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Microbial Contamination of the Hen's Egg: A Review

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ABSTRACT

The hen's egg is susceptible to microbial attack in a number of ways. The yolk or the albumen may be contaminated before the egg is laid. After the egg has been laid the possibility exists of microbial penetration from the outside. In this review, both these possibilities are discussed together with the defences, both physical and chemical, that the egg has against microbial contamination. Most eggs contain no bacteria when they are laid and only become contaminated subsequently. The shell membrane offers the best protection against bacterial penetration, but once inside the egg their growth and multiplication is slowed due to the viscous nature of the egg white proteins, their pH, and the bactericidal properties of lysozyme and conalbumen.

MICROBIAL CONTAMINATION OF THE HEN'S EGG

Microbial contamination, and the subsequent results of this contamination of eggs intended for human consumption or hatcing purposes, have attracted the attention of several workers (e.g. *14,15,18,36,37,45,48,49,51,56, 59,71,86*). As early as 1939, Haines (*45*) observed that the egg is equipped with physical and chemical defences against microbial infection, and it has been suggested that these defences have been developed to protect the developing embryo. Contamination and rotting of eggs take place when such physical and chemical defences become overloaded (*25*). Because of the importance of these aspects the natural and chemical defences against bacterial penetration will be discussed.

Contamination before laying

The mechanism and level of microbial contamination of the egg contents are not well known. This is due to the technical difficulties of sampling the internal contents of the egg. It is difficult to find methods which ensure the sterilization of the shell of the egg (*4,14,18,42,44*). Even when sterilization is possible, contamination with airborne microorganisms may affect the results (*18*).

Many workers suggested that the egg can be easily contaminated within a short period after laying (e.g. *63*). Bacteria isolated on newly laid eggs are mainly *Micrococcus* spp. which grow poorly at body temperature (*66*) suggesting that they may be contaminants. An attempt was made by Harry (*50*) to clarify this confused situation. He killed hens and examined their ova. He was able to recover bacteria from 43% of the ova. The most common bacteria isolated were *Lactobacillus* sp. and *Micrococcus* sp. It is therefore possible that the *Micrococcus* sp. isolated by Miller and Crawford (*66*) had originated from the ova and were not accidental contaminants from the shell or the air. Board (*14*), and MacLaury and Moran (*64*) citing Rettger (*78*), maintain that there are egg-transmitted organisms which can invade the yolk in the ovary, such as some of the salmonellae. Gordon and Tucker (*43*) were able to demonstrate that *Salmonella* spp. have the ability to pass from the alimentary canal, via the blood, to the ovaries. Also, it has been suggested that in some instances, disease-causing bacteria may be excreted from the ovarian blood supply or may infect the tissue of the ovary itself (*50*). Other workers (*7,65*) have been unable to convincingly demonstrate transovarian transmission of *Salmonella* either by oral or intravenous inoculation. This conflict may be due to level of contamination, for it has been shown (*30*) that high levels of *Salmonella* in the food were required for them to become established in the gut. In this respect, contamination of drinking water was a more effective route for transmission (*30*).

Transovarian transmission has been definitely established in some species, however. These include *Staphylococcus aureus* and *Pasteurella haemolytica* (*65*) although the same workers were unable to show transmission of *Listeria monocytogenes* or *Pseudomonas aeruginosa*. Harry (*50*) found that contamination of the oviduct occurred from the cloacal region and the contaminants were dominated by *Micrococcus, Streptococcus,* and coli-aerogenes organisms. This author commented that the practice of artificial insemination (A.I.) appeared to introduce the risk of cloacal contamination unless care was taken in the collection and introduction of the semen. The possible explanation of cloacal contamination is that the microorganisms may be carried to the oviduct when the oviduct contracts (*81*).

It seems that most eggs receive their first load of contamination at oviposition and it may be considered, therefore, that the major (but not all) contamination of the egg is of external origin. The generalization that roughly 90% of newly-laid eggs are free from microorganisms is now generally accepted, and indeed the true figure may be even higher (*18,25*).

Contamination after laying

Once the egg has been laid, it is usually moistened and becomes soiled at the same time. The presence of dirt in the surrounding environment adds to the number of contaminating organisms (*20,51,82*). The only pathway through which bacteria can get into the interior part of the egg is via the pores. As the cuticle is moist at this stage, microbial invasion of the shell could conceivably occur (*18*). The humid condition promotes growth of molds on the surface of the shell (*91*). Growth of hyphae of molds facilitates the enlargement of pores, which helps the entry of bacteria into the egg (*110*). It has been reported by several investigators (*39,46*) that the shell is pervious to microorganisms such as *Escherichia coli, Salmonella paratyphi, Serratia marcescens* and *Pseudomonas aeruginosa*. Haines and Moran (*46*), for example, were able to draw *Saccharomyces allipsodeus* and species of *Pseudomonas* from the interior to the exterior side of the shell.

The degree of egg contamination appears to be a function of the cleanliness of the surface onto which they are laid (*51*), and the manner in which the eggs are handled after laying, which produces the greatest effect when the eggs become cracked (*29*). The level of contamination on the shell of eggs collected with gloved hands was less than that when the eggs were handled normally (*82*). According to Board (*14*), the level of contamination varies with the standard of hygiene. Environmental conditions such as temperature (*19,57*) and length of storage (*41*), as well as any treatment they receive such as washing (*62,67,88*) also exert an effect.

Contamination on the shell's surface also varies, both quantitatively and qualitatively in different geographical areas. See, for example, papers referring to India (*11,75*), Europe (*9,24*), North (*29,67*) and South (*61*) America. Despite this diversity, the bacteriology of spoiled eggs has remained relatively constant over time, geographical location and husbandry methods. This suggests that the major factors influencing the selection of rot-producing bacteria are intrinsic to the egg (*18*). The microflora of the egg shell is dominated by gram-positive bacteria which may originate from dust, soil or feces (*12,18*).

Rotten eggs normally contain a mixed infection of gram-negative bacteria and few gram-positive organisms. Some of the most common contaminants are members of the genera *Alcaligenes, Pseudomonas, Proteus* and *Aeromonas* (*18,48,76*) (see Table 1). This indicates that gram-negative bacteria are well equipped to overcome the antimicrobial defences of the egg. According to Board (*18*), the internal properties of the eggs favor growth of organisms which are gram-negative, have a relatively simple nutrition requirement and have the ability to develop at low temperatures.

TABLE 1. *The microflora of the hen's egg.*

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

[a]The more plus signs, the more frequent the occurrence (adapted from Board, *18*).

DEFENCE AGAINST BACTERIAL ATTACK

Two types of defences are involved in protection of egg contents from the invading microorganisms. These are physical defences and chemical defences.

Physical defences

The natural physical defences of the egg are the shell, the cuticle and the two shell membranes. The shell and the associated membranes may be considered to be the parts which act as the biggest barrier to penetration by bacteria and viral contaminants. Bacteria are the most important of these contaminants. The four physical defences will now be discussed in more detail.

The cuticle. Once the outer shell is formed, a protein layer is deposited over the surface of the shell, which is named the cuticle (bloom) (*96*). The cuticle is about 0.01 mm thick (*94*). The cuticle is an important covering that acts to interfere with bacterial invasion by closing the pores resulting in a reduction in the permeability of the shell (*35*). According to Fromm (*35*), abrasion of the shell, washing, humidity, ambient temperature and temperature changes influence the permeability after oviposition. Once the cuticle is removed, bacteria gain the ability to cross the shell more rapidly than before. Removal of the cuticle by abrasion of the shell (*35*), or by chemical treatment with E.D.T.A. (*103*) increases the permeability of the shell to bacteria. Similarly, eggs removed from the uterus before the cuticle had formed, and shell-less eggs, spoiled much faster than normal eggs (*103*). Some naturally-laid eggs are also without a cuticle (*17*), and this has commercial implications beyond the experimental manipulation of the cuticle. Vadehra et al. (*103*) concluded that the protection provided by the cuticle lasted at least 96 h after the eggs were laid. Moran and Hale (*68*) described the cuticle as a layer on the outer surface of the shell composed mainly of mucin cells. They found that the organic material which formed the outer cuticle was present in the lower part of the oviduct. Simkiss (*93*) was able to show the presence of protein in the cuticle, but a more detailed chemical analysis was reported by Wedral et al. (*109*). Recently two species of *Pseudomonas* have been isolated that can digest the cuticle (*21*). It is thought that these bacteria then allow yeasts to colonize the eggshell by utilizing bacteria as a food source, since the yeasts on their own could not grow on a cuticle substrate. Factors that affect the penetration of the cuticle include: (*a*) *Deposition of organic matter*. This varies among breeds, varieties, and among eggs laid by individual hens (*70*). (*b*) *Egg handling*. Careless handling and rubbing of the eggs may destroy the cuticle (*27,35,46,94*). Tyler (*100*) stated that, not only does removal of the cuticle encourage bacterial invasion, but it also results in a markedly increased loss of water from the internal contents of the eggs. The cuticle may be easily damaged shortly after oviposition. Such damage is more frequently noted when the birds are kept on wire floored cages, or have a chance to damage the egg by their claws or beaks (*102*). (*c*) *Length of storage*. As the egg ages, drying of the cuticle results in its shrinking, which may leave the pores more exposed (*89*). (*d*) *Fumigation*. Formaldehyde reacts with cuticle protein and removes some of the cuticle (*6,106*). (*e*) *Storage temperature*. As the temperature of the egg holding room increases, the quicker is the loss of cuticle (*70*). (*f*) *Egg cleaning*. Washing of soiled eggs in water, by hand or with a cloth, produces no apparent change in the structure of the cuticle, but may remove some of it.

The shell. The shell of a hen's egg is another barrier against bacterial penetration. The shell has numerous pores (7,000-17,000) per egg. These pores are not evenly distributed over the shell but more are found at the broad end than at the narrow end (*90,105*). The diameter of the pores varies throughout the shell. Haines and Moran (*46*) found the pore has a diameter of 13 μm at the top and 6 μm at the bottom. According to North (*70*), some pores are malformed and these are many times the diameter of most invading bacteria, which allows for easy penetration. Shell formation starts after the egg enters the isthmus when deposition of the mammilia takes place (*98*). The rest of the shell formation takes place in the shell gland over a period of 18-20 h.

Taylor and Martin (*97*) observed that the shell thickness is influenced by nutritional, environmental and hereditary factors. The shell of the first and last egg laid were thicker than those of eggs in the middle of the clutch (*10*). This observation was explained by the longer time the shell spend in the shell gland. The importance of shell thickness as protection against the invading microorganisms was studied by Sauter and Petersen (*86*). These authors showed that the shell thickness has a major effect on the ability of bacteria to pass through the egg shell. As the specific gravity of the egg is directly related to the shell thickness, it was used as a criterion for classifying the eggs. The resultant bacterial penetration of each group is shown in Table 2. It seems that shell thickness is more important in resisting shell penetration than is time. Only 21% of good quality shells had been penetrated after 24 h, while for poor quality shells this figure had been exceeded in less than 30 min.

TABLE 2. *The relationship between shell quality and bacterial penetration.*[a]

Specific gravity of Eggs	Shell quality	% Bacterial penetration of shell After 30 min	After 60 min	After 24 h
1.070	poor	34	41	54
1.080	average	18	25	27
1.090	good	11	16	21

[a]From Sauter and Peterson (*86*).

When considering bacterial penetration of the shell, Ferdinandov (*33*), quoted by Zagaevsky and Lutikova (*115*), reported that the egg shell is susceptible to bacterial penetration within a short time after laying. It was concluded that because the freshly laid egg cools after laying and that the yolk and white contract more than does the shell, water and microorganisms are drawn into the pores at this time (*33,64*). Vadehra et al. (*104*) observed that the blund end of the egg is more susceptible to spoilage than the other regions. Since the shell is thickest at the blunt end (*101*), shell thickness does not seem to determine which part of the egg is penetrated. They suggest that the suction effect when the air cell is formed and also, possibly, better environmental conditions in this region once the bacteria have penetrated, account for these results. They tentatively reject the hypothesis that the mechanism for bacterial penetration involves a permeability effect. The blunt end of the shell contains more pores and is more permeable than other regions (*90,105*), but penetration involves crossing the membranes as well as the shell. Other authors have reported a poor correlation between shell porosity and bacterial spoilage (*58,77*) so the suction effect when the air cell is formed would appear to be the major factor. Several authors have investigated bacterial penetration of the shell by immersing eggs in bacterial suspensions. They have identified a number of factors that affect penetration: (*a*) *The temperature differential between the egg and bacterial suspension* (*22,46,63*). Very little invasion of the egg will take place without a temperature differential (*64*). (*b*) *The number of organisms in the suspension*. The higher the number of bacteria, the greater the penetration (*22,28,53*). (*c*) *The period of immersion* (*22,53,84*). The longer the time, the higher the number of bacteria that penetrate. (*d*) *The treatment of the shell*. Contamination was increased when the shell was rubbed with material, such as cheese cloth, sandpaper or steel wool. Wiping the shell with cloth or brush moistened with bacterial culture will result in contamination of the egg's contents (*112*). (*e*) *Shell thickness*. Thin shells offer less resistance than thick ones (*73,86*).

Once the micro-organisms penetrate the shell to the shell membrane, they will be protected from any externally applied bactericide by proteinaceous material in the shell membrane (*79*). Thus disinfection probably cannot be expected to cure or correct the faults which are due to bad management.

The shell membrane. There are two membranes below the shell and they are held firmly together, except at the blunt end of the egg where they separate to enclose the air space. The inner membrane lies immediately over the albumen and the outer membrane is attached to the true shell. Moran and Hale (*68*) found that the outer membrane possessed three layers of fibers, while the inner membrane consisted of only two indistinct layers. For a chemical analysis of the membranes see Wedral et al. (*109*).

Many investigators have attempted to measure the thickness of the two membranes and their results are summarized in Table 3. For more details see the review by Simons (*95*). The results obtained differ not only from egg to egg, but also from region to region, within the same egg. Hence, Romanoff and Romanoff (*81*) stated that membrance thickness varies between the blunt end, the equatorial region and the pointed end. Hays and Sumbardo (*54*) reported that the membranes contain pores, the inner membrane containing more than the outer one. According to the observation of many workers, among them Haines and Moran (*46*) and Garibaldi and Stokes (*39*), the shell membane acts as a bacterial filter. It has also been suggested that it contains antibacterial substances (*25*). Many investigators demonstrated that the egg shell membranes were more impenetrable to bacteria than was the shell. Lifshitz et al. (*60*) reported that the inner shell membrane was the most effective in preventing bacterial penetration of the egg contents, the shell ranked second and the outer shell membrane was the least important. However, Garibaldi and Stokes (*39*) and Florian and Trussel (*34*) showed that the membranes are more important than the shell itself in preventing penetration of bacteria into the egg. These authors were able to show that speed of penetration of the shell membrane depends on the type of invading bacteria. The time taken to penetrate ranged between 1 and 4 d.

TABLE 3. *Thickness of egg-shell membranes according to various authors.*

Authors	Reference	Thickness (μm)		
		Outer membrane	Inner membrane	Total
Hays and Sumbardo	*54*	50	14	64
Romankewitsch	*80*	-	-	60-114
Moran and Hale	*68*	30-36	40-48	70-84
Wolken	*113*	-	-	60-70
Balch and Tyler	*8*	-	-	102-114

Although it is known that bacteria penetrate the shell via the pores, little is known about the mode of penetration through the inner shell membrane. Proteolysis of the membrane is thought to take place (*13,18,39,53*). Brown et al. (*26*) found some zones of hydrolysis surrounding the bacteria located in the membrane and such observations tend to support the theory that enzymes are actively involved in the penetration process. Wedral et al. have shown that most enzymes which they investigated produced no detectable changes in the permeability of the inner shell membrane to *Salmonella typhimurium* or radioactive lysine (quoted in *108*). They also faound that penetration of the inner membrane by bacteria produced no permanent increase in its permeability (*108*).

It seems from the study of the physical defences of the egg, that none of them possess bactericidal properties. For this reason, under certain conditions, bacteria are able to find their way into the egg contents.

Chemical defences

Although the albumen is not in direct contact with the microflora of the outer surface of the shell, it is liable to get contaminated. Contamination of the albumen occurs when the cuticle, shell and shell membranes fail to prevent microbial invasion. In addition to the chemical defences of albumen mentioned below, Board (*18*) reported other defence mechanisms. These are the viscous nature of the proteins which hinder movement of bacteria to the yolk and the organization of the chalazae in the albumen of fresh eggs which hold the yolk away from the sides of the shell membrane. Yadav and Vadehra (*114*) came to similar conclusions in that they thought that the physical structure of the egg white proteins determines its resistance to bacterial growth as well as its apparent lack of available water and nutrients. Changes during storage, such as the thinning of albumen, weakening of the chalazae and the vitalline membrane including the virtual removal of the outer layer (*85*) will tend to conteract these defences.

The main defence against microbial growth in the albumen is chemical (*16,23,55,72*). The chemicals involved are normally naturally occurring compounds although it has also been demonstrated that antibiotics fed to hens are rapidly incorporated into both the albumen and the yolk of their eggs (*2*). Of the naturally occurring compounds, lysozyme, conalbumen and the alkaline pH appear to be of primary importance (*16*), but ovomucin and ovinhibitor may also be involved (*23,74*).

The alkaline pH. The pH of the albumen of a hen's egg immediately after laying is typically 7.6 - 7.9; that is near the optimum for growth for most saprophytes, but there is a gradual increase in the pH value upon storage. This increase is due to loss of carbon dioxide. After about 1 week at room temperature, the pH increases to over 9 (*81*). This is beyond the maximum tolerated by many common micro-organisms.

When growth of bacteria in egg white at pH 9.1 - 9.3 was compared with growth in egg white adjusted to the pH in the egg at laying time, it was found by Garibaldi (*38*) that several strains of bacteria were unable to grow or survive at pH 9.1. Some survived if the pH was adjusted to 7.9, but there were several species of bacteria studied, including *Pseudomonas fluorescens, Proteus vulgaris* and *Alcaligens* sp. which were unable to grow, even at the more favorable pH. The author commented that there is some other factor still operating at this lower pH that causes destruction of the tested organisms.

It would appear that an egg white pH of over 9 has a germicidal effect on some of the invading microorganisms and the sensitivity of bacteria to this germicidal effect varies from one species to another.

Lysozyme. Lysozyme was reported by Board (*16,18*) and Geoffrey and Bailey (*40*) to have lytic action on the bacterial cell wall. It is capable of hydrolysing the (1-4) linkage between N-acetylneuramine and N-acetylglucosamine in the bacterial cell wall. This observation was demonstrated in vitro. Lysozyme may also act on some organisms because of the basicity of the molecule. It has been postulated (*69*) that a rise in pH to about pH 8 renders the bacterial cell more negatively charged so that in the presence of positively charged molecules, like lysozyme, a complex is formed agglutinating the cells. However, according to Board (*18*), no research workers have yet provided evidence that lysozyme activity would be of such importance in the defence against microbial attack within the egg itself. Furthermore, from some in vitro trials it was established that there are some microorganisms that can resist the activity of lysozyme, e.g. some naturally occurring strains of *S. aureus* (*47*), especially spore forming organisms, e.g. *Clostridium tyrobutyricum* (*107*). It is widely accepted, however, that the absence of gram-positive organisms in rotten eggs is due to their sensitivity to lysozyme (*83*). Indeed, some workers have even cautioned against the use of albumen derived from egg white in microbiological studies, because of the possibility of contamination with lysozyme (*3*).

Conalbumen. Many varieties of conalbumen were found in the eggs of birds (*5*). It represented about 10% of the total egg white solids (*1*). The activity of the conalbumen is dependent on hydrogen ion concentration (*38*). Conalbumen, the protein moieties of which are identical to those of transferrin, acts against microbial infection of the egg by depriving the microorganisms of Fe^{++} ions, which prevents their multiplication (*31*). Feeney et al. (*32*) noted different degrees of inhibition between tested microorganisms. *Micrococcus* spp. were more sensitive than *Bacillus* spp. and *Bacillus* spp. were more sensitive than gram-negative species. Schade (*87*) and Theodor and Schade (*99*) reported that the rate of growth was a function of the iron or conalbumen concentration in media. When water was artificially contaminated with iron salts and used for washing eggs, it resulted in a high incidence, and fast rate, of rotting of the washed eggs (*22*). These findings indicate that chelation of iron by conalbumen is an important factor in the egg's defence against microbial invasion.

However, when the invading microorganisms succeed in penetrating the physical barriers, they rapidly decrease in number (*81*). This may be attributed to the albumen component and inability of bacteria to utilize the proteins of the albumen. Albumen does become infected during this period but contaminants fail to multiply (*18*). Growth of bacteria in the albumen does not occur until the bacteria reach the yolk (*92*). According to Board (*12*) and Board and Ayres (*19*), renewed multiplication occurs when the yolk makes contact with the inner shell membrane. Board (*18*) reported that the albumen, under improper handling and adverse conditions, loses its rigidity, resulting in freedom of yolk movement. Such physical changes are associated with changes in some of the chemical defences of albumen (*111*).

Thus, in conclusion, it can be seen that the egg has a range of mechanisms, both physical and chemical, to protect the contents from bacterial invasion. In general, in the absence of gross mishandling by man, these defences are remarkably successful in preventing bacterial spoilage.

REFERENCES

1. Alderton, G., W. H. Ward, and H. L. Fevold. 1946. Identification of the bacteria inhibiting, iron-binding protein of egg white as conalbumen. Arch. Biochem. 11:9-13.
2. Anhalt, G., S. Wenzel, and P. Conrad. 1976. Modelluntersuchungen zur Rückstandsbildung im Hühnerei nach therapeutischer Verabreichung antibakteriell-wirksamer Substanzen. Arch. Lebensmittelhyg. 27:201-205.
3. Ashton, D. H., A. A. Wilson, and G. M. Evancho. 1975. Identification of a component of crystalline egg albumen bactericidal for thermophillic aerobic sporeformers. Appl. Microbiol. 30:821-824.
4. Ayres, J. C., and B. Taylor. 1956. Effect of temperature on microbial proliferation in shell eggs. Appl. Microbiol. 4:355-359.
5. Baker, C. M. A. 1968. The proteins of egg white. p. 67-108. *In* T. C. Carter (ed). Egg quality. A study of the hen's egg. Oliver and Boyd, Edinburg.
6. Baker, J. R., and D. A. Balch. 1962. A studyy of the organic material of hen's egg shell. Biochem. J. 82:352-361.
7. Baker, R. C., J. P. Goff, and E. J. Mulnix. 1980. *Salmonella* recovery following oral and introvenous inoculation of laying hens. Poult. Sci. 59:1067-1072.
8. Balch, D. A., and C. Tyler. 1964. Variation in some shell membrane characteristics over different parts of the same shell. Br. Poult. Sci. 5:201-215.
9. Baur, E. 1976. Ein Beitrag zum Keimgehalt von frischen Hühnereiern. Fleischwirtschaft 56:1237-1239.
10. Berg, L. R. 1945. The relationship of clutch position and time interval between eggs to egg shell quality. Poult. Sci. 24:555-563.
11. Bhargava, D. N., U. Mandokhot, R. S. Panghal, K. G. Naryan and I. P. Singh. 1975. Quantitative and qualitative microflora on the poultry egg shell surface. Indian J. Poult. Sci. 10:19-23.
12. Board, R. G. 1964. The growth of gram-negative bacteria in the hen's egg. J. Appl. Bacteriol. 27:350-364.
13. Board, R. G. 1965. The properties and classification of the predominant bacteria occurring in rotten eggs. J. Appl. Bacteriol. 28:437-453.
14. Board, R. G. 1966. The course of microbial infection of the hen's egg. J. Appl. Bacteriol. 29:319-341.
15. Board, R. G. 1968. Microbiology of the egg: A review. p. 133-162. *In* T. C. Carter (ed). Egg quality. A study of the hen's egg. Oliver and Boyd, Edinburgh.
16. Board, R. G. 1969. The microbiology of the hen's egg. Adv. Appl. Microbiol. 11:245-281.
17. Board, R. G. 1975. The microstructure of the cuticle-less shell of the eggs of the domestic hen. Br. Poult. Sci.16:89-91.
18. Board, R. G. 1977. The microbiology of eggs. p. 49-64. *In* W. J. Stadelman and O. J. Cotterill (eds.). Egg science and technology. AVI Publishing Co., Inc., Westport, Connecticut.

19. Board, R. G., and J. C. Ayres. 1965. Influence of temperature on bacterial infection of the hen's egg. Appl. Microbiol. 13:385-364.
20. Board, R. G., J. C. Ayres, A. A. Kraft, and R. H. Forsythe. 1964. The microbiological contamination of egg shells and egg packing materials. Poult. Sci. 43:584-595.
21. Board, R. G., S. Loseby, and V. R. Miles. 1979. A note on microbial growth on hen egg-shells. Br. Poult. Sci. 20:413-420.
22. Brant, A. W., and P. B. Starr. 1962. Some physical factors related to egg spoilage. Poult. Sci. 41:1468-1473.
23. Brendl, J., and S. Klein. 1976. Some natural substances in egg white as components of the antimicrobial barrier in the egg. (In Czech). Prumysl Potravin 27:467-469.
24. Brodnanova, O. 1976. Microbiology of shell eggs. (In Slovakian). Hydrinarsky Priemysel 18:83-89.
25. Brooks, J. and D. I. Taylor. 1955. Eggs and egg products. Spec. Rep. Food Invest. Bd. No. 60. H.M.S.O., London.
26. Brown, W. E., R. C. Baker, and H. B. Naylor. 1965. The role of the inner shell membrane in bacterial penetration of chicken eggs. Poult. Sci. 44:1323-1327.
27. Brown, W. E., R. C. Baker, and H. B. Naylor. 1966. Shell treatment as affecting microbial egg spoilage. Poult. Sci. 45:276-279.
28. Brown, W. E., R. C. Baker, and H. B. Naylor. 1966. Egg spoilage as affected by the route of exposure. Poult. Sci. 45:279-283.
29. D'Aoust, J. Y., P. Scotland, and C. J. Randall. 1980. *Salmonella* in "grade cracks" shell eggs. Can. Inst. Foods Sci. Technol. J. 13:184-187.
30. Dhillon, A. S., A. J. Maurer, R. H. Deibel, and R. W. Haller. 1974. Feeding of different levels of *Salmonella* to chickens. Ind. J. Poult. Sci. 9:103-107.
31. Donovan, J. W., R. A. Beardslee, and K. D. Ross. 1976. Formation of monoferric ovotransferrins in the presence of chelates. Biochem. J. 153:631-639.
32. Feeny, R. E., E. D. Ducay, R. S. Sliva, and L. R. Macdonnell. 1952. Chemistry of shell egg deterioration: The egg white proteins. Poult. Sci. 31:639-647.
33. Ferdinandov, V. V. 1944. Quoted by Zagaevsky and Lutikova (1944).
34. Florian, M. L. E., and P. C. Trussell. 1957. Bacterial spoilage of eggs. IV. Identification of spoilage organisms. Food Technol. 11:56-60.
35. Fromm, D. 1963. Permeability of the hen's egg shell. Poult. Sci. 42:1271.
36. Furuta, K., and S. Muruyama. 1981. Bacterial contamination of eggs during incubation and hatching and of fluffs of newly-hatched chicks. Br. Poult. Sci. 22:247-254.
37. Furuta, K., and K. Watanabe. 1978. Studies of the disinfection of hatching eggs. III. Hatchability of eggs disinfected by formaldehyde or certain kinds of disinfectant solution. Jap. Poult. Sci. 15:25-30.
38. Garibaldi, J. A. 1960. Factors in egg white which control growth of bacteria. Food Res. 25:337-344.
39. Garibaldi, J. A., and J. L. Stokes. 1958. Protective role of shell membranes in bacterial spoilage of eggs. Food Res. 23:283-290.
40. Geoffrey, P., and C. J. Bailey. 1975. The action of hen and goose lysozyme on the cell wall peptidoglycan of *Micrococcus lysodeikticus*. Biochem. Soc. Trans. 3:1212-1214.
41. Gesche, E., and A. M. Schuler. 1979. Bacterial flora of fresh eggs and eggs stored for four weeks (In Spanish). Alimentos 4:11-13.
42. Gillespie, J. H., and W. J. Scott. 1950. Studies in the preservation of shell eggs. IV. Experiments on the mode of infection by bacteria. Aust. J. Appl. Sci. 1:514-530.
43. Gordon, R. F., and J. F. Tucker. 1965. The epizootology of *Salmonella menston* infection of fowls and the effect of feeding poultry food artificially infected with *Salmonella*. Br. Poult. Sci. 6:251-264.
44. Hadley, P. B., and D. W. Caldwell. 1916. The bacterial infection of fresh eggs. Bull. Rhode Isl. Agric. Exp. Stn. No. 164:1-70.
45. Haines, R. B. 1939. Microbiology in the preservation of hens eggs. Rep. Food Invest. Bd. 47. H.M.S.O., London.
46. Haines, R. B., and T. Moran. 1940. Porosity of, and bacterial invasion through the shell of the hen's egg. J. Hyg., Camb. 40:453-461.
47. Hara, S., and Y. Matsushima. 1967. Studies on the substrate specificity of egg white lysozyme. 1. The N-acyl substituents in the substrate mucopolysaccharides. J. Biochem. 62:118-125.
48. Harry, E. G. 1957. The effect on embryonic and chick mortality of yolk contamination with bacteria from the hen. Vet. Rec. 69:1433-1439.
49. Harry, E. G. 1961. Surface disinfection of poultry buildings by formaldehyde and sulphur dioxide. Vet. Rec. 73:522-526.
50. Harry, E. G. 1963. Some observations on the bacterial content of the ovary and oviduct of the fowl. Br. Poult. Sci. 4:63-70.
51. Harry, E. G. 1963. The relationship between egg spoilage and the environment of the egg when laid. Br. Poult. Sci. 4:91-100.
52. Hartung, T. E., and W. J. Stadelman. 1962. The influence of metallic ions on the penetration of the egg shell membrane by *Pseudomonas fluorescens* Poult. Sci. 41:1590-1596.
53. Hartung, T. E., and W. J. Stadelman. 1963. *Pseudomonas fluorescens* penetration of egg shell membranes as influenced by shell porosity, age of eggs and degree of bacterial challenge. Poult. Sci. 42:147-150.
54. Hays, F. A., and A. H. Sumbardo. 1927. Physical characters of eggs in relation to hatchability. Poult. Sci. 6:196-200.
55. Kato, S., I. Suzuki, T. Ismii, and N. Yano. Effect of proteolytic enzyme on the antibacterial property of egg albumen. Agric. Biol. Chem. 41:727-731.
56. Kiefer, E. 1976. Mikrobiologie die Eier und Eiprodukte. Arch. Lebensmittelhyg. 27:218-223.
57. Kinner, J. A., and W. A. Moats. 1981. Effect of temperature, pH and detergent on survival of bacteria associated with shell eggs. Poult. Sci. 60:761-767.
58. Kraft, A. A., L. E. Elliott, and A. W. Brant. 1958. Shell quality and bacterial infection of shell eggs. Poult. Sci. 37:638-643.
59. Lancaster, J. E., and W. E. Crabb. 1953. Studies on disinfection of eggs and incubators. II. The value of formaldehyde gas with particular reference to the concentration resulting from the addition of formalin to potassium permanganate. Brit. Vet. Jour. 109:390-397.
60. Lifshitz, A., R. C. Baker, and H. B. Naylor. 1964. The relative importance of chicken egg exterior structures in resisting bacterial penetration. J. Food Sci. 29:94-99.
61. Lopez de Herrera, A. I. and L. G. Manrique. Bacterial counts and identification in eggs. (In Spanish). Revta. Inst. Colomb. Agropecu. 12:489-503.
62. Lorenz, F. W., and P. B. Starr. 1952. Spoilage of washed eggs. I. Effect of sprayed versus static water under different washing temperatures. Poult. Sci. 31:204-214.
63. Lorenz, F. W. P. B. Starr, M. P. Starr, and F. X. Ogasawara. 1952. The development of *Pseudomonas* spoilage in eggs. 1. Penetration through the shell. Food Res. 17:351-360.
64. MacLaury, D. W., and A. B. Moran. 1959. Bacterial contamination of hatching eggs. Kentucky Agric. Expt. St. Bull. 657:3-27.
65. Matthes, S., and J. Hanschke. 1977. Experimentelle Untersuchungen zur Übertragung von Bakterien über das Hühnerei. Berl. Munch. Tierärztl. Wschr. 90:200-203.
66. Miller, W. A., and L. B. Crawford. 1953. Some factors influencing bacterial population of eggs. Poult. Sci. 32:303-309.
67. Moats, W. A. 1980. Classification of bacteria from commercial egg washers and washed and unwashed eggs. Appl. Environ. Microbiol. 40:710-714.
68. Moran, T., and M. P. Hale. 1936. Physics of the hen's egg. 1. Membranes in the egg. J. Exp. Biol. 13:35-40.
69. Ng, H., and J. A. Garibaldi. 1975. Death of *Staphylococcus aureus* in liquid whole egg near pH 8. Appl. Microbiol. 29:782-786.
70. North, M. O. 1978. Commercial chicken production manual. AVI Publishing Co. Inc., Westport, Connecticut.
71. North, M. O. 1981. Egg quality is a major hatchability factor. The Jamesway Letter 2(1):1-4.
72. O'Leary, J., and F. F. Busta. 1974. Effect of food components on growth of *Bacillus stearotheromophilus*. J. Food Sci. 39:1157-1160.

73. Orel, V. 1959. The *Pseudomonas* spoilage of eggs laid by individual hens. Poult. Sci. 38:8-12.
74. Osuga, D. T., and R. E. Feeney. 1974. Avian egg white proteins. *In* I. E. Leiner (ed). Toxic constituents of animal foodstuffs. Academic Press, New York.
75. Panda, P. C. 1975. Salmonella contamination of shell eggs. Z. Bakteriol. Parasitenk. Infektionskrank. Hyg. II. 129:753-756.
76. Reid, W. M., T. A. Macy, F. M. Boyd, A. L. Kleckner, and S. C. Schmittle. 1961. Embryo and baby chick mortality and morbidity induced by a strain of *Escherichia coli*. Poult. Sci. 40:1497-1502.
77. Reinke, W. C., and R. C. Baker. 1966. Relationship between carbon dioxide permeability and bacterial penetration in chicken egg shell models. Poult. Sci. 45:1327-1334.
78. Rettger, L. F. 1913. The bacteriology of the hen's egg, with special reference to its freedom from microbic invasion. Storrs Agr. Expt. Sta. Bull. 75:191-213. (Quoted from MacLaury, D. W., and A. B. Moran 1959).
79. Rizk, S. S., J. C. Ayres, and A. A. Kraft. 1966. Disinfection of eggs artificially inoculated with *Salmonella*. 1. Application of several disinfectants. Poult. Sci. 45:764-769.
80. Romankewitsch, N. A. 1932. Die biomorphologischen Besondderheiten der Structur der Membrana Testae des Hühnereies. Z. Zellforsch. Mikroscop. Anat. 16:208-216. (Quoted by Simons, P.C.M. 1971).
81. Romanoff, A. L., and A. J. Romanoff. 1949. The avian egg. Wiley and Sons. New York.
82. Rosser, F. T. 1942. Preservation of eggs. 11. Surface contamination on egg shell in relation to spoilage. Can J. Res. 20D:291-296.
83. Salton, M. R. J. 1957. The properties of lysozyme and its action on microorganisms. Bacteriol. Rev. 21:82-99.
84. Sanz, P. B., D. I. Panuiga, and I. J. Tormo. 1973. Resistance of the external structures of eggs to microbial penetration. (In Spanish). Anales de Bromatologia 25:275-295.
85. Sato, Y., and T. Kato. 1978. Changes of chalazae and vitelline membrane during storage of chicken egg. Intern. Congr. Food Sci. Technol. Abstr. P168.
86. Sauter, E. A., and C. F. Petersen. 1974. The effect of egg shell quality on penetration by various salmonellae. Poult. Sci. 53:2159-2162.
87. Schade, A. L. 1963. Significance of serium iron for the growth, biological characteristics, and metabolism of *Staphylococcus aureus*. Biochem. Z. 338:140-148.
88. Scheibner, G., and F. Fehlhaber. 1977. Untersuchungen über die Inaktivierung von Mikroben auf der Schahenoberflache aussortierter Hühnereies. Mh. Vet. Med. 32:226-230.
89. Schmidt, W. J. 1961. Über das Schleimhautchen der Vogeleischale, insbezondere bei Phoenicopterus, Aptenodytes und Spheniscus. J. Orn. Lpz. 102:456-467. (Quoted by Simons, P.C.M. 1971).
90. Schoorl, P., and G. Mos. 1968. Porositeit-gewichtsverlies en porienaantal - bij kippe-en eende-eieren. Pluimveeonderzoek, Communication no. 155. Central Institute for Poultry Research 'Het Spelderholt', Beekbergen, the Netherlands. (Quoted by Simons, P.C.M. 1971).
91. Sharp, P. F., and G. F. Stewart. 1936. Effect of relative humidity on growth of molds on eggs in storage. Mem. Cornell Univ. Agric. Exp. Stn. No. 91:1-11.
92. Sharp, P. F., and R. Whitaker. 1927. The relation of hydrogen ion concentration of egg white to its germicidal action. J. Bacteriol. 14:17-46.
93. Simkiss, K. 1958. The structure of the egg shell with particular reference to the hen. PhD Thesis, University of Reading, U.K.
94. Simkiss, K. 1968. The structure and formation of the shell and shell membranes p. 3-25. *In* T. C. Carter (ed.). Egg quality. A study of the hen's egg. Oliver and Boyd, Edinburg.
95. Simons, P. C. M. 1971. Ultrastructure of the hen's egg shell and its physiological interpretation. PhD Thesis, Agricultural University, Wageningen, The Netherlands.
96. Szielasko, A. 1913. Die Bedeutung der Eischalenstruktur der Vogel fur die Systematik. J. Orn. Lpz. 6:52-117. (Quoted by Simons, P.C.M. 1971).
97. Taylor, L. W., and J. H. Martin. 1929. Factors influencing thickness of egg shell. Poult. Sci. 8:39-44.
98. Taylor, T. G. 1970. How an egg shell is made. Sci. Am. 222:89-95.
99. Threodore, T. S., and A. L. Schade. 1965. Carbohydrate metabolism of iron-rich and iron-poor *Staphylococcus aureus*. J. Gen. Microbiol. 40:385-396.
100. Tyler, C. 1945. The porosity of egg shells and the influence of different levels of dietary calcium upon porosity. J. Agric. Sci. Camb. 35:168-176.
101. Tyler, C. 1961. Studies on egg shell. XVLL. Variation in membrane thickness and in true shell nitrogen over different parts of the same shell. J. Sci. Fd. Agric. 12:470-475.
102. Tyler, C., and N. Standen. 1969. The artificial production of translucent streaks on egg shells and various factors influencing their development. Br. Poult. Sci. 10:359-369.
103. Vadehra, D. V., R. C. Baker, and H. B. Naylor. 1970a. Role of cuticle in spoilage of chicken eggs. J. Food Sci. 35:5-6.
104. Vadehra, D. V., R. C. Baker, and H. B. Naylor. 1970b. Infection routes of bacteria into chicken eggs. J. Food Sci. 35:61-62.
105. Walden, C. C., I. V. F. Allen, and P. C. Trussell. 1956. The role of egg shell membranes in resisting the entry of microorganisms. Poult. Sci. 35:1190-1196.
106. Walker, J. R. 1944. Formaldehyde. Reinold, New York.
107. Wasserfall, F., and M. Teuber. 1979. Action of egg white lysozyme on *Clostridium tyrobutyricum*. Appl. Environ. Microbiol. 38:197-199.
108. Wedral, E. M., D. V. Vadehra, and R. C. Baker. 1971. Mechanism of bacterial penetration through the eggs of *Gallus gallus*. 2. Effect of penetration and growth on permeability of inner shell membrane. J. Food Sci. 36:520-522.
109. Wedral, E. M., D. V. Vadehra, and R. C. Baker. 1974. Chemical composition of the cuticle and the inner and outer shell membranes from the eggs of *Gallus gallus*. Comp. Biochem. Physiol. 47B:631-640.
110. Weston, W. A. R. D., and E. T. Halnan. 1927. "Black spot" of eggs. Poult. Sci. 6:251-258.
111. Wilcox, F. H. 1955. Evidence for an association between lysozyme level and the quality of egg white. Poult. Sci. 34:1170-1172.
112. Wilson, J. E. 1945. Infected egg shells are a means of the spread of salmonellosis in chicks and ducklings. Vet. Rec. 57:411-413.
113. Wolken, J. J. 1951. Structure of hen's egg membranes. Anat. Rec. 111:79-84.
114. Yadav, N. K., and D. V. Vadehra. 1977. Mechanism of egg white resistance to bacterial growth. J. Food Sci. 42:97-99.
115. Zagaevsky, J. S., and P. O. Lutikova. 1944. Sanitary measures in the egg breaking plant. U.S. Egg Poult. Mag. 50:17-20, 43-46, 75-77, 89-90, 121-124.