laboratory studies, Hinton and Linton (1988) have also shown that the inclusion of organic acids in feed can protect young chicks from feed-borne salmonella infections. These compounds are active in the crop. Recent work (Humphrey, 1991) demonstrated that acid treatment of feed limited the horizontal spread of *S. enteritidis* in flocks of growing broilers, presumably by interfering with the faecal/oral route of transmission.

Chicks can be infected at hatching as a result of vertical transmission from infected parent flocks. Such infection can spread easily to other chicks in the hatchery (Cox *et al.*, 1990). This can be minimized by frequent egg collection and improved hatchery hygiene. Elimination of salmonellas from breeder birds is more difficult. Attempts to do so usually involve treatment of the birds over a week or more with one or two antibiotics such as tetracycline and neomycin at therapeutic levels. Even with a light salmonella infection, however, Smith (1978) showed that, although neomycin treatment reduced the incidence of chickens shedding *S. typhimurium*, the incidence of caecal infection reverted to its original state when treatment ceased. Medication of birds to reduce or eliminate salmonella infection is likely to have a marked, disruptive effect on the normal intestinal microflora and, once the treatment is discontinued, the birds are very susceptible to reinfection. Evidence suggests that the problem could be minimized by quickly re-establishing a mature gut flora through oral administration of cultured caecal contents from an appropriate donor bird (Seuna and Nurmi, 1979; Seuna *et al.*, 1980). Recent use of this combined treatment for breeder birds in the UK is showing promising results (Mead, 1991), and there is increasing interest in the 'competitive exclusion' principle as a means of reducing flock infection in various countries. Evidence is also accruing that the technique could be applied to minimize horizontal transmission of salmonellas in poultry flocks. Improved on-farm hygiene and treatments such as chlorination of drinking water (Poppe *et al.*, 1986) may also help to limit salmonella infections.

(b) Control in the packing station and in retail outlets

Normally eggs are screened at the packing station and cracked and/or faecally contaminated ones removed. As the entry of salmonellas into egg contents (Vadehra *et al.*, 1969) may be enhanced by cracks in the shell, it is important that such eggs do not enter the shell egg market. They can, however, be sent for processing and manufacture of egg products.

   Many of the larger egg producers in the United Kingdom hold eggs at 8–12°C both before and during distribution. This will maintain egg quality (Williams, 1992) and minimize the growth of salmonellas in egg contents (Bradshaw *et al.*, 1990; Humphrey, 1990a). The delay of 2–3 weeks in the growth of salmonellas in the contents of artificially (Stokes *et al.*, 1956) and naturally contaminated eggs (Humphrey *et al.*, 1991b) suggests that it may not be necessary for eggs to be held under refrigeration in retail outlets although this should not be discouraged. What is of greater importance is that more attention is paid to shelf life. In a small survey of 44 retail outlets in the Exeter area in 1991, eight – generally large supermarkets – gave eggs a shelf-life of less than 14 days; in 16 smaller shops, the shelf-life was between 15 and 21 days. In twenty other establishments, shelf-life either exceeded 21 days or was not indicated on the packaging. In seven shops no packing date was given. This disparity in the age at which eggs can be sold should be addressed by the retail and egg industries. As age-related changes in egg contents which appear to permit the growth of salmonellas in albumen (Humphrey *et al.*, 1991b) may have started to take place while the eggs were held in retail outlets, refrigeration is to be encouraged once eggs have been purchased (Anon, 1988a).

(c) Control in the home

The importance of eggs as a human food and the large number consumed each day means that even a low incidence of salmonella contamination may be significant. The potential hazard from contaminated egg contents is related, in part, to the number of salmonellas present. To minimize the risk, particularly to vulnerable groups of people, consumers should follow the advice issued by the United Kingdom Chief Medical Officer (Anon, 1988a). Raw eggs should not be used in foods such as mousse and mayonnaise; eggs should be purchased as fresh as possible and should be stored under refrigeration in the home; vulnerable groups such as the old, the very young or the infirm should only receive eggs which have been cooked until the white and yolk are solid. These guidelines were issued at a time when yolk contents were believed to be an important site of contamination in eggs. Although this is now considered to be less likely, this advice on cooking is still pertinent. *S. enteritidis* can be isolated from albumen (Humphrey *et al.*, 1991b) but evidence is accumulating which suggests that the outside of the vitelline membrane may also be an important site of contamination with *S.*

enteritidis in intact eggs (Gast and Beard, 1990a). This area will only receive minimum heat treatment in the light cooking of whole eggs. Heat resistance of any salmonellas present will be increased by exposure to the alkaline conditions of the albumen (Humphrey *et al.*, 1991d) and, in older contaminated eggs, there is the possibility that large populations of cells may be present (Humphrey *et al.*, 1991b).

5.6 OTHER PATHOGENS

Laying hens can be colonized with some potential human pathogens other than salmonellas.

   Campylobacter jejuni is commonly associated with poultry (Doyle, 1984) and there is thus the possibility that eggshells can become contaminated as a result of intestinal carriage of this important human pathogen. Doyle (1984) infected laying hens at 20 weeks of age. Of 226 eggs from hens faecally excreting *C. jejuni*, the organism was isolated from two shell surfaces but no egg contents. Egg penetration studies revealed that the organism would not penetrate into the contents of eggs but could be isolated occasionally from the inner shell membranes of refrigerated eggs. These latter observations agree with those of Acuff *et al.* (1982) who examined turkey eggs. Neill *et al.* (1985) immersed hens' eggs in a suspension of *C. jejuni* and, although the organism was recovered from shell membranes, all yolk and albumen samples were *Campylobacter*-negative.

   Hanninen *et al.* (1984) studied the behaviour of *C. jejuni* in liquid egg. At 37°C the organism grew slowly in either egg yolk or homogenized whole egg. At 20°C, *C. jejuni* died slowly in these materials. Death was rapid in egg albumen irrespective of temperature. Given the apparent lack of penetration by *C. jejuni*, the demonstrated ability to grow in samples of yolk at 37°C is likely to be of only limited practical importance.

   Listeria monocytogenes has been isolated from chickens and from the surfaces of eggshells (Nitcheva *et al.*, 1990) although, as yet, not from egg contents. The organism has been isolated, with high frequency, from samples of eggs collected at processing plants (Leasor and Foegeding, 1989) and is capable of growth in whole liquid egg even at refrigeration temperatures (Khan *et al.*, 1975). *L. monocytogenes* can also survive the spray drying of egg powder (Brackett and Beuchat, 1991) and has been shown to be able to grow in egg nog containing 7% ethanol after prolonged incubation at 22°C (Notermans *et al.*, 1990). The public health significance of these observations has yet to be assessed.

   Yersinia enterocolitica can also be found in chicken faeces (Norberg, 1981) and the bacterium is reported to be able to survive in egg wash water (Southam *et al.*, 1987). Studies designed to facilitate contamination of the

shell membrane (Amin and Draughon, 1990) demonstrated that, although no *Yersinia* could be detected in egg contents immediately after inoculation, storage for up to 14 days at 10°C resulted in high levels of contamination in a number of eggs. However, it has yet to be shown that eggs are an important vehicle for infection with *Y. enterocolitica.*

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The microflora of the alimentary tract and avian pathogens: translocation and vertical transmission

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6.1 INTRODUCTION

The relationship of the hen's egg to human and animal health depends to some extent on its microbial content, more specifically on the microbiology of the freshly laid egg. Subsequent to this microbial contamination may occur from the environment. This may generally soil the surface of the shell, but most infectious agents involved in disease enter the egg while it is warm, or as it is cooling, or do so during egg formation.

Microorganisms from the normal flora of the alimentary tract of poultry may be involved in egg spoilage and deterioration. Some of these may be avian pathogens whose spread by this route is of considerable epidemiological importance in transmission from one generation to the next. Some pathogens may incidentally affect humans in which case public health issues are also involved. All these components and other microorganisms which become components of the microbial flora of the egg require a number of microbial determinants, sometimes coinciding with host factors, to allow transfer to the egg to occur.

6.2 GENERAL STRATEGIES FOR VERTICAL TRANSMISSION AND SURVIVAL IN THE TISSUES

Microorganisms can be transmitted into the laid egg by several routes; organisms so transmitted may or may not survive until the egg hatches.

The part of the egg in which a microorganism is deposited depends largely on whether it becomes localized in the ovary, oviduct or cloaca. The ability of organisms to become localized in these organs and remain viable depends on their possession of characteristics which prevent killing by complement, prevent opsonization and phagocytosis, allow penetration and survival within living cells themselves or allow survival and multiplication in the alimentary tract, primarily the distal regions.

Microorganisms enter the tissues as a result of infection. This must happen relatively frequently with commensal organisms present normally in the alimentary tract but, generally, no disease ensues. However, many pathogens enter the tissues as a normal part of the infection and disease process. In the laying hen, such organisms may immediately become localized in the reproductive tract. However, this may occur later as a result of disease or in the case of chronic infection may occur in younger birds as the ovaries develop at sexual maturity. Bacterial cells become localized in the ovary as a result of passage through large lacunae present in the walls of ovarian blood vessels which normally allow passage of nutrients into developing ova (Griffin *et al.*, 1984). *Salmonella* organisms may enter this way. However, avian viruses may penetrate ovarian cells themselves, even the germ cells, in this case as DNA copies integrated into the germ cell genome.

The oviduct may become infected by haematogenous spread or vertically from the cloaca. The relative contribution of these is currently difficult to assess. Whether microorganisms become situated in the albumen or shell membrane depends largely on whether the infundibulum or the shell gland becomes infected. Microorganisms (bacteria, viruses or bacteriophage) whose natural environment within the host is the lower alimentary tract may contaminate the egg surface as it passes through the cloaca. Organisms deposited on the surface of a freshly laid egg, either from the cloaca or nest box, may be drawn into the shell as the egg contents cool and contract, absorbing surface moisture.

Infection of ovary or oviduct is unlikely to occur *via* the peritoneal cavity since in the healthy animal and in infected birds in the early stages of disease few microorganisms will be present. This situation may change in later stages of disease but by this time a hen may have ceased to lay.

For microorganisms to localize in the reproductive tract and survive in the egg presupposes possession of characteristics allowing survival in the relatively inimical environments of the blood and tissues, alimentary tract, albumen and egg shell. The full details of microbial virulence determinants that allow organisms to survive the host's various defence mechanisms are adequately covered by Mims (1985) and need not be dealt with in depth here. Survival in the blood can involve anti-opsonic and antiphagocytic mechanisms, antigenic mimicry and variation and serum resistance. Each mechanism itself may be multifactorial. Serum resistance, for example is

mediated not only by lipopolysaccharide and capsular antigens which are generally chromosomally mediated in bacteria but also by the plasmid-mediated *traT* and *iss* genes present on some F-like plasmids and the ColV plasmid respectively. The conditions in the alimentary tract such as extremes of pH and Eh, bile acids and salts and presence of volatile fatty acids are inimical to many bacteria and the genetic determinants of microorganisms whose normal habitat is the gut have not yet been characterized.

Within the egg itself albumen, but not yolk, is iron-depleted. Few microorganisms can survive and grow in the albumen and this depends to some extent on their exact location in the albumen (Chapter 3). To survive on the surface or in the eggshell microorganisms must be able to withstand desiccation.

6.3   THE NORMAL MICROFLORA OF THE ALIMENTARY TRACT OF THE CHICKEN

The following is a short review of the microflora of the alimentary tract of the healthy fowl, the factors affecting its composition and ways in which it affects the presence of pathogens (mainly bacteria) in the gut.

The composition of the flora can conveniently be described according to the anatomical structure of the digestive tract. However, many microbial types are present throughout the length of the gut.

Food is swallowed whole and is stored in the crop where a predominantly lactic acid semi-batch fermentation takes place. The Eh is fairly high so that ingested obligate anaerobes generally die. In the healthy bird, other non-enteric organisms also do not thrive. Thus, in comparison with the caeca, the crop flora is relatively simple. The predominant bacteria are lactobacilli producing mainly lactic and acetic acids such that the pH of the crop contents is generally 4–5, and as a consequence less aciduric bacteria do not normally grow to such high numbers (Table 6.1). A number of metabolic taxa can be identified (Fuller, 1973) including *Lactobacillus salivarius*, *L. fermentum* and a type resembling *L. acidophilus*. Eyssen *et al.* (1965) and Fuller and Turvey (1971) found large numbers of lactobacilli adhering to the squamous epithelium of the crop. This adherent flora became established within days of hatching. The numbers of lactobacilli decrease with starvation but sufficient remain to inoculate fresh food entering the crop. *Escherichia coli* is present in the crop in fairly low numbers, possibly maintained under commercial conditions by the ingestion of litter, contaminated by faeces. *Enterococcus faecalis* subsp. *liquefaciens* and subsp. *zymogenes*, *Ent. faecium*, *Ent. avium* and *Ent. gallinarum* are less aciduric than lactobacilli and are also present in lower numbers. There is no evidence for extensive colonization of the epithelium by these organisms

Table 6.1 The numbers of major bacterial components in the alimentary tract of chickens

Bacterial group	Log$_{10}$ median viable count (of 5–7 birds) in contents of						
	Crop	Gizzard	Small intestine			Caeca	Faeces
			Section 1	Section 2	Section 3		
E. coli	1.7	–	2.0	1.7	2.7	5.6	6.1
Clostridia	–	–	(-)	(-)	(-)	9.0	2.0
Enterococci	4.0	3.7	4.0	4.0	4.2	6.7	6.5
Lactobacilli	8.7	7.3	8.0	8.2	8.6	8.7	8.5
Yeasts	2.7	–	1.7	–	–	2.0	1.7
Non-sporing obligate anaerobes	–	–	–	–	–	10.0	9.0
Anaerobic streptococci	–	–	–	–	–	10.0	8.7

– =log$_{10}$<1.0
(-) = may or may not be present.
From Smith (1965a).

but quantitative bacteriology indicated relatively low level colonization of the epithelium by *E. coli* (Barrow *et al.*, 1988).

The pH of the proventriculus and gizzard is very low (pH 1–2) and microbial survival depends on acid tolerance. Little multiplication of organisms occurs in the duodenum because of the relatively high rate of flow of the very liquid contents. However, colonization of duodenal villi by *Ent. hirae* may result in growth depression in chickens (Fuller *et al.*, 1981). In addition to the other microorganisms present in the small intestine (Table 6.1), a filamentous organism, similar to *Arthromitis*, may be seen embedded in the surface of epithelial cells disrupting the brush borders (Fuller and Turvey, 1971). Its activities and interaction with the host are unknown. *Clostridium perfringens* may occasionally be isolated from the small intestine where it splits fatty acids.

The caeca are filled with a thick viscous fluid containing no food particles: the caeca of animals given oral antibiotics may contain more liquid contents. In these organs the highest bacterial counts (10^{11}/g) and the most complex microflora exist. Smith (1965b) attributed this to the slow rate of flow, the kinetics of bacterial growth resembling batch culture. Most of the microorganisms present are obligate anaerobes, there being more than 200 strains present in the highest dilutions of caecal contents of chickens more than 4 weeks of age. Gram-positive, anaerobic cocci, including peptostreptococci, comprise up to 30% of the total viable count. Other major components include Gram-negative, non-sporing rods (20% of the total) such as the Bacteroidaceae. This important group includes *Bacteroides hypermegas* (now reclassified as *Megamonas*), *B. microfusus* and many other types distinguished by morphology, biochemical activity and fermentation products. Few of these can be assigned to known species. Gram-positive, non-sporing rods, including several types of *Eubacterium*, comprise up to

16% of the total count. Bifidobacteria including *Bif. gallinarum* and other types which clearly differ from human strains are present at 10^9–10^{10}/g of contents. The major clostridia are present at greater than 10^9/g, many of them strict anaerobes. Species isolated include *Clostridium malenonominatum, C. symbiosum* and helically coiled organisms.

Budding bacteria, including *Gemmiger formicalis*, are also present at 10^9–10^{10}/g. These organisms are identified morphologically because they cannot yet be cultured on solid media under conventional anaerobic conditions (Mead, 1989).

Facultative anaerobes include members of the Enterobacteriacae such as *E. coli, Citrobacter, Salmonella, Proteus* and *Klebsiella* which are frequently present but in lower numbers. Smaller numbers of other organisms such as the aerobe, *Pseudomonas*, and yeasts may be found throughout the gut from time to time.

The mechanisms whereby these bacteria are maintained in the caeca are poorly understood. A layer of bacteria, hundreds of cells thick, is present embedded in the mucus lining the epithelium. They are intimately associated with the gut wall but whether this is epithelial adhesion in the strictest sense is a matter of semantics. Such a thick layer which allows rapid colonization of fresh contents when drawn into the caecal lumen is thought to be of significance in the protection against pathogens afforded by the normal flora of the adult. The mechanism of colonization by potential pathogens such as *Campylobacter, Clostridium* and *Salmonella* is poorly understood (Barrow *et al.*, 1988). The slow rate of turnover is thought to contribute to colonization by the latter but there is little evidence for extensive adhesion being a prerequisite. Chromosomal colonization determinants can be recognized in *Salmonella*.

The extent to which viruses are a normal part of the gut flora is difficult to discern. A number of pathogenic viruses may use the gastrointestinal route as the major route of infection or may multiply there, e. g. infectious bronchitis virus, Newcastle disease virus, adenoviruses, egg drop syndrome virus. Some, such as rotaviruses, astroviruses and reoviruses, are associated primarily with enteritis and diarrhoea. In addition to the production of disease some of these, e. g. Newcastle and reoviruses, show variants which produce no apparent clinical disease. Whether or not they can be classified as commensals may depend on whether their release from the cell depends on cell lysis or a non-destructive method such as budding from the cell membrane or into the endoplasmic reticulum.

Bacteriophages are undoubtedly present when susceptible host bacteria are present in the gut. They may be ingested with food but multiplication in the gut does not necessarily affect the numbers of the bacterium. This is the general situation in the alimentary tract of healthy chickens. In addition, a number of factors such as disease, age and diet can affect the composition of the gut flora.

Detailed analyses of the intestinal flora of avian species other than chickens have yet to be made. The composition and significance of the crop flora of some of these birds are unknown. Considerable populations of greater than 10^{10}/g have been found in the caecal contents of turkeys, ducks, guinea-fowl and pheasants. Microscopical observations indicate that the proportion of the predominant types vary. For example in 13-week-old turkeys only 36% of the population enumerated microscopically were cultivable. Of these, 42% were Gram-positive rods, 32% were Gram-positive or variable cocci or coccobacilli, 16% were spirilla and 11% were Gram-negative rods. Some types such as *Megamonas hypermegas*, budding bacteria and uric acid-utilizing bacteria, have been found in many types of the above birds. In contrast some species appear to be specific for individual host species such as *Fusobacterium necrogenes* in the duck and unidentified, large, motile, curved rods found in pheasants. Whether these differences are real or are the results of differences in age or diet in the birds examined is not known.

There is considerable evidence that the highly complex, normal flora of the caeca exert considerable protection against the establishment of microbial pathogens which preferentially colonize the caeca, such as *Salmonella* and *Campylobacter*. Newly hatched chicks which have little or no intestinal flora are much more susceptible than adults to oral infection with these organisms. This can be remedied by administering suspensions or cultures of caecal contents from the adult to chicks and is known as competitive exclusion.

6.4 FACTORS AFFECTING THE COMPOSITION OF THE FLORA

A number of naturally occurring and artificial factors are able to affect the composition of the flora. These factors include age, the immune response, diet and orally administered antibiotics.

Although the alimentary tract of the healthy newly hatched chick is usually sterile it rapidly becomes colonized by facultative anaerobic bacteria, particularly coliforms and streptococci; some clostridia may be present also. Lactobacilli soon displace these types as the dominant organisms in the crop and small intestine. Obligate anaerobes appear after about a week when the conditions in the caeca become favourable for their establishment. The immediate source of such strictly anaerobic organisms in the chicken is not known. The caecal flora does not stabilize until 4–6 weeks after hatching by which time obligate anaerobes are numerically predominant (Smith, 1965a; Mead, 1989).

The importance of the immune response in affecting the components of the intestinal flora is largely unknown. Humoral responses are likely to be

more effective than cell-mediated responses. Although secretory and systemic responses can be detected following infection with normal flora components the response to some bacteria, particularly the more invasive members of the Enterobacteriaceae, is much stronger than towards other, more numerically dominant, organisms such as the lactobacilli and Gram-negative anaerobic rods. Whether invasiveness is the major determinant of immune responsiveness or whether the cell components of some organisms are able to induce a degree of tolerance is unclear. Some human strains of *E. coli* share blood group antigens and may thus be at an advantage over other strains in a host possessing these antigens.

The most obvious changes in the flora induced by dietary change occur at the anterior end of the tract; little change seems to occur in the caeca (Smith, 1965b). Increased carbohydrate stimulates the saccharolytic lactobacilli whereas diets artificially enriched in protein suppress the lactobacilli allowing coliforms, clostridia and streptococci to increase in numbers in the crop. Vitamin-producing bacterial strains may increase in number when vitamin-deficient diets are used.

Antibiotics may be administered in the feed or water for chemotherapy, chemoprophylaxis or for growth stimulation (broilers only). Major groups of microorganisms may be affected. Antibiotic-sensitive microorganisms may be replaced by different resistant types which may enter the food chain. In addition plasmid-mediated antibiotic resistance may transfer from antibiotic-resistant members of the normal flora such as *E. coli* to pathogens such as *Salmonella* under the selective pressure of the presence of the antibiotic. Many of the susceptible organisms affected by antibiotics are mildly inhibitory to *Salmonella* organisms *in vivo*. Their elimination or reduction thus allows increased *Salmonella* multiplication with associated increases in faecal excretion (Smith *et al.*, 1985).

6.5 TRANSLOCATION OF THE INDIGENOUS GUT FLORA

The ability of investigators to isolate normal non-pathogenic intestinal microorganisms from extra-intestinal tissues in different animal species has been recorded many times (for review see Berg, 1992). This has included the isolation of a number of different bacterial species including lactobacilli, enterococci, coliform bacteria and *Bacteroides* from mesenteric lymph nodes, liver, spleen, peritoneal cavity and other organs from rodents, cats, dogs and rabbits.

Work by Fuller and Jayne-Williams (1968) demonstrated bacterial contamination of the peritoneal cavity and yolk sac infections in 38% and 23% respectively of 121 conventional chickens examined during the first 5 days of life. Whether or not the chicks were fed had no major effect. Considerable

variation, related to incubator hygiene, was observed between different batches of chickens. The most frequently isolated organisms were streptococci followed by, in decreasing order, micrococci and coliform organisms (mainly *E. coli*). The micrococci were considered to be characteristic of the chicken gut prior to feeding. Lactobacilli were less frequently isolated and this was thought to be related not to bacterial numbers in the gut but rather to the rate of destruction in the tissues. When experimental infections were made with bacterial strains isolated from avian tissues or from the guts of conventional birds, *E. coli* was isolated more frequently from the tissues than were streptococci or lactobacilli.

Neither the eggshell nor the unhealed navel were considered to be major routes of infection. The main route was considered to be invasion through the mucosa or through lymphoid tissue or *via* migratory phagocytic cells. Histological examinations failed to demonstrate any evidence of invasion although the bacterial numbers involved may have been too small to allow demonstration by this method.

Further investigations by these authors (Fuller and Jayne-Williams, 1970) revealed that the phenomenon was restricted to the first week after hatching. Clearance of streptococci from the livers of intravenously inoculated chicks was greater in 4- and 7-day-old chicks than in newly hatched birds. It was concluded that this increase in resistance may have been related to the maturation of the reticuloendothelial system. Similar experience has been obtained with *Salmonella* infection in chickens where resistance to oral and parenteral infection in chicks increased greatly during the first days of life (Barrow *et al.*, 1987). This was attributed largely to maturation of the reticuloendothelial system (Karthigasu *et al.*, 1965).

On this basis, it seems unlikely that translocation of normal components of the intestinal microflora occurs extensively in adult laying hens to the extent where microorganisms can be cultured from the tissues. Under normal conditions the reticuloendothelial system of the healthy adult chicken results in rapid clearance of microorganisms from the blood (Barrow and Lovell, unpublished findings). Adult chicken serum is also highly bactericidal to many members of the gut flora. The localization of viable microorganisms in the ovary or oviduct of the healthy laying hen must therefore be a rare event. By contrast, the same types of organisms, present in large numbers in the alimentary tract, may be deposited on the egg surface during passage through the cloaca. Some organisms may also ascend the genital tract and contaminate the shell gland, although the extent to which this happens is unknown.

The main elements of host resistance which prevent translocation in the healthy adult are the presence of the complex microflora in the alimentary tract, the existence of an intact mucosa and a mature and functional immune system (Berg, 1992).

The complex intestinal microflora, which is established at 4–6 weeks of

age, exerts a considerable inhibitory effect on colonization by opportunistic and obligate pathogens. Its alteration or removal by antibiotic administration (see below) allows pathogens to occupy niches not usually available to them. Chemotherapeutic antibiotics allow selection for antibiotic-resistant organisms which may colonize the gut and which, at least in young birds, have been demonstrated to invade the tissues (Smith, 1970). Such antibiotics may be used to treat a number of clinical and subclinical conditions including intestinal salmonellosis. With the increase in incidence of *S. enteritidis* in the national flock there is a desire, following the introduction of legislation requiring bacteriological monitoring, to eliminate this organism. Substantial reductions in incidence are claimed by a combination of antibiotic therapy (chlortetracycline or the quinolone, enrofloxacin) and movement to new premises followed by re-establishment of the gut flora by oral inoculation of a competitive exclusion mixture. Such antibiotics will also affect other organisms including, probably, bacteria which are themselves inhibitory to *Salmonella* colonization. In contrast with the calf, it is unusual to find multiple antibiotic resistance in *Salmonella* organisms in poultry. Therefore, unless antibiotic-resistant organisms are present (Smith and Tucker, 1975), the number of potentially translocating bacteria in the gut of such animals will probably be reduced, even though translocation resistance is itself reduced. The extent of isolation of bacteria from the tissues of chickens treated with antibiotic/competitive exclusion mixture would be an interesting study.

In general growth-promoting antibiotics are not used in laying hens. Since the 1970s (Report, 1969) chemotherapeutic antibiotics may not be used for growth stimulation purposes. As a consequence new antibiotics have been developed for this purpose which stimulate growth but to which *Salmonella* and *E. coli* are inherently resistant. However, by virtue of this characteristic some of these antibiotics promote colonization and increase faecal shedding and, possibly, tissue invasion by salmonellas. Some related antibiotics used for treatment of *Mycoplasma* infections have similar effects. How the use of such antibiotics might affect translocation by such potential pathogens is another area where information is unavailable.

Berg (1992) has shown through a number of studies that disruption of the integrity of the intestinal mucosa of rodents increases bacterial translocation. A number of avian infectious diseases occur which potentially have similar effects. Coccidiosis (*Eimeria* infection) is a protozoal disease affecting the alimentary tract which causes extensive mucosal sloughing and submucosal haemorrhage resulting in severe diarrhoea associated with varying degrees of mortality. It is well recognized that the tissue damage and pathological changes in intestinal function can lead to colonization by pathogenic bacteria such as *Clostridium perfringens* resulting in necrotic enteritis, or *S. typhimurium* which is known to be invasive. The extent to which *Eimeria*, particularly the species affecting the lower alimentary tract

such as *Eim. tenella*, *Eim. brunetti* and *Eim. mitis* promote bacterial translocation to internal tissues is not known. However, the knowledge that viral damage in the respiratory tract can lead to invasion by avian pathogenic *E. coli* serotypes, which are not otherwise invasive, suggests that bacteraemia following *Eimeria* infection occurs to some extent at least.

All the major components of the cellular and humoral immune system of rodents have been demonstrated to be involved in resisting bacterial translocation (Berg, 1992). It seems unlikely that the systems would be greatly different in poultry, despite the differences in the structure of the cellular immune system and in the immunoglobulin types. In chickens a number of viral diseases including infectious bursal disease (IBD, Gumboro disease), Marek's disease and other lymphoproliferative conditions have an immunosuppressive component. In the case of IBD this is fairly transient and generally occurs soon after chicks become infected early in life so the immunosuppression does not extend into the laying period. However, the other diseases may induce long-term immune suppression. The effect of this on subsequent bacterial infection or translocation has not been investigated but Marek's disease is known to increase susceptibility to coccidial infection (Biggs *et al.*, 1968).

In summary there is little evidence for extensive translocation of the commensal intestinal microflora in laying hens. However, a number of factors may come into play affecting either the gut flora itself, the mucosa or the immune system which would potentially increase the probability of translocation taking place.

6.6 VERTICAL TRANSMISSION OF AVIAN PATHOGENS

6.6.1 Bacterial infection

A small number of bacterial types of economic and zoonotic significance to the poultry industry are thought to be transmitted vertically. These include a number of salmonellas including *S. gallinarum* and its variant *S. pullorum*, *S. enteritidis*, *S. typhimurium*, *S. arizona* and some others, a number of important *Mycoplasma* spp. and possibly *Mycobacterium avium*.

Salmonella gallinarum and its variant *S. pullorum* (more correctly referred to as *S. gallinarum-pullorum*) are virulent host-adapted avian pathogens of considerable economic significance world-wide. Both produce clinical disease, primarily of the reticuloendothelial system, *S. gallinarum* in chickens of all ages and *S. pullorum* mainly in young birds. Following oral infection the highly invasive bacteria penetrate the mucosa. Whether this is primarily *via* the mucosa or also *via* M cells is unknown. Both organisms possess a large-molecular-weight plasmid which is essential to the subsequent bacterial multiplication in the reticuloendothelial system. However, the

plasmids also contribute with a chromosomally mediated component towards the invasion/colonization process. *Salmonella* organisms are resistant to the bactericidal effects of serum and spread through the tissues and between organs could thus occur both extra- as well as intracellularly, the latter probably occurring in macrophages. Unlike the *Salmonella* serotypes more frequently associated with food-poisoning, *S. gallinarum-pullorum* are not shed extensively in the faeces except early and late in disease in the same way that *S. typhi* is in man.

Considerable evidence has been produced that vertical transmission is extensive and is as important to the spread of infection as horizontal infection. Relatively recent investigations have supported early epidemiological studies which suggested this. Hall *et al.* (1949) found that 50% of hens which were serologically positive to *S. gallinarum* produced infected eggs and that in one batch of 906 eggs 32.6% produced infected chicks which died within the first 6 months of life. Similar data can be found for *S. pullorum*.

Although *S. pullorum* has been shown to penetrate the shell after laying this route of infection is thought to be of little significance. In most cases it is thought that contamination of the ovum occurs after ovulation. Among the gross lesions that characteristically occur in laying hens chronically infected with either of these organisms are grossly misshapen and discoloured ova which may lead to peritonitis following impaction of the oviduct. The organism can usually be cultured from such lesions but since many of these ova will be unlikely to yield fertile eggs and because many other tissues, including the oviduct, are infected, it seems possible that the majority of eggs will be infected either in the yolk membrane or during deposition of the albumen and shell and not primarily through the presence of *Salmonella*-infected yolk.

Eggs from some infected hens carry agglutinating antibodies in the yolk which are thought to allow longer survival than in control eggs. Such antibodies may prevent embryonic mortality in infected eggs and thus may increase vertical transmission.

Vertical transmission of *Salmonella* serotypes other than these two is more unusual. This is probably accounted for by the fact that, generally, such 'paratyphoid' salmonellas are less virulent and invasive for poultry. Thus the two serotypes which account for most cases of food-poisoning associated with consumption of eggs, namely *S. typhimurium* and *S. enteritidis* (particularly phage types 4, 6 and 8) are usually more virulent for chickens than other serotypes. Their invasiveness can lead to localization in the oviduct and ovary as occurs with *S. gallinarum-pullorum*. However, they are also shed in the faeces in relatively high numbers for several weeks and eggshell contamination may occur during passage through the cloaca or by faecal contamination (Barrow and Lovell, 1991). Differences in behaviour between *Salmonella* strains is known to occur. The association

between *S. enteritidis* and egg infection is dealt with in greater detail in Chapter 5.

The faecal carrier is known to be important in eggshell contamination. In this case both invasive and less-invasive serotypes will be involved with equal frequency such as *S. senftenberg* and *S. heidelberg* (Board *et al.*, 1964; Smyser *et al.*, 1966). As the egg cools these and other organisms may be absorbed through the shell (Chapter 3). *S. typhimurium* organisms, smeared onto the surface of chicken eggs, can penetrate and multiply in the egg, although the rate of transmission is thought to be low. Eggshell defects rather than shell thickness affect *Salmonella* penetration.

In addition, *S. arizonae* (or *S. enterica* subsp. *arizonae* as it is now called) is a significant poultry and public health problem in countries outside the United Kingdom, particularly in North America. Turkeys are particularly involved with reduced hatchability and mortality in poults. These organisms resemble *S. enteritidis* and *S. typhimurium* in their virulence for poultry in that tissue invasion and faecal shedding can occur. *S. arizonae* has been isolated from ovaries. Vertical transmission has been reported and demonstrated, and faecal contamination of eggshells also occurs.

Since faecal contamination is thought to be a major cause of egg contamination by *Salmonella* it is not surprising that other members of the Enterobacteriaceae, particularly *E. coli*, can also be isolated from eggs. Between 0.5 and 6% of eggs from normal hens contain *E. coli*. In one study serotypes pathogenic for poultry accounted for 43 out of 245 isolates from dead embryos (Harry, 1964). Since this shows that non-pathogenic strains produce more mortality, how these figures relate to the incidence in faeces would be interesting. The relative virulence of pathogenic and non-pathogenic *E. coli* strains for chicks and embryos would also have been useful information from this study. Although faecal shedding is obviously important, salpingitis, sometimes following air sac infection, may also be involved. The yolk sac of the embryo is the focus of infection. Many embryos die before hatching, particularly late in incubation. Hatched chicks may thus already have *E. coli*-infected yolk sacs leading to neonatal mortality from 'mushy-chick disease'. However, other organisms including *Proteus* and enterococci may also be involved, suggesting involvement of the gut flora. Eggs can be infected artificially by dipping into *E. coli* broth cultures. A higher incidence of infection is obtained with eggs at 18 days of incubation compared with dipping freshly incubated eggs but how this relates to the infections observed naturally is unclear.

A number of serologically unrelated mycoplasmas, including *M. gallisepticum*, *M. meleagridis*, *M. synoviae* and *M. iowae* are of economic importance to the poultry industry mainly because of mixed infections involving viruses such as Newcastle disease or because of secondary bacterial infections. They are all known to be transmitted vertically though the literature pertinent to each varies considerably.

Mycoplasma gallisepticum (MG) produces a chronic respiratory disease in turkeys and chickens. Mortality is generally only considerable in outbreaks complicated by other pathogens such as *E. coli*, Newcastle disease virus (NDV) or infectious bronchitis virus (IBV). Horizontal transmission is known to be important in disease spread but vertical transmission is common. Venereal transmission may also be involved since MG can be isolated from the semen of infected males. Experimental egg transmission has been successfully carried out (Benton *et al.*, 1967; Glisson and Kleven, 1984). It is also possible to culture the organism from fresh eggs, in which case more isolations are made from the vitelline membrane than from older embryos.

Mycoplasma meleagridis is primarily a pathogen of turkeys and produces air sacculitis, skeletal abnormalities, poor growth and decreased hatchability. The organism is transmitted both vertically and horizontally but the former is important in perpetuating infection. According to the literature infection of the female reproductive tract occurs primarily by insemination with infected semen. That this route is undoubtedly important is suggested by the fact that egg infection does not occur in hens where the organism is found only in the upper respiratory tract or sinuses and is minimal in hens with infected air sacs but inseminated with clean semen. Infection of hens by this route is thought to be able to produce air sacculitis in 10–25% of young poults. Individual egg-transmission rates vary between 10 and 60%. Although there is no regular pattern to transmission of infection, it generally starts low soon after lay, reaches a maximum mid-season and gradually declines towards the end of lay. It has not been possible to relate this to insemination.

Mycoplasma meleagridis persists in the genital tract for longer periods than do other mycoplasmas. The main site of infection from eggs is thought to be the uterus and vagina (Mohamed *et al.*, 1966) and the ovary is thought not to be involved in vertical transmission. The organism has been isolated from the shell membrane (10–12%) and from the vitelline membrane–yolk (2–4%) of eggs from naturally infected turkeys (Ghazikhanian *et al.*, 1980), where the counts of mycoplasma organisms can be as high as 10^5 per membrane. Thus the main site where infection takes place appears to be in the area of the fimbria or magnum.

Poults hatched from infected eggs may contain mycoplasmas in a number of tissues including the bursa of Fabricius and cloaca from which they may be shed. Cloacal infection may persist to maturity and semen taken from such males will contain mycoplasmas. The organisms remain localized in the cloaca/phallus and do not ascend to the *vas deferens* or testes. The submucosal gland is thought to be the main site of infection (Gerlach *et al.*, 1968).

The organism may also infect the oviduct of unmated females as a result of endogenous infection early in life. Infection rates of 20–60% have been

reported for vaginal sampling (Matzer and Yamamoto, 1974, 1975). The importance of this route of infection has therefore probably been largely overlooked in the past.

Mycoplasma synoviae (MS) generally produces subclinical upper respiratory disease in chickens and turkeys which may progress to clinical manifestations in the presence of NDV or IBV. It can also produce more severe disease including synovitis. Both horizontal and vertical transmission are important, the latter having been demonstrated in naturally and experimentally infected chickens. It is possible to demonstrate infection serologically in chickens and offspring in the absence of clinical signs. When breeder flocks become infected during lay the egg transmission rate is highest in the first 4–6 weeks after infection and may reduce and cease thereafter but sporadic transmission may also occur subsequently. The organisms can be isolated from the trachea of hatched birds. Dead-in-shell embryos and infertile eggs also result from artificial infection.

Mycoplasma iowae produces reduced hatchability and embryo mortality in turkeys and is known to be transmitted vertically. Venereal infection is again important. Other species such as *M. gallinarum* and *M. anseris* and *M. cloacale* in geese are also thought to be transmitted vertically.

A number of measures have been used to reduce *Mycoplasma* infections, particularly the vertical transmission of *M. gallisepticum* and *M. synoviae*. These include dipping warm eggs in cold solutions of antibiotics such as erythromycin and tylosin. Such measures may reduce but will not eliminate infections and may affect hatchability. However, they have helped to produce MG-free flocks. Antibiotics may also be inoculated into the air space. Heating eggs to 46.1°C also inactivates both MG and MS (Yoder, 1991). In view of the contribution of infected males to vertical transmission it is not surprising that using non-infected males for insemination should be an important component in the control of *Mycoplasma* infections in chickens and turkeys.

Mycobacterium avium causes avian tuberculosis in poultry, producing mortality and severe loss of productivity. Its prevalence is higher in North America than in Europe but it is anyway only found extensively where flocks are reared for long periods of time allowing prolonged exposure to infection. Since large caseous lesions may develop in many organs including the lungs and intestine large numbers of bacteria may be shed which may stay viable in soil and litter for a considerable period of time. Because of the great persistence of the organism and of prolonged exposure it is unclear whether vertical transmission is of any real significance. Experimental studies can readily demonstrate that artificially infected eggs will hatch and that the chickens hatched from such eggs will be infected with *Mb. avium*. However, whether this occurs naturally has never been demonstrated. Several records exist of hatching eggs from naturally infected hens without disease appearing in the chicks (Fitch and Lubbenhausen, 1928;

Feldman, 1938; Francis, 1958). The incidence of infected eggs appears to be low. Figures of 8% of 175 eggs (Bojarski, 1968), 3.55% of 899 eggs (Fritzsche and Allam, 1965) and 0.31% of 650 eggs have been quoted, all obtained from naturally infected flocks. This compares with 8 of 24 (33%) eggs from artificially infected hens.

6.6.2 Viral infections

Among the Retroviridae it is perhaps surprising that whereas Marek's disease, a lymphoproliferative disease of world economic significance, is not transmitted vertically, the avian leukosis/sarcoma viruses are, and by more than one route (Payne and Purchase, 1991). These related viruses belong to the family Oncovirinae. They are characterized by possessing the enzyme reverse transcriptase allowing formation of a DNA provirus from its RNA which may subsequently become integrated into the host genome. The viruses induce a variety of neoplasia. Many of them such as the avian myeloblastosis virus (AMV) and the sarcoma viruses carry specific oncogenes that can cause rapid neoplastic transformation and tumour development within a few days or weeks. The lymphoid leukosis viruses (LLV) are exceptional in lacking transformation genes, and tumour development takes many weeks or months. Transformation is believed to be by viral activation of cellular genes homologous to virus-transforming genes. Virtually all normal chickens carry complete or defective DNA proviral genetic sequences of one particular low pathogenicity type of leukosis virus in their cell genome. These occur in both somatic and germ line cells and are transmitted vertically in Mendelian fashion. This is sometimes referred to as genetic transmission of leukosis virus. Spontaneous virus may be produced from such endogenous virus if a complete viral genome is present and fully expressed virus may be transmitted vertically or horizontally.

Exogenous LLV may be transmitted horizontally, when the infection is in the viraemic phase, and vertically. Although only a small minority of chicks are infected vertically this is important since it maintains infection from one generation to the next. Horizontal transmission within each generation is also important since it maintains a sufficiently high rate of vertical transmission. A minority of hens are viraemic and transmit virus to their progeny. Congenitally infected embryos develop tolerance and become viraemic without circulating antibody. Young hens (up to 18 months of age) transmit at a higher rate than older hens of 2 to 3 years of age. Not surprisingly the genetics of the host and of the virus strain affect the extent of shedding and congenital infection. In the case of the cock, virus budding has been seen on all structures of the reproductive organs except germinal cells. Thus spread *via* the cock is venereal. Congenital infection of embryos is strongly associated with shedding of virus with egg albumen and the presence of virus in the vagina. These are highly correlated with

viraemia. Virus replicates in the albumen-secreting glands of the oviduct and also in the various cell types in the ovary but not in the follicular cells or ovum. Thus transovarial infection does not seem to be important.

Not all infected eggs produce infected embryos or chicks. Whether this is as a result of antibody neutralization or thermal inactivation is unclear. Virus replication can be demonstrated in many of the organs in infected embryos. Infected chicks then shed virus in faeces and saliva.

Endogenous leukosis viruses are usually transmitted in the germ cells of both sexes. Many are genetically defective and incapable of giving rise to infectious virus. However, some are not and may be expressed in an infectious form in embryos or hatched chicks. In this form they are then transmitted similarly to exogenous viruses although most chickens are genetically resistant to such exogenous infection. Endogenous viruses have little or no oncogenicity but may influence the bird's response to infection by exogenous leukosis virus.

Endogenous virus infection can be eradicated from flocks by producing eggs from hens selected because they are non-virus-shedders or non-viraemic. Rearing chickens in isolation and maintaining good hygiene can prevent infection in the period immediately after hatching when the chickens are most susceptible. In addition, the frequency of alleles that encode cellular susceptibility/resistance to infection may vary greatly between commercial lines and it is possible to artificially select and breed for resistance to different subgroups. However, in this latter case a single viral mutation may conceivably overcome this resistance.

Another group of retroviruses of turkeys and chickens, the reticuloendotheliosis viruses, are also transmitted both horizontally and vertically. These cause acute reticulum cell neoplasia, a runting disease syndrome and chronic neoplasia of lymphoid and other tissues. The transforming gene has been identified and is believed to be derived from related sequences in the chicken chromosome. The virus is transmitted horizontally by contact and may be isolated from faeces, body fluids and litter. Viral shedding probably occurs during bouts of active viraemia but is also influenced by host species and virus strain. Contact infection rarely results in clinical disease.

Vertical transmission occurs at very low rates. Isolation of virus, albeit at a low rate, from embryos produced by tolerantly infected turkey hens has been reported (Witter, 1991). Albumen samples from such hens may contain viral antigen but not infectious virus. Vertical transmission from non-tolerantly infected chickens is not common. The role of the male in vertical transmission has not been satisfactorily proved. Some experiments indicate a higher rate of infected progeny are produced in previously non-exposed turkey hens inseminated with infected semen (McDougall *et al.*, 1980) whereas others (albeit using hens from a previously infected flock) have not found this to be the case. Proviral DNA can also be demonstrated

in chicks from matings between viraemic males and non-viraemic females.

The relative role of horizontal and vertical transmission in the field is not known. Neither appears highly efficient but horizontal exposure may be more common. Since horizontal infection, especially at an older age, is less likely to induce clinical disease than vertical infection, this may account for the relative infrequency of clinical disease.

A number of other types of tumours whose aetiology is unknown can affect both ovary and testis but no information is available on their vertical transmission.

Diseases produced by Newcastle disease virus (NDV) and other related paramyxoviruses vary enormously in the type and severity of the disease they produce. Strains of NDV vary considerably in their virulence. Clinical signs vary from acute, lethal disease in chickens of all ages with haemorrhagic lesions in the digestive tract or with respiratory and neurological signs to less severe disease with deaths in young birds and also to inapparent or asymptomatic infections. Serologically different paramyxoviruses produce respiratory diseases in turkeys and other birds which may be of little economic interest.

Vertical transmission *via* the embryo has not been adequately demonstrated and its true significance is not clear. Experimental infection with virulent viruses is usually complicated by cessation of laying after infection. Infected embryos have been reported but these generally die. Virus may also penetrate the shell after laying, complicating a true assessment of transovarian transmission. Experiments with less virulent strains have yet to be carried out. Infected chicks hatched from eggs infected with less virulent viruses do not necessarily cause death of the embryo. It is not always clear how the embryos become infected naturally but the LaSota vaccine, for example, has been shown to be present in most of the reproductive organs after vaccination.

Avian encephalomyelitis is caused by a picornavirus which infects the central nervous system of birds of all ages, but primarily young birds. The disease, which can result in mortality exceeding 50%, results in a progressive ataxia in chicks and blindness in adults. It is considered that naturally occurring avian encephalomyelitis is an enteric infection. Virus is shed in the faeces for a period of several days and because it is quite resistant to environmental conditions it may be transmitted between houses and farms. Dams infected in lay may show reduced egg production and eggs laid during the period show decreased hatchability due to retardation of embryonic development and embryo death.

Vertical transmission is an important means of dissemination. The infection occurs extensively. In the 1960s and 1970s more than 50% of breeder flocks in North America had been exposed to the virus by 5 months of age and by 13 months the incidence of serologically positive birds was 96%. In susceptible breeder flocks infected after sexual maturity a variable propor-

tion of eggs are found to contain virus. Experimental work has shown that most infected embryos and chicks come from eggs laid during the period 5–13 days after infection of breeders with high embryo death during the last three days of incubation. Chicks can also become infected in the incubator.

Adenoviruses produce a number of well and poorly defined diseases depending on the type of virus involved. They can be associated with decreased egg production, reduced food consumption, inclusion body hepatitis and respiratory disease. Types are defined by serum neutralization. Type 1 viruses have not been well defined as primary pathogens but there is increasing evidence for their role as secondary pathogens associated with other diseases such as chicken anaemia agent or infectious bursal disease. Some other well-known agents such as the egg drop syndrome virus and other similar duck viruses partially show the group I antigen.

Horizontal spread of adenoviruses occurs *via* faeces and nasal secretions but vertical transmission is also very important and may be detected by cell cultures from embryos and young chicks obtained from infected flocks. Although maximum virus excretion is found at 4–6 weeks of age, a second peak occurs around peak egg production, possibly induced by the stress and hormone levels associated with egg production.

Egg drop syndrome 1976 (EDS '76) is also an adenovirus infection the causative agent of which has not been completely classified. It results in greatly reduced egg production by apparently healthy birds with frequent occurrence of soft-shelled eggs and eggs showing other shell changes and weaknesses. It is thought that it was possibly introduced into chickens through a contaminated vaccine. Sporadic outbreaks may be the results of infection by waterfowl. Following experimental oral infection a viraemia occurs together with replication in the nasal mucosa. Further replication occurs in lymphoid tissue throughout the body especially in the spleen and thymus. The infundibulum is consistently infected. At 7–20 days postinfection the virus undergoes further extensive replication in the pouch shell gland and to a lesser extent in other parts of the oviduct. This is associated with an inflammatory response which results in soft-shelled eggs. The presence of virus in the faeces probably results from oviductal exudate.

Waterfowl may spread EDS, fowl of all ages being susceptible. However, as originally observed the main mode of spread is probably vertically *via* the embryonated egg. Transmission is efficient albeit at a low frequency. Chicks infected *in ovo* may not excrete virus or develop haemagglutination inhibitory antibody until the flock has reached extensive egg production. The virus is then 'unmasked' and excreted, resulting in an apparently rapid viral spread due to multiple infective loci. Both normal and abnormal shelled eggs laid in the period of viral growth in the pouch shell gland contain virus in the contents and on the shell.

Reoviruses have been isolated from a variety of tissues from chickens affected by arthritis–tenosynovitis, infectious stunting syndrome, respiratory disease and enteric disease. They are, however, also found in clinically normal chickens. Horizontal transmission occurs as a result of respiratory and intestinal involvement. However, vertical transmission has also been clearly demonstrated. Virus can be demonstrated in chicks from eggs laid 2–3 weeks after experimental infection. In this case egg transmission rates were low (1–2%). Reoviruses can also be isolated from chicken embryo fibroblast cell cultures prepared from embryonated eggs derived from experimentally infected hens.

Parvoviruses are thought to contribute to the runting and stunting syndrome seen in broiler chickens and are also known to be responsible for goose influenza and other syndromes including enteritis and hepatitis. Fowl parvovirus spreads horizontally *via* intestinal secretions but is also thought to be able to transmit the virus vertically because parvovirus particles can be demonstrated in embryo fibroblast cultures. Goose parvovirus is also transmitted by both routes. Older birds may become carriers and transmit the virus through the eggs to susceptible goslings in the hatchery but exactly how this happens is not known.

6.7 SUMMARY

For microorganisms to be transmitted vertically a number of factors are required which allow survival and persistence in the alimentary tract of the host or which enable the microorganism to penetrate and survive in the tissues allowing localization in the egg-producing organs. Commensal members of the normal gut flora of poultry are less likely to have virulence determinants enabling them to invade egg-producing organs than they are likely to be able to colonize the alimentary tract. The normal gut flora of chickens is highly complex and not yet fully understood. Some major components cannot as yet be cultivated. For poultry species other than the fowl even less information is available on its composition. The gut flora is affected by a number of factors including host age, diet, immune response and the oral administration of antibiotics. There is some evidence for translocation of components of the gut flora to the tissues in very young chickens. Although under normal conditions it is likely to be a very rare event in adults some practices common in the poultry industry may increase the possibility of its occurrence, including antibiotic usage which may reduce the inhibitory effects of the gut flora, coccidial infection which may disrupt mucosal integrity and the immunosuppressive effects of some avian viral infections. A number of bacterial and avian pathogens of considerable economic and public health significance are routinely transmitted vertically by incorporation

into host DNA, or deposition either within the egg during its formation or on the egg as it passes through the cloaca.

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Contaminants of liquid egg products

W.J. STADELMAN

7.1 INTRODUCTION

In most instances the liquid contents of a newly laid shell egg are free of bacterial contaminants. The eggshell, however, is exposed to numerous contaminants (Chapter 4). The shell, shell membranes, and bacterial inhibitors in the albumen (Chapter 3) protect the white and yolk of the egg from the general contaminants of the environment. When the shell and membranes are removed, this protection is gone. The yolk of the egg is an excellent medium for many bacteria so either liquid whole egg or egg yolk allows rapid bacterial growth if the temperature of a product is appropriate. Galyean *et al.* (1972) found that the lytic activity effect of lysozyme was lost when 10% of yolk or more was mixed with the albumen.

The contaminants of liquid egg products are generally the result of contaminants within the shell egg, the cleanliness of shells of eggs being broken, and the small shell particles that often drop into the liquid even though these are filtered out later. The age of eggs at the time of breaking also affects the levels of microbial contaminants. When eggs are held for several days or weeks prior to breaking some organisms will have penetrated the shell membranes and be present in the albumen. For these reasons the microbial flora of eggshells and penetration of organisms through the shell and its membranes must be considered when reviewing contamination of liquid egg products. A further source of contamination may be the equipment in the breaking room (Zagaevsky and Lutikova, 1944a).

7.2 EGGSHELL EGG CONTAMINATION

The eggshell and membranes serve as a primary barrier to bacterial

contamination of the contents of the egg (Chapters 1 and 4). Stuart and McNally (1943) found that the shell membranes had bactericidal activity. Walden et al. (1956) reported that shell membranes also physically restrained bacterial invasion of the egg contents.

At the time of laying, the egg temperature is over 41°C. The cooling and drying of the moist egg surface, especially the cuticle, and continued cooling of the contents to ambient temperatures results in development of a negative pressure in the egg. This sucks bacterial contaminants on the surface through the pores of the shell. Haines and Moran (1940) reported that if the temperature of the egg is higher than the medium in which it is immersed, including air, bacteria are readily drawn through the shell by simple suction as the egg cools down. Contaminants on the eggshell (Chapter 4) are, therefore, potential contaminants of the liquid egg products. The problem of measuring bacterial contamination on eggshells was investigated by Gentry and Quarles (1972), who found that washing eggs in water in sterile plastic bags was an efficient method of recovering shell contaminants.

The handling of eggs such that condensation forms on the shell surface is referred to as sweating. Its effect on bacterial invasion of the eggshell was studied by Fromm and Margolf (1958), Williams and Yung (1955) and Forsythe et al. (1953) all of whom investigated the effects of washing eggs on bacterial content of intact eggs. Sweating was found to cause a slight increase in bacterial invasion but its contribution to contamination was much less than that of washing of eggs. Forsythe et al. (1953) emphasized that methods used in washing eggs were of the greatest importance in egg processing. This emphasis has led to improvements in egg washing procedures as recommended by Forsythe et al. (1953) and Zagaevsky and Lutikova (1944a), such that, in many countries, all eggs are washed in order to reduce the numbers of bacteria on the shell surface. It is essential to pay particular attention to the cleanliness of the wash water as Listeria monocytogenes has been reported to persist in wash water (Brackett, 1988).

Research on improved methods for washing of eggs, to minimize loss from rotten eggs in storage, was initiated during the Second World War. Later Pino (1950) showed that eggs could be cleaned by a hot detergent-soak followed by a hot-water rinse. Soiled eggs cleaned in this way did not lose interior quality but there was some loss due to cracking of shells. Wash water temperatures of 50–60°C were suitable for washing eggs and minimal spoilage occurred during storage (Starr et al., 1952). Lorenz et al. (1952) found wide variation among ranches washing eggs with regards to egg spoilage.

The level of contamination of the shell egg contents has also been related to the porosity of the shell (Fromm and Monroe, 1960). Haines and Moran (1940) observed that Pseudomonas spp. moved through the shell more efficiently than Saccharomyces ellipsoideus when a suction equivalent to that of cooling of eggs was applied.

In a study of the penetration of pseudomonads into egg contents Hartung and Stadelman (1963) found that shell membranes from eggshells of varying porosities did not differ in their resistance to penetration by *Pseudomonas fluorescens*. The incidence of bacterial penetration increased with age of the egg and with elevated numbers of bacteria on the eggshell. These results confirmed a report by Brooks (1960).

About 12% of shell eggs less than seven hours old contain bacteria (Wolk *et al.*, 1950) and it was concluded that the penetration and survival of bacteria in shell eggs are favoured by elevated holding temperatures. Studies at 9°, 25° and 35°C showed maximum bacterial activity at 25°C.

Pennington (1910) and Goresline (1941) reported that fresh eggs from healthy hens may contain bacteria. Whether the bacteria enter the egg during its formation or penetrate the shell at the time of laying or afterwards are questions on which opinions vary. As yet, this question has not been adequately resolved. Snoeyenbos *et al.* (1969) reported that *Salmonella pullorum* and *S. gallinarum* have long been known to localize in the ovary of chickens. They also found *S. enteritidis, S. heidelberg, S. typhimurium*, and *S. typhimurium* var. *Copenhagen* in the ovaries and the peritoneum of laying hens. Barnhart *et al.* (1991) collected ovaries aseptically from hens at processing plants and observed that *S. heidelberg* was the most frequently isolated serovar but 13 other species of *Salmonella* were also found. *S. enteritidis* was found in ovaries of hens in only one of the 42 flocks sampled. From 1982 to 1987 the number of food-borne illnesses attributed to *S. enteritidis* increased sixfold in Wales and England. Raw or partially cooked eggs were one of the main sources of food infection (Anon., 1988). Coyle *et al.* (1988), reporting on four *S. enteritidis* food poisoning outbreaks in Wales, identified the sources of the organism in eggs of *S. enteritidis*-infected hens. Hinton *et al.* (1989) reported on the ease of infecting young poultry with *S. enteritidis* by contaminated feed. As rodents are usually found in poultry production facilities and feed mills they may be vectors through which feed is contaminated. Winter (1942) reported that the source of infection of most black rot eggs was dirt on the shell or in the water used in cleaning eggs. The predominant organisms causing black rots were *Alcaligenes, Escherichia* and *Proteus*.

The contamination of egg contents by bacteria frequently leads to the development of rots. Dockstader (1952) found that most rotten eggs in commercial breaking stock had cracked shells. It is common practice to use cracked eggs and eggs with deformed shells as breaking stock. The most common spoilage organism was *Pseudomonas* spp. Other genera of bacteria found to be commonly responsible for spoilage were 'Paracolobactrum', *Alcaligenes, Achromobacter, Flavobacterium, Micrococcus, Streptococcus* and *Bacillus*. Dockstader (1952) considered these along with pseudomonads to be primary invaders. Any microorganism found in shell eggs must be considered to be a potential contaminant of liquid egg products. Florian

Table 7.1 Differentiation of spoilage organisms according to their ability to penetrate and infect shell eggs (Florian and Trussell, 1957)

Primary invaders
   Pseudomonas fluorescens
   Alcaligenes bookeri [a]
   Paracolobactrum intermedium[a]
   Proteus melanovogenes
   Pseudomonas putrefaciens
   Proteus vulgaris [a]
   Alcaligenes metalcaligenes
   Flavobacterium invisible
   Alcaligenes faecalis
   Proteus vulgaris [b]
Secondary invaders
   Achromobacter indeterminate
   Achromobacter liquefaciens
   Achromobacter cloacae
   Alcaligenes bookeri[b]
   Alcaligenes recti
   Coli-aerogenes indeterminate
   Escherichia freundii
   Escherichia intermedium
   Flavobacterium indeterminate
   Flavobacterium lactis
   Paracolobactrum aerogenoides
   Paracolobactrum intermedium[b]

[a] or [b] indicate different strains.

and Trussell (1957) classified bacteria as primary and secondary invaders of shell eggs (Table 7.1). All of the organisms were Gram-negative, rod-shaped bacteria.

Many of the early investigators found a lag of 10–20 days between infection of the shells of newly laid eggs and the occurrence of microorganisms in the egg contents (Zagaevsky and Lutikova, 1944b; Orel, 1959; Brooks, 1960; Fromm and Monroe, 1960) (Chapter 3). Board (1964) postulated that the lag was due to time required for the yolk to migrate to near the inner shell membrane. He presented further evidence on the lack of available iron in albumen as a deterrent to bacterial growth.

Walden *et al.* (1956) reported that the blunt end of the shell contains more pores than other sections of the shell. Kraft *et al.* (1958) found that shell porosity appeared to be a useful index for determining susceptibility of eggs to bacterial infection. Similarly, Vadehra *et al.* (1970) found that infection of shell eggs after production is more readily achieved by contaminating the blunt end of the egg

The early work on the microbiology of shell eggs and egg products concentrated on spoilage organisms. Winter (1942) included *Salmonella pullorum* in his list of bacteria found in black rots. During the last 25 years most of the published results of microbiological studies with eggs have

dealt with food-borne pathogens, such as *Salmonella* and, more recently, *Listeria*. Baker *et al.* (1980) reported that very few *Salmonella* were found on eggshells. Rizk *et al.* (1966) attempted, without success, to eliminate *Salmonella* from eggs by applying disinfectants to shell. By using quaternary ammonium compounds in the wash water, they were able to get rid of cells on the surface but not those that had already penetrated to the shell membrane. This disinfection of the shell would likely reduce numbers penetrating the shell at a later time. Similar results and conclusions were reported by Williams and Dillard (1973).

In a review of earlier literature Zagaevsky and Lutikova (1944b) concluded that as eggs of all grades, including 'cracks' and 'dirties', are used in producing liquid eggs, the liquid products will likely be contaminated with soil and faecal bacteria. The names of bacteria listed in these early reports may have been changed in recent literature following more detailed taxonomic characterization. Trussell (1955) reported that 4.9% of eggs from nine farms harboured bacterial contaminants. He listed the causes of contaminated eggs to be, in descending order of importance, poor washing methods, heavy visible soiling of eggs, and infection of eggs by nesting materials. Forsythe *et al.* (1953) reported that washing methods can be improved so as to reduce rather than increase bacterial infection of eggs.

The fact that egg albumen is able to kill, or at least inhibit growth of some bacteria was reported by Zagaevsky and Lutikova (1944c) (Chapter 3). Garibaldi (1960) identified conalbumin as one of the inhibitory substances present in the albumen. It prevents growth of Gram-negative spoilage bacteria. Board (1964) indicated that the Gram-negative bacteria were the predominant organisms in hens' eggs, and Brooks (1960) and Elliott (1954) identified pseudomonads as the primary invaders of shell eggs. Both Garibaldi (1960) and Board (1964) found that addition of iron salts to egg white overcame the inhibitory effect of ovotransferrin (conalbumin). Zagaevsky and Lutikova (1944c) described the effects of a bactericidal agent in egg albumen but did not identify it as lysozyme.

In an investigation of the potential of eggs as carriers of food-borne pathogens, primarily salmonellas Cox *et al.* (1973) fed three species of *Salmonella* to laying hens. *Salmonella* were found on about 10% of the eggshells, but none of the egg contents contained *Salmonella*. Forsythe *et al.* (1967) also reported no egg contamination when *Salmonella* were fed to layers. When *S. anatum* was inoculated directly into the ovary none of the eggs produced was contaminated. When *S. pullorum* was inoculated into the ovary, the ovary became infected and *S. pullorum* was isolated from eggs. Mundt and Tugwell (1958) used six species of *Salmonella* in two feeding and two intravenous injection trials. From a total of 1728 eggs produced none of the egg contents was infected even though salmonellas were found on the shells.

Stokes *et al.* (1956) found that egg contents could be contaminated with

Salmonella by immersing warm eggs in cold aqueous suspensions of *S. oranienburg, S. montevideo, S. typhimurium, S. gallinarum* and *S. pullorum*. The eggs were then stored at 29°C for 3–4 weeks, after which all strains, both motile and non-motile, penetrated the shell membranes and multiplied to populations as high as one billion cells/ml of egg contents. From the above review it is apparent that bacterial contaminants of liquid egg are very varied.

7.3 LIQUID EGG CONTAMINANTS

Mallman and Churchill (1942) proposed a rapid method for determining bacteria in liquid whole eggs or yolks. The procedure was proposed for determining bacterial counts on products during processing. Wrinkle *et al.* (1950) have reported on a number of microorganisms which were found in samples of liquid egg before and after freezing (Table 7.2). Pasteurization reduced the number of genera present but the same genera were predominant in both raw and pasteurized products. Barnes and Corry (1969) found the predominant organisms in raw liquid albumen to be *Pseudomonas, Acinetobacter* and *Serratia* (formerly *Enterobacter*) *liquefaciens*. Only one Gram-negative rod (unidentified) was found to survive pasteurization of albumen at 57.2°C for 3 min.

Garibaldi *et al.* (1969) obtained samples from a number of egg breaking plants located in eight different states of the United States. Of 287 samples, 95% contained less than one *Salmonella*/g. The highest level of *Salmonella*

Table 7.2 Distribution of principal genera of bacteria in whole egg samples (Wrinkle *et al.*, 1950)

Genera	Unpasteurized		Pasteurized	
	Before freezing (%)	After freezing (%)	Before freezing (%)	After freezing (%)
Achromobacter	0.0	1.5	....	....
Aerobacter	3.0	0.0	....	....
Alcaligenes	25.1	20.0	4.0	8.3
Bacillus	7.4	2.0	8.3	83.5
Chromobacter	1.6	1.6	....	....
Egerthella	1.0	0.0	....	....
Escherichia	5.7	6.8	....	....
Flavobacterium	20.0	26.9	4.0	0.0
Gram-positive cocci	5.3	3.5	2.5	0.0
Proteus	15.6	18.1	4.0	8.3
Pseudomonas	7.4	16.0	....	....
Salmonella	1.0	0.0	....	....
Streptothrix	0.0	2.3	....	....

Values shown are percentages of total colonies isolated and identified from seven samples of unpasteurized and four samples of pasteurized whole egg before and after freezing.

contamination was 110 colony-forming units per gram. Shafi *et al.* (1970) found that *Micrococcus*, *Streptococcus* and *Bacillus* were the predominant flora in commercially pasteurized frozen eggs with an average total plate count of 410 colony-forming units per gram. Miller and Winter (1950) observed a 99% kill of bacteria in liquid whole egg following pasteurization at 60–61°C for 4 min.

Several species of *Listeria*, including the psychrotrophic pathogen *L. monocytogenes*, have been found in raw liquid eggs (Leasor and Foegeding, 1989). Sionkowski and Shelef (1990) reported that *L. monocytogenes* grew well in liquid whole egg or yolk. Liquid albumen at pH values of 7.0, 8.0 and 8.9 killed *L. monocytogenes* cells inoculated into raw product. *Salmonella enteritidis* grew in egg yolk (Bradshaw *et al.*, 1990). When the organism was inoculated into eggs from *S. enteritidis*-infected hens the organism grew at a slower rate than when injected into the yolk of a normal hen. When one *S. enteritidis* positive egg is blended with normal eggs the organism can grow at a rapid rate if subjected to temperature abuse. *Yersinia enterocolitica*, another psychrotrophic pathogen, has been found in egg wash water (Southam *et al.*, 1987) and Amin and Draughon (1990) reported that eggs immersed in a suspension of *Y. enterocolitica* became infected with this organism.

Zagaevsky and Lutikova (1944a) emphasized the need for strict sanitation in the egg breaking room. Their data showed great benefits in reducing microbial counts of raw liquid egg by cleaning and sanitizing eggs prior to breaking, by keeping equipment clean and sanitized, and by keeping the temperature in the egg breaking room at 12°C rather than 25°C. Zagaevsky and Lutikova (1944c) found that when storage temperatures of shell eggs were maintained at 0.5°C mould growth on eggshells and shell membranes was inhibited.

Pasteurization requirements vary between countries. Cunningham (1990) summarized the variations (Table 7.3). Shrimpton *et al.* (1962) found that α-amylase inactivation temperature was more than adequate to kill salmonellas in egg products. Their conditions of pasteurization varied from 61.1°C for 1 min to 65.5°C for 5 min. All the treatments destroyed salmonellas but 64.4°C for 2.5 min was necessary to inactivate α-amylase. Garibaldi *et al.* (1969) reported that with the low numbers of salmonellas found in raw commercially broken liquid egg, the US requirement of 60°C for 3.5 min gives an excellent margin of safety in eliminating salmonellas. Johns and Bèrard (1945) found some liquid egg samples were heavily contaminated with spoilage organisms prior to drying. The majority of such instances were the result of including eggs that appeared normal at the time of breaking even though these had high counts.

DeBord (1925) found that spray drying of eggs resulted in a big reduction in the total number of viable bacteria, and in some cases a complete loss of the *coli-aerogenes* group of organisms. Goresline *et al.* (1951), in a study of

Table 7.3 Pasteurization times and temperatures required by laws in several countries for liquid whole egg magma (Cunningham, 1990)

Country	Time(s)[a]	Temperature (°C)
Australia	150	62
China	150	63
Denmark	90–180	65–69
England	150	64
Poland	180	68
USA	210	60

[a]Times are for the average particle. With the higher temperatures turbulence within the mix must be increased to minimize heat damage to the eggs.

the effects of spray drying and pasteurization at 60°C for varying lengths of time on destruction of total bacteria and *Salmonella*, concluded that flash pasteurization at temperatures up to 62.5°C did not kill all serotypes of *Salmonella*. A flash temperature of 60°C with a 3 min holding time killed all species of *Salmonella* likely to be found in liquid whole egg products.

In samples of liquid whole egg collected after pasteurization at 65°C for 3 min (Payne *et al.*, 1979), many of the surviving organisms were found to be related to several groups of *Microbacterium lacticum*. Other unidentified cocci and coccobacilli and *Bacillus* spp. constituted the remaining viable microflora. None of the survivors grew at 5°C but several, which grew rapidly at 10°C and 15°C, were the most heat resistant and had a doubling time of 6–8 h at 15°C.

Osborne *et al.* (1954) found that *S. senftenberg* strain 775W was 10–20 times as heat resistant as the other strains of *Salmonella* studied. Corry and Barnes (1968) determined heat resistance of *S. typhimurium* and *S. senftenberg* 775W in egg albumen. With both organisms the heat resistance was lowered as the pH of the albumen increased. In albumen with a pH of 9.1, the D value at 57.8°C was 126–144 s for *S. senftenberg* 775W compared to 7.5 s for *S. typhimurium*. The heat resistance of several strains of *Salmonella* was studied by Anellis *et al.* (1955), who were primarily interested in thermal resistance of the organisms in acid-treated egg products. Heat resistance was greater for all strains in egg at pH 5.5 than at pH 8.0. With usually observed pH levels in egg products, the times listed in Table 7.3 allow a margin of safety even for *S. senftenberg* 775W.

The heat resistance of *Enterococcus* (formerly *Streptococcus*) *faecalis* (White, 1953), was found to be influenced by the age of the culture of three strains and that the log survivor/time graph was not always straight over its whole length. Old cultures were the least heat resistant. The heat resistance decreased with age of cultures from 30 min to 2 h. There was then an increase in resistance to 5.5 h followed by a decrease at 8 h to a steady value to 48 h, the oldest culture tested. Even with the youngest cultures 60°C for 3.5 min would destroy the organisms.

Foegeding and Leasor (1989) reported on heat resistance of *L. monocytogenes* in liquid whole egg. The minimal pasteurization requirement in the United States (Table 7.3) would reduce populations by 2–3 log cycles. Since *L. monocytogenes* is a psychrotropic pathogen, an increase in pasteurization time or temperature might be advisable when the egg product is to be used in uncooked products. Doyle *et al.* (1987) reported that minimum pasteurization requirements for milk would not eliminate *L. monocytogenes*. Foegeding and Stanley (1990) suggest designing an ultra-pasteurization process so as to render raw egg products free of *L. monocytogenes*. York and Dawson (1973) have reported a usable shelf-life of pasteurized eggs of 12 days when stored at 2°C and 5 days when stored at 9°C. Ball *et al.* (1987) described an ultrapasteurization system linked to aseptic packaging to yield whole egg products with significantly extended shelf-life of 3–6 months at 4°C.

Erickson and Jenkins (1992) found that *L. monocytogenes* would grow in pasteurized whole egg when the product was held at 12.8°C. At 6.7°C the organism was maintained at inoculation levels over the 14-day test period whereas at 2°C the population decreased 1 log cycle over 14 days. Erickson and Jenkins (1992) further reported that *Y. enterocolitica* and other psychrotrophs, such as *Aeromonas hydrophila*, survived or even multiplied in liquid whole egg even when held at 2°C. Payne and Gooch (1980) found that *Enterococcus* (formerly *Streptococcus*) *faecalis* grew in raw and pasteurized whole egg but not in albumen. Pasteurization of albumen at 57.2°C for 2.5 min resulted in only a single log cycle reduction whereas in whole egg pasteurized at 64.4°C for 2.5 min there was a 2 log-cycle reduction.

Wilken and Winter (1947) reported that egg albumen could be pasteurized to destroy 99% of the 'standard plate count' bacteria and all of the coliforms at a temperature of 56.7°C for 1.6 min. In order to get similar results with egg yolk the requirements were increased to 61.1°C for 2 min. Winter (1952) reported that pasteurization improved the keeping quality of whole egg, egg white, and egg yolk when held under refrigerated conditions. He recommended pasteurization temperatures of 60°C for 4 min or 61.1°C for 2 min for whole egg or yolk and 56.7°C for 2 min for egg white. These conditions destroyed 99% of the viable bacteria and all the coliforms and *Salmonella*.

Notermans *et al.* (1991) attempted to eliminate *Listeria* from egg products by adding 25% by weight of sucrose. At 4°C *Listeria* survived in yolk, egg white and whole egg. At 20–22°C in whole egg there was a significant decrease in *Listeria* but this was followed by an increase in numbers indicating adaptation by the surviving organisms. Notermans *et al.* (1990) evaluated the bactericidal properties of advocaat. This is an alcoholic drink consisting of 68% egg yolk, 25% sugar and 7% alcohol. After 3 weeks at 22°C the viable counts of *Salmonella, Staphylococcus,* and *Listeria* were reduced by three log cycles; however spoilage organisms had increased by

three log cycles. When stored at 4°C a similar reduction in the pathogenic bacteria was achieved after 7 weeks with no increase in spoilage bacteria.

Ultrapasteurization and aseptic packaging has been suggested as a means of extending the refrigerated shelf-life of whole egg products (Ball *et al.*, 1987). Foegeding and Stanley (1987) found that such ultrapasteurized products contained *Bacillus circulans* and a species of *Pseudomonas* that grew well at 4°C, and *Ent. faecalis*, a common contaminant in liquid raw eggs, grew rapidly at 10°C. Similar results might be expected with any heat-resistant organism.

7.4 SUMMARY

It is apparent that a very wide range of organisms could be present in liquid egg products. Minimum pasteurization time and temperature requirements have been developed to kill all pathogens. They also destroy about 99% of spoilage organisms. There are indications that some of the pathogenic organisms might have developed strains that are more heat resistant than originally determined.

Discussing the control of *Salmonella*, Bryan (1968) suggested the importance of feed ingredients, feed manufacturing, farms, hatcheries, animal-transporting, holding facilities, abattoirs, and food-processing plants as significant steps in food production, processing and distribution where such contamination might be acquired. In so doing, he presented the basis of the HACCP (hazard analysis of critical control points) concept in the control of microbial contamination.

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Effects of processing on the microbiology of eggs

R.C. BAKER AND C. BRUCE

8.1 INTRODUCTION

It is important to discuss the effects of processing when considering the microbiology of eggs. The subject can be examined from a historical as well as a technical point of view. A look at the future of egg processing, while based on trends, current conditions, and educated guesses, can also serve a useful purpose.

First, the term 'processed' eggs should be defined. In this chapter, the basic premise is that any table egg that does not go to market in its shell is a processed egg. However, also included in this chapter are the proper techniques for washing shell eggs. This is an important process for improving the safety of shell eggs or processed eggs.

In the last 30 years the percentage of table eggs being processed in the United States has increased from about 9% in 1960 to more than 25% in 1990 (Figure 8.1). Aho (1991) quoting Arthur Papetti of Papetti's Hygrade Egg Products, Inc., noted that 'breaking eggs will represent 50% of total egg production by the year 2000'. Aho calculates that with shell egg consumption now at 180 and processed egg products at 50 eggs per year, a fifty-fifty split would mean perhaps 115 eggs in each category. Evidence for this potential growth in processed egg products is indicated by the increasing demand by householders for convenient and safe eggs as a result of the *Salmonella* problem and by institutional users as they meet food safety regulations and strive to cut labour, storage, and garbage disposal costs. A pasteurized product has great appeal for both these markets. Looking into the future, one must also consider the possibility of pathogens other than *Salmonella* becoming a problem (Chapter 5).
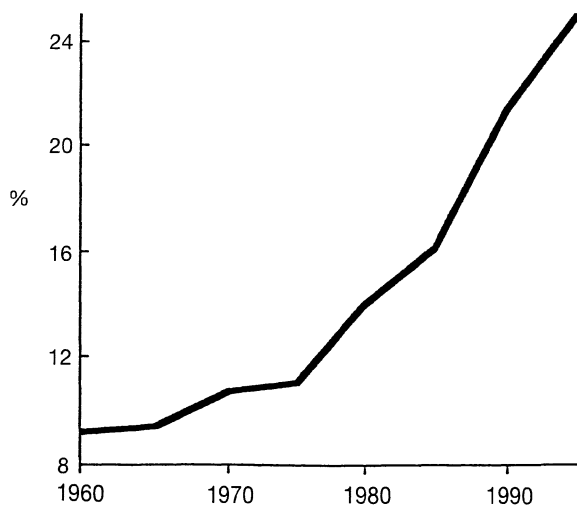
Figure 8.1 Breaking eggs as a percentage of total table egg production. (Source: Aho, 1991.)

8.2 EGG WASHING

The washing of eggs on a large scale began in the United States in the 1940s. Before that time, washing of eggs entailed either soaking very dirty eggs in water for several hours and then wiping the dirt off the shells with a cloth or using a wet cloth to get rid of the dirt. The water used for soaking was usually colder than the eggs which caused the egg to contract and thus allowed bacteria to be pulled across the shell, resulting in contamination of some of the washed eggs. Thus, the washing process was thought by some to be counterproductive. However, when properly done, it is an excellent way to help control microbial problems. Today virtually all commercial eggs in the United States are washed but this is not the case in all countries.

The washing process was improved once it was recognized that it was essential to use water with a temperature higher than that of the eggs. The warmer water caused the eggs to expand thereby creating an outward pressure and the bacteria were not sucked in. With today's washing techniques, and the added step of using a sanitizer, the contents of eggs are rarely contaminated during the washing process. Indeed, it is very rare for an addled egg to be found.

Because of the extreme importance of proper egg-washing procedures, educational programmes to urge their application have been sponsored by industry organizations throughout North America. Poultry specialists at Cornell University have developed the 'Ten Commandments of Washing Eggs'; these have been printed as a poster and distributed widely to egg producers:

1. *Wash all eggs.* Previously, producers were encouraged to wash only the dirty eggs, but they found that it took too much time to sort clean from dirty, it was accepted that it was faster and easier to wash all the eggs. That is now the recommended practice.

2. *Wash eggs soon after lay.* There are two reasons for this caution. First, the dirt, especially faecal material, on eggs is much easier to remove when it is fresh. If the bacteria in moist faecal material are allowed to remain on the shell for several hours they will enter the shell pores where they will be protected from the action of the sanitizers used in the wash water. Second, any dirt, especially faecal material, that invades the pores will stain the shells and cause down-grading of the egg.

3. *Wash water temperature must be at least ten degrees higher than that of the egg.* If this rule is not observed, the egg will contract when plunged into colder water. A pressure differential will be created such that bacteria will be sucked in through the pores.

4. *Wash with an approved sanitizer-detergent following manufacturer's directions.* Washing an egg with plain water or with water containing a detergent is not adequate. A sanitizer must be used with the detergent. There are many sanitizers available, but the most popular in the United States is chlorine. Iodine and quaternary ammonium compound are also used. The sanitizer is necessary to kill microbes including pathogens.

5. *Rinse all washed eggs with approved sanitizers following manufacturer's directions.* Usually the bacteria on the shells of eggs are destroyed in the washing operation. However, if the eggs are not washed promptly, making bacteria more difficult to reach, this rinsing with sanitizer is effective in destroying bacteria that have survived the previous wash–rinse step. Furthermore, this rinse forms a residual film that protects the egg for a short time from bacteria which may come in contact with the shell following the washing operation.

6. *Check residual of sanitizer in wash water daily.* The residual amounts of sanitizer in the wash water can be checked using a kit available from the manufacturer of the sanitizer. Sanitizers are effective for only a short period especially if the wash water becomes dirty. They combine with organic material such as faecal matter and become ineffective as a bactericide. Check the residual amount of sanitizer often and, if it is low, then more sanitizer should be added or the water changed.

7. *Change wash water as often as needed.* The wash water should be changed each day and more frequently if it becomes dirty. The contamination of the wash water will depend on the number of dirty eggs and the extent of their soiling. Clean eggs result from clean wash water.

8. *Clean and sanitize the egg washer daily.* It is vital to clean and sanitize the egg washer at the end of each day. If this is not done the bacterial load will build up and an effective washing procedure cannot be accomplished.

9. *Water supply with a high iron content must have the iron removed.* There is always the possibility that a few bacteria may get into an egg. If this occurs, the bacteria probably will not multiply in the albumen because they are deprived of iron by conalbumin (ovotransferrin) contained in the albumen (Chapter 3). However, if the wash water contains iron, the bacteria will use that which accompanies the contaminants across the shell. A correlation has been seen between elevated numbers of spoiled eggs and high iron content of water in some areas of the United States (Baker, 1973). The United States Department of Agriculture (USDA) suggests that water with an iron content in excess of 2 p.p.m should not be used unless deironized.

10. *Place washed and dried eggs in the cooler immediately after packaging.* There are two reasons for refrigerating eggs immediately after they are washed, air dried, and packaged. Microbes, both pathogenic and those causing spoilage, grow and multiply faster at ambient temperatures. Further, refrigeration slows the rate of loss of egg quality.

8.3   PRESERVATION OF EGGS

Success in preserving eggs depends on three steps. First, an attempt is made to prevent spoilage organisms such as pseudomonads from entering an egg. Should they do so, it is important to prevent or retard their growth. The second vital step is to prevent pathogenic bacteria from entering eggs. As with spoilage organisms, it is very important to inhibit their growth. A third necessity in egg preservation is to maintain egg quality. This is done by preventing carbon dioxide and water loss from the egg. Eggs give off carbon dioxide acquired in the oviduct. With the loss of carbon dioxide from the albumen, the pH of the albumen becomes more alkaline. When an egg is first laid, the pH of the albumen is about 7.2 but as carbon dioxide is lost by diffusion, the pH goes up gradually and when it reaches about 8.5, thick albumen changes to thin resulting in observable lower quality. It is important to prevent loss of carbon dioxide from the egg or at least to retard it. Loss of water from eggs lowers quality because, as it is lost, the air cell enlarges to compensate for the reduction in albumen volume. The size of the air cell is an indicator of the commercial quality of shell eggs; the bigger the air cell, the lower the quality.

8.3.1   Methods of preserving shell egg quality – traditional

An early method of egg preservation was the use of sodium silicate (water glass). The eggs were placed in large containers such as crocks and the water glass (which had the appearance and consistency of egg albumen)

was added to fill the container. The water glass was an unfavourable medium for the growth of microbes and, because of its viscosity, it retarded carbon dioxide and water loss from the eggs by blocking the pores in the egg shell. Eggs could be kept in water glass for up to six months.

Another method used for long-term storage of eggs was refrigeration at about 0°C. At this temperature very little carbon dioxide loss occurs and egg quality is maintained for several months. A third method involved oiling the surface of the shell and then refrigerating. The eggs were dipped in a colourless, odourless, tasteless mineral oil. Consumers in those days did not like the oily shine on the shells but it was a way of having eggs to eat in the winter months.

Over the years fewer and fewer eggs have been oiled. Dipping the eggs in mineral oil went out of fashion because of the shine. For many years the size of most egg farms was small and the eggs went to market only about once a week. To help maintain quality many eggs were oiled using an oil emulsion or spray to cut down on the amount of oil on the shell. With a very thin layer of oil, the shells did not have an objectionable shine. With this treatment plus refrigeration, it was not difficult for consumers to receive good quality eggs. Today, most large egg farms in the United States move eggs from farm to supermarket, restaurants, etc., every day and thus the eggs can reach the consumer when only two-days old. Because of this change only eggs that move long distances to market are oiled.

8.3.2 Methods of preserving shell egg quality – contemporary

(a) Modified atmosphere storage

As stated earlier, carbon dioxide loss is a major cause of loss of egg quality. Loss of carbon dioxide can be drastically reduced by packing in a carton that is an effective barrier to carbon dioxide diffusion. The egg will lose carbon dioxide for a short period of time but only until the carbon dioxide in the egg and in the carton are in equilibrium. This method maintains egg quality without the need for refrigeration.

Workers at Cornell University packed eggs in cartons made of rigid poly-styrene laminated with Saran® thus allowing the eggs to create a modified atmosphere as they gave off carbon dioxide. The eggs were then market tested in New York State. The market test was successful but most of the test consumers continued to place the cartons in their refrigerators out of habit. This same technique should be successful in countries without adequate refrigeration.

(b) Refrigeration

World-wide, refrigeration is the most popular method of preserving egg

quality. It retards carbon dioxide loss thus helping to maintain egg quality and slows the growth of pathogenic bacteria. In the United States 13°C was selected for storage because it is effective in maintaining egg quality and eliminates condensation on the shell when the eggs are removed from refrigeration. The relative humidity recommended for egg refrigerators is 70–75%.

During the past few years *Salmonella enteritidis* associated with whole eggs and egg dishes has caused serious illness in many people in the United States and in many other countries (Chapter 5). It has been shown that this organism will grow and multiply in eggs stored at 13°C but not in those stored at 7°C (Agger, 1989). At the time of writing more American states are making it mandatory that eggs must be stored at 7°C as they move through commercial channels. This means that they will now be kept at the same temperature as other perishable foods.

8.3.3 Liquid egg characteristics

Liquid whole egg is a blend of egg albumen and yolk. In natural proportions, it contains 23–25% solids. However, if a buyer wants lower solids, extra egg white can be added or, if the request is for higher solids, extra yolk can be added. The bacterial quality of raw liquid whole egg is usually not as good as that of raw white or raw yolk alone. However, it is much better than many other food products that have a good reputation for low total plate counts (Cotterill, 1986a). If the initial total bacterial count is greater than 5000 per gram, processing conditions are less than optimal and plant sanitation, operating procedures, and the quality of the eggs being broken should be checked. Pasteurization will reduce counts. If very fresh eggs are broken, then counts of less than 100 per gram in a pasteurized product can be expected. Pasteurized whole egg liquid is used by bakeries, candymakers, and eating establishments of many kinds. This product is now being retailed to household consumers in the United States in quarts, pints, and half pint amounts.

8.3.4 Liquid egg pasteurization

Pasteurization of liquid egg was first used in the United States in the early 1930s. It is now required by USDA regulations for all liquid, frozen, and dried whole egg, yolk, and white unless otherwise treated to reduce the number of viable salmonellas to very low levels. The early operations were small and used batch-type pasteurizers which had been developed for milk pasteurization. Later it was noted that plate-type high temperature, short time (HTST) milk pasteurizers could be used. The unit (Figure 8.2) consisted of a plate heat-exchanger with sufficient holding tubes to maintain a temperature of 60°C for 3.5 min. Today plate pasteurizers are very common
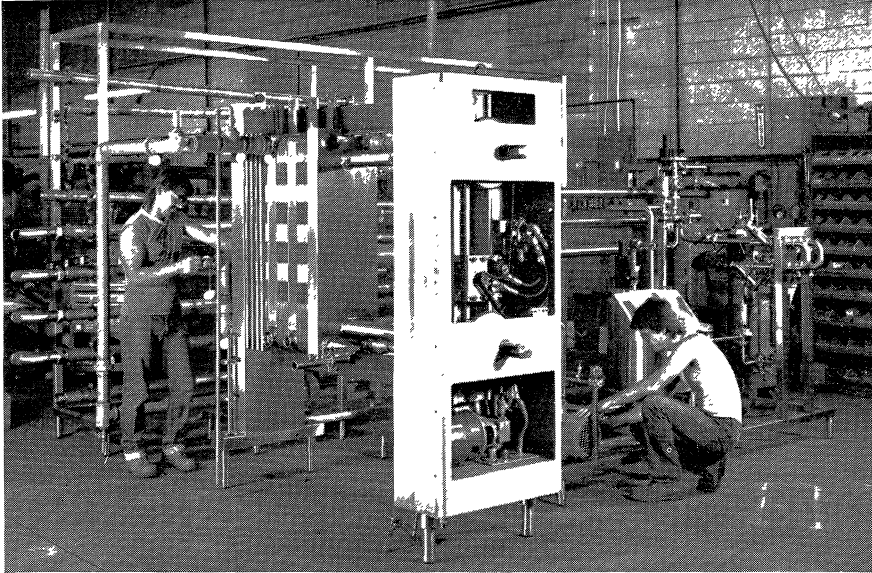
Figure 8.2 Egg pasteurization system. Courtesy APV Crepaco Inc.

but the temperature and time combination used varies in different countries. In the United States, the Department of Agriculture requires that liquid whole egg be heated to at least 60°C and held for not less than 3.5 min (Table 8.1), whereas in the United Kingdom the recommendation is 64°C for 2.5

Table 8.1 USDA pasteurization requirements

Liquid egg product	Minimum temperature requirements (°C)	Minimum holding time requirements (min)
Albumen (without use of chemicals)	57	3.5
	56	6.2
Whole egg	60	3.5
Whole egg blends (less than 2%	61	3.5
added non-egg ingredients)	60	6.2
Fortified whole egg and blends (24–38%	62	3.5
egg solids, 2–12% added non-egg ingredients)	61	6.2
Salt whole egg (with 2% or more salt added)	63	3.5
	62	6.2
Sugar whole egg (2–12% sugar added)	61	3.5
	60	6.2
Plain yolk	61	3.5
	60	6.2
Sugar yolk (2% or more sugar added)	63	3.5
	62	6.2
Salt yolk (2–12% salt added)	63	3.5
	62	6.2

Source: Regulations Governing the Inspection of Eggs and Egg Products (Anon, 1991a)
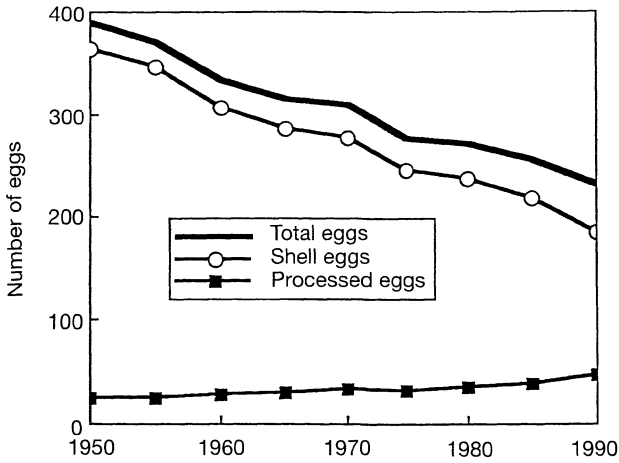
Figure 8.3   Egg per capita consumption. (Source: Aho, 1991.)

min. The purpose of egg pasteurization is to destroy as many microbes as possible, without damage to the functional properties of the liquid egg. Table 8.1 shows USDA pasteurization time–temperature requirements.

8.3.5   Pasteurization of egg white

Liquid egg white has a solids content of close to 12%. The pH of egg white can vary from 7.6 to 9.3 with a range of 8.4–9.2 being common. The bacterial quality of liquid egg white is usually very good and is dependent upon the quality of broken eggs, the sanitation of the plant, and the handling practices used. The contents of most shell eggs are sterile when broken and egg albumen is not a good medium for the growth of microbes.

Care must be taken to avoid impairment of the functional properties of egg white by pasteurization. For the pasteurization to be effective in destroying pathogens, especially salmonellas, either the pH should remain the same and time–temperature adjustments be made, or the pH should be changed to the level required for a different combination of time and temperature. There are three different processes used for pasteurizing liquid egg white: (1) the lactic acid–aluminium sulphate (pH 7) process; (2) the hydrogen peroxide process; and (3) the vacuum process.

(a)   Lactic acid–aluminium sulphate process

With this method, egg white can be pasteurized at the same temperature that is used in the United States for whole egg. Stabilization of the egg albumen is accomplished by adding an aluminium sulphate solution to the egg white before heating. The solution contains one ounce of aluminium sulphate to one pound of 25% (v/v) lactic acid. About 6.5 pounds of this

solution is used for 1000 pounds of liquid egg white. When pasteurized the pH of the liquid albumen should be about 7. The process is protected by a government patent but is available for licence at no charge.

(b) Hydrogen peroxide process

Armour and Company hold a patent on this process which combines the use of heat and hydrogen peroxide. Liquid egg white is heated to 52°–53°C and held at this temperature for 1.5 min. Sufficient hydrogen peroxide (10% solution) is then added to give a concentration of 0.075–0.10% in the egg white. The hydrogen peroxide is allowed to react at this temperature for 2 min. The albumen is then cooled and catalase added to destroy the hydrogen peroxide.

(c) Vacuum process

A third method approved by USDA for pasteurizing egg white uses a vacuum. With this method the HTST plate pasteurizer is equipped with a vacuum chamber in which 17–20 inches of vacuum are applied to the egg albumen before heating at 57°C for 3.5 min.

8.3.6 Whole egg pasteurization

As stated earlier the temperature and time recommended for whole egg pasteurization in the United States is 60°C for 3.5 min. According to Cunningham (1986) it appears to make little difference which *Salmonella* serotype (other than *S. senftenberg*) is used as the test organism when checking for the effectiveness of this time–temperature combination. A prime purpose for pasteurizing whole egg, and egg products in general, is to create a wholesome product by eliminating pathogenic bacteria. Primary concern is with salmonellas because of their long history of association with eggs and salmonellosis in humans (Chapter 5). Fortunately salmonellas are relatively easy to destroy by heat. Baker (1990) showed that *S. enteritidis* is destroyed by pasteurization at 60°C for 3.5 min.

8.3.7 Pasteurization of egg yolk

Liquid egg yolk in the pure form from fresh eggs has about 51.9±0.1% solids. The amount of water migrating from the albumen into the yolk as the egg ages is a major factor affecting the final percentage of egg yolk solids. The solids level in yolk produced by egg breaking machines can be affected by many factors, such as the equipment, age of eggs, or size of eggs. Normally the solids content is in the range 46 to 48% depending on the amount of egg white adhering to the yolk. However, most liquid yolk is standardized by the addition of albumen to a 43–44% solids level. Most egg

yolk is purchased by bakeries, candy makers, and mayonnaise manufacturers.

Salmonellas are more heat resistant in yolk than in whole egg because of the lower pH and higher solids content. This means that yolk should be pasteurized at a higher temperature or for a longer time at the same temperature as compared to whole egg. As yolk is less sensitive to heat in terms of impairment of functional properties, there is a greater tolerance to temperature limits in the operation of plate-type pasteurizers. Additives such as sugar or salt increase the thermal resistance of microbes in egg yolk.

8.4   EGG BREAKING

The difference between shell and liquid egg as far as microbial contamination is concerned should be borne in mind. As stated earlier in this book (Chapter 3), shell eggs are well protected against microbial infection thereby assuring the safe development of the chick embryo and guaranteeing the survival of the species. However, when the shell and the membranes are removed and the albumen is mixed with the yolk, there is no impediment to microbial growth. In fact, liquid whole egg and liquid yolk are ideal media for microbial growth making sanitation and temperature control vitally important. The three egg products produced by an egg-breaking plant are liquid whole egg, liquid egg white, and liquid egg yolk.

In the past liquid egg products provided an outlet for surplus eggs but today many eggs are produced specifically for this purpose. According to statistics obtained from USDA/National Agricultural Statistics Service for the year starting 1 October 1990, there were 1 123 014 000 dozen eggs processed by breakers creating 1 404 403 000 pounds of various edible liquid egg products. Figure 8.3, showing the trend of *per capita* egg consumption in the United States between 1950 and 1990 indicates a dramatic decline in the use of shell eggs and a modest rise in processed eggs. It is the latter that is predicted to rise in the future.

There has been a tremendous increase in the production and consumption of liquid fresh egg. Some of this increase reflects the shift to its use instead of the frozen product. Many users of liquid egg find the fresh product more convenient to use and also more economical. Years ago it was felt that liquid egg was too perishable for institutional users such as bakeries, confectioners, and restaurants, but now that liquid egg is pasteurized it has about the same shelf life as milk. Modern equipment speeds the breaking and separation process (Figure 8.4) and reduces production costs. Refrigerated transport allows quick distribution of fresh liquid egg
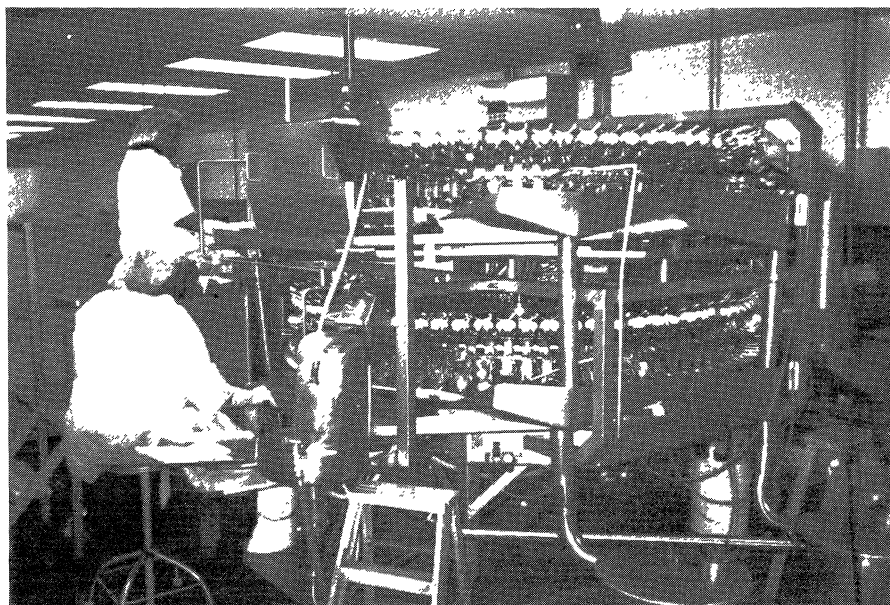
Figure 8.4 Egg breaker, and separator. Courtesy, Sanovo Engineering A/S.

products. The use of HTST to achieve commercial 'sterility' standards and the use of sterile containers has limited or eliminated problems with shelf life.

USDA has strict requirements for cooling times and temperatures for liquid egg products as shown in Table 8.2.

8.5 LONG-TERM PRESERVATION OF LIQUID EGG

8.5.1 Freezing liquid egg

Frozen egg products are usually marketed as ingredients for use in other foods. About 29% of the total liquid production is frozen. This is considerably less than was the case just ten years ago. In some egg products major textural changes are caused by freezing. Large reductions in bacterial counts also result from freezing. Functional properties are normally only slightly affected. If raw egg yolk is frozen and stored without the addition of sodium chloride or sucrose, its viscosity increases and gelation occurs. It also has an undesirable appearance. After a period of time, all fluidity is lost and the gelled yolk will not combine as a normal liquid with other ingredients. Usually, sodium chloride or sucrose is added at the 10% level but smaller amounts may be used depending on the intended use of the

Table 8.2 Minimum cooling and temperature requirements for liquid egg products (Unpasteurized product temperature within 2 hours from time of breaking)

Product	Liquid (other than salt product) to be held 8 h or less	Liquid (other than salt product) to be held in excess of 8 h	Liquid salt product	Temperature within 2h after pasteurization	Temperature within 3h after stabilization
Whites (not to be stabilized)	13°C or lower	7°C or lower		7°C or lower	
Whites (to be stabilized)	21°C or lower	13°C or lower		13°C or lower	a
All other products (except products with 10% or more salt added)	7°C or lower	4°C or lower		If to be held 8 h or less, 7°C or lower. If to be held in excess of 8 h, 4°C or lower	If to be held 8 h or less, 7°C or lower. If to be held in excess of 8 h, 4°C or lower
Liquid egg product with 10% or more salt added			If to be held 30 h or less, 18°C or lower. If to be held in excess of 30h, 7°C or lower	18°C or lower[b]	

[a]Stabilized liquid whites shall be dried as soon as possible after removal of glucose. The storage of stabilized liquid whites shall be limited to that necessary to provide a continuous operation.
[b]The cooling process shall be continued to assure that any salt product to be held in excess of 24 h is cooled and maintained at 7°C or lower.
Source: Regulations Governing the Inspection of Eggs and Egg Products (Anon, 1991a).

material being frozen. Egg yolk gelation is affected by rate and temperature of freezing as well as by the storage temperature and time. Cotterill (1986b) found that the freezing point of egg yolk is about -1°C, but gelation does not occur until a temperature of -6°C is attained. Liquid whole egg also undergoes gelation when frozen and thawed but much less drastically than egg yolk. It is generally agreed that the functional properties of whole egg are not altered to any great extent by the freezing process and only minor changes occur in the freezing of raw egg white.

8.5.2 Egg dehydration

Removing a large proportion of water from food stops the growth of microorganisms as well as retarding undesirable chemical reactions. Dehydration has been used as a preservation method of food for centuries but egg dehydration did not come into being in the United States until 1930. Now about 8% of egg products are dehydrated.

Egg drying differs from most food dehydrating operations in that glucose must be removed before drying takes place in order to obtain a high quality product. If this is not done, carbonyl-amine browning can take place giving the egg product an undesirable colour and flavour. According to Bergquist (1986) the problem of solubility of spray-dried egg whites (because of clumping) has been overcome with instant egg white, an agglomerated product, which disperses and dissolves rapidly in water.

Dried egg products are used in many foods and they have the following advantages (Bergquist, 1986). They:

- Can be stored at low cost and require less space than shell or liquid egg;
- Incur lower transportation costs;
- Are easy to handle in a sanitary manner;
- Are not susceptible to bacterial growth when held in storage;
- Allow for precise control over the amount of water used in a formulation;
- Have good uniformity;
- Make possible the development of new convenience foods.

8.5.3 Methods of drying liquid egg

Spray drying is the most common method of drying egg products. In this process the liquid is finely atomized into a stream of hot air. The enormous surface area created by atomization allows water evaporation to take place rapidly.

Pan drying, an old method, is still used for producing egg white

products. When dried in pans to a moisture level of 12–16%, the end product is a flake-type material. Products containing fragments with dimensions of 1.5 to 12.5 mm are considered flakes and smaller particles are usually called granular egg white. Pan-dried egg white can also be milled to a fine powder.

Belt drying is another method of producing dried whole egg and dried yolk but has little commercial use today. The liquid can be spread as a thin film on a continuous aluminium belt which moves through a hot air stream.

A newer method of drying egg products is freeze drying. With this technique, water is removed from the product while it is in the frozen state. This is done by first freezing the product and then subjecting it to a high vacuum. Heat is supplied to the product while it is drying. The quality of the end product is excellent. This method is expensive and is not as popular commercially in the United States as it is in some European countries.

8.6 MICROBIOLOGICAL CONCERNS IN DEVELOPING NEW MARKET FORMS

In recent years new convenience egg products designed to meet the needs of the home and institutional markets have been introduced. If eggs are to be consumed in greater volume, they must be offered to both markets in new and enticing forms. Given the concern for egg safety, one of these enticements may be pasteurization. For a long time pasteurized liquid egg products have been available to food manufacturers and institutional caterers. Frozen and dried products have also made an impact on these markets. Part of the reason for their success is that they eliminate many microbiological problems for the user, while being economical, convenient, easy to handle, and easy to use.

8.6.1 No cholesterol and low cholesterol egg blends

Perhaps the newest of these convenience products on the institutional and retail markets are the 'egg substitute' and low-cholesterol products. These novel products can be used to make scrambled eggs, omelettes, custards and cakes. Low cholesterol eggs are also sold as cooked, ready-to-eat products such as scrambled eggs, hard cooked eggs, and omelettes. The egg substitute products use ingredients such as vegetable oil, non-fat milk powder, soy protein, gums, food colouring, artificial flavours, minerals and vitamins as a yolk replacement to eliminate animal fat and cholesterol. In other products some of the yolk has been replaced by egg albumen (Baker

and Darfler, 1977) thus lowering the cholesterol content without destroying the functional properties. It is also possible to remove cholesterol from liquid egg, but this is an expensive system.

8.6.2 Scrambled eggs

Partially prepared scrambled eggs first appeared on the market as a frozen mix of whole raw liquid eggs and seasoning. These were intended for use in restaurants, hotels, and hospitals. The mixture was first thawed and then the scrambled eggs were cooked. Later, scrambled egg mixes were frozen in 'boilable' plastic bags which could be placed in hot water for cooking. Before the egg was completely coagulated, the bags were removed from the water and egg material in the bag was kneaded into smaller particles by hand. The scrambled egg material could go directly from the bags into serving containers. With the advent of pasteurization, consumers seem to prefer pasteurized liquid fresh egg. More recently fully cooked scrambled eggs have been marketed, especially for institutional use. These can be sold frozen or they can be packaged hot (82°C) and have about a 45-day shelf life if stored at 2°C. With pasteurization and good sanitation, bacteria, especially pathogens, should not be a problem.

One word of caution about using liquid egg. Carbon dioxide can be easily lost thus raising the pH of the mixture. When this happens, iron in the yolk is released and reacts with hydrogen sulphide released from sulphur amino acids in the albumen during an overly long cooking period or during a long serving period when the eggs are kept at about 63°C. This results in the development of an unacceptable greenish-grey coloration. Fortunately there are acceptable ingredients (Table 8.3) that can be added to prevent this reaction. If the pH is lowered, the iron will be chelated or bound so it will not react with hydrogen sulphide to form the green–grey colour.

Table 8.3 Conditions for prevention of green–grey discoloration in cooked liquid whole eggs

Chelator	pH of liquid egg and chelator	Concentration (w/w)
Acetic acid	6.42±0.07	0.19%
Citric acid	6.83±0.03	0.17
Na_2EDTA	8.40±0.01	0.029
Lactic acid	5.74±0.02	0.27
Malic acid	6.53±0.04	0.22
Monosodium phosphate	7.08±0.02	0.34
Propionic acid	6.47±0.03	0.26
Succinic acid	6.15±0.02	0.27

Source: Gossett and Baker (1981).

Gossett and Baker (1981) determined the optimum conditions needed to prevent this discoloration (Table 8.3).

8.6.3 Omelettes

Omelettes first appeared on the market as a frozen raw product. This was soon followed by pasteurized liquid mixes of different kinds. Cooked, frozen, omelettes were not popular when introduced because of their poor texture after thawing. So, although the freezing protected the safety of the product, the texture problem had to be solved. It was determined that this resulted from the freezing operation wherein ice crystals broke up the protein structure causing an inferior texture. Now, small ice crystals formed at the low temperatures that are produced with cryogenic techniques produce omelettes much improved in texture. O'Brien *et al.* (1982) found that some gums, such as xanthan and kayara, or sodium tripolyphosphate could be used to improve the texture of thawed, frozen omelettes.

8.6.4 Hard-cooked eggs

Hard-cooked eggs can be classed as a convenience food but not a new one. In the past 20 years, a very large volume has been produced. Some hard-cooked eggs are sold in the shell but sales of peeled eggs are greater. They may be sold without further treatment, or, to insure a good shelf life, packed in an organic acid solution to control bacteria, and either sodium benzoate or potassium sorbate added to inhibit fungal growth. The organic acid most commonly used is citric and the concentration varies from 0.15% to 0.5% or even higher.

Microbiological aspects must be considered in the production of high quality hard-cooked eggs. If they can be machine peeled (deshelled) the possibility of contamination is less than with hand shelling. In order to prevent shell breakage it is important to use eggs with sound shells and to avoid putting cold eggs into boiling water. Fresh eggs do not peel easily so it is wise to age the eggs before processing. Very fresh eggs contain large amounts of free carbon dioxide in the albumen producing a pH of about 7.2. As carbon dioxide is lost from eggs, the pH increases. When it gets to about 8.7 there is little, if any, chemical bonding between the inner shell membrane and the cooked albumen, thus the eggs peel easily.

The unattractive greenish-black discoloration sometimes seen on the surface of cooked egg yolks can be prevented. It is caused by the formation of ferrous sulphide at the interface of the yolk and the albumen. This happens when free iron in the yolk reacts with hydrogen sulphide coming from the sulphur-containing amino acids in the albumen. It does not occur if fresh eggs are used because the iron is not free to react. However, fresh

eggs peel poorly. Cooking at a temperature below 100°C will help prevent the discoloration.

Rapid cooling of the cooked eggs is very important and should be done in clean, cold tap water or iced water to prevent microbial contamination. During the cooking operation, hydrogen sulphide is produced as a gas in the albumen but due to the heating, the outward pressure prevents the gas from contact with the yolk. When cooking has ceased, the egg cools and contracts, pulling the hydrogen sulphide inward to make contact with the yolk. With rapid cooling, the hydrogen sulphide does not have time to react with the yolk and the discoloration will be minimal.

In some processing plants the shells of cooked eggs are removed by hand. There are also many machines used for deshelling hard-cooked eggs. With one machine the shells are cracked by rollers. The cracked shells are then removed from the cooked albumen by a high velocity air jet or by water under pressure. Another machine uses revolving, flexible rubber fingers to remove the shells. Microbial infection and spoilage can be a problem if good sanitation is not practised. An egg cooked in its shell is, for all practical purposes, sterile but can be reinfected by hands, air, water, and rubber fingers during the shelling operation. If the cooked eggs are placed in a preservative as suggested above, a long shelf life should result. A further safeguard against reinfection of the surface of the hard-cooked egg is to immerse the egg in boiling water or expose it to steam before it is packaged. A storage temperature of about 0°C should be used.

If hard-cooked eggs are to be chopped or diced it is not necessary to cook the eggs in their shells, eliminating both the deshelling and microbiological problems. The eggs can be broken into large trays and then cooked by steam. If they are diced or chopped while hot and then packed hot (82°C), the possibilities of contamination are limited thus giving a long shelf life when properly refrigerated. This method of production allows extra egg albumen to be added before cooking, resulting in a lower cholesterol content in the final product.

8.6.5 Hard-cooked egg roll

In the past, slices of hard-cooked egg were popular garnishes on salads. As eggs were marketed fresher and fresher, peeling became a problem and this, along with high labour costs, discouraged the use of hard-cooked egg slices. To solve the problem, product developers at Cornell University created and test marketed in 1963 the prepackaged hard-cooked egg roll (Figure 8.5). With this roll there is no peeling problem and the product has a long shelf life because the cooking is done in a polypropylene tube which is not removed until the hard-cooked eggs are consumed. The cooking temperature (93°C) for a cooking time of 20 min prevents greening of the

Figure 8.5 Hard cooked egg roll. Courtesy, New York State College of Agriculture and Life Sciences at Cornell University.

'yolks'. This time–temperature combination will destroy all microbes (Jack, 1964).

8.7   SAFE PREPARATION OF EGGS AND EGG DISHES AT HOME

The concern for safe processing of eggs carries over to householders whose awareness of microbiological problems has been heightened by the media in the past few years. Consumers are concerned about the eggs they prepare at home as well as dishes containing eggs, particularly those in which the eggs are only partially cooked or not cooked at all. First of all consumers need to be reminded to buy eggs wisely. The best indicators of freshness are dated cartons, refrigerated display, and rapid turnover of supplies. The next step is refrigeration of the eggs at about 7°C in the home. Quality is lost rapidly at room temperature and microbes, if present, could proliferate. A rule-of-thumb that is sometimes suggested to homemakers is that eggs should not be left at room temperature for more than 2 h.

It may seem elementary, but food handlers need constant reminders about hand washing with soap when working with food. The possibilities for cross-contamination (touch raw chicken then slice hard cooked eggs for

the salad, for instance) without washing between tasks or poor personal hygiene habits such as covering the mouth when coughing and then not washing provide excellent opportunities for introducing bacteria into food. Clean utensils and surroundings are also important. Storage temperature of foods containing non-cooked or lightly cooked eggs are of concern. These foods should be promptly chilled when made and not allowed to stand at room temperature until served. A good scenario for bacterial growth is a bowl of egg nog standing at room temperature while a party goes on and on.

Another rule-of-thumb regarding the destruction of salmonellas in cooked egg is that if the yolk and albumen are coagulated, they are safe. Although an oversimplification, it serves some purpose in the home kitchen.

Baker (1990) tested eggs prepared by common home methods to determine the thermal death time of *S. enteritidis*. According to this study, when using an uncovered electric frying pan set at 121°C, scrambled eggs should be cooked for 1 min to be free of *S. enteritidis*. Using this same temperature, the cooking time for fried eggs will vary depending upon whether the pan is covered and the eggs turned or not (sunnyside) during cooking. In a covered pan, the cooking time was 4 min; for sunnyside, 7 min and for turned eggs, 3 min on one side and 2 min on the other. For complete kill when poaching in boiling water, 5 min was needed and 7 min for eggs boiled in the shell (Table 8.4). Variables that make it impossible to state thermal death times for composed egg dishes unless they are individually tested, are the amounts and types of other ingredients. Acid, sugar, and salt are common ingredients that change the pasteurization requirements.

8.8 SUMMARY

Egg processing is a relatively new enterprise compared to other agricultural

Table 8.4 Time required to destroy *Salmonella enteritidis* in cooked eggs

Cooking method	Time needed for complete kill (min)	Final temp. (°C)
Scrambling	1	74
Poaching	5	75
Boiling	7	75
Frying:		
Covered	4	70
Sunnyside	7	64
Turned over	3+2	61

Source: Baker (1990).

commodity processing practices. Producers apparently thought that eggs could not get much more convenient and, until consumers made their wishes known, they saw no reason to undertake product development. Now that it is evident that institutional and home users want to save time, labour, and clean-up effort, the incentives to develop new market forms have increased. Even stronger motivation for buying processed egg products is the assurance that pasteurized products diminish the concern regarding pathogenic bacteria.

Shelled eggs have good protection against microbial growth. Contamination is not easy if recommended steps are followed. However, we know it is possible for eggs in the shell to become infected; *S. enteritidis* is a prime example. Processing eggs, whether by pasteurization or cooking, greatly reduces the chances of infection. Good sanitation and refrigeration should give a good shelf life. It must be remembered, however, that these processed products can become reinfected by careless handling. In distribution and in storage by consumers, they must be treated like any other perishable food.

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