Antibacterial Properties of an Egg

Pages with reference to book, From 185 To 187

Altaf Ahmed (Department of Microbiology, The Aga Khan University Hospital, Karachi.)

Introduction

Reports of outbreaks of salmonella enteritidis food poisoning associated with consumption of hen eggs or egg products have appeared with increasing frequency all over the world[1-3]. The main causative organism was salmonella enteritidis phage type IV. Spread of infection was from an important new source, contents of intacthen eggs. The proportion of eggs that are infected internally is very low indeed but because millions of eggs are consumed daily the number of human infections represent an important public health problem. In the United States of America, late in 1986, veterinary investigators demonstrated that trans-ovarian infection with salmonella enteritidis phage type VIII can lead to human food poisoning from shell eggs3. As eggs are a major portion of every day diet, there are many studies on the process of Infection of shell egg and its natural defence systems. The first systematic investigation on microbial deterioration of eggs intended for human consumption was conducted by Gayon in 1873[4]. Haines In 1939 stated that egg is equipped with physical and chemical defence against microbial infection and suggested that, these have evolved to protect the embryo during. incubation4. Brooks and Taylor in 1955[4] considered that the rotting of market eggs. occurs when the defence are overloaded. In 1966 Board discussed the, events which take part In rotting of an egg4.. In, his view there were two 'ways of infections of an egg, congçnital and extra-genital.

Congenital

It is known since a long time that organisms placed In the oviduct remain viable for 48 to 72 hours[4] and that salmonellals one of the organisms that can infect ovaries. There is abundant evidence that salmonella spp. passes from alimentary canal to, reach the ovaries via blood stream[4].

Extra-genital Infection

Brooks and Taylor[4] in 1955 observed that less than 1% of naturally clean eggs rot after prolonged storage. It was also noted that gram negative bacteria are detected most frequently in contents of Incubated eggs[5,6]. This observation indicates that' gram negative 'bacteria are better equipped to overcome the antimicrobial defence of egg compared to gram positive ones. This is probably due to the fact that the cell wall of gram negative bacteria has an outer membrane which resists action of lysozyme and also' because large protein molecules' cannot penetrate this outer membrane.

Major Defence Mechanism of Egg

There are two major components of antimicrobial defence system in egg:

1. Physical dçfence provided by egg integument.
2. Chemical defence present In albumen.

1. Physical defence

A. The Sheli

The shell of a domestic hen's egg contains 7000-17000 pores with diameters In the range '9-35 micrometers (Figure la).

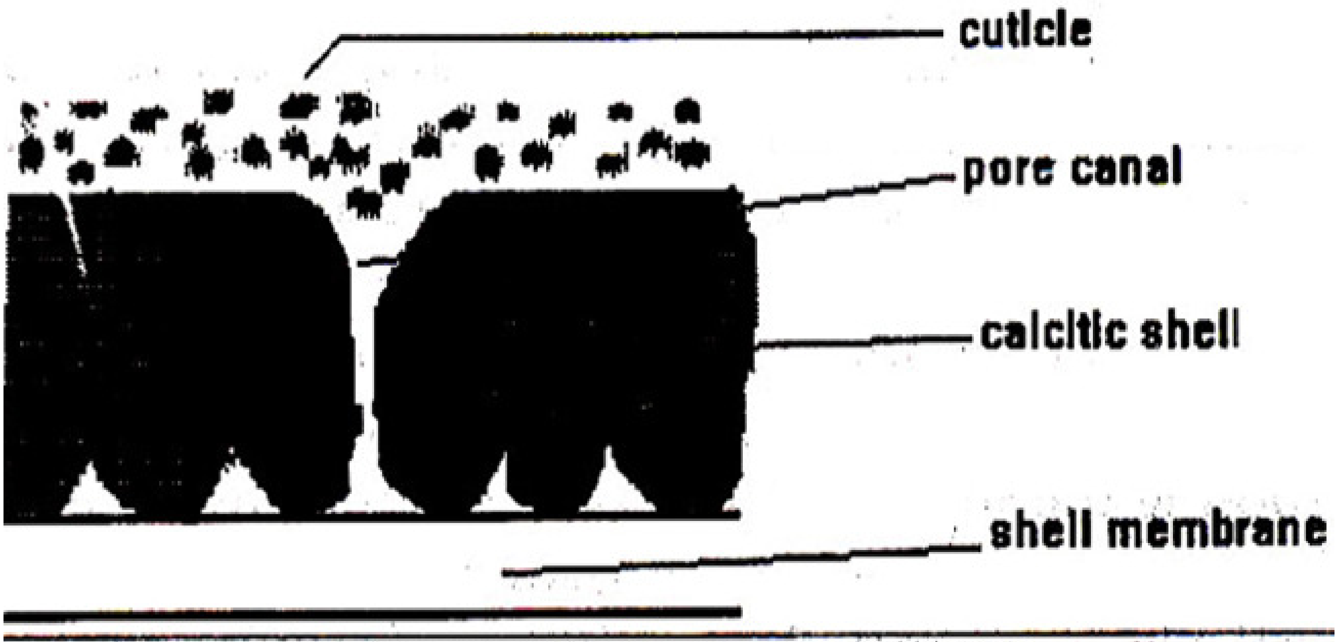

Figure 1 (a). Longitudinal section of egg shell.

Microorganisms at outer surface of shell can penetrate the shell barrier if the warm eggs are suddenly Immersed in cold water. As the contents of eggs contract more than its shell, a pressure difference develops which draws the bacteria inside alongwith water through these pores. Although cuticle on the surface of an egg provides good protection from water it is usually destroyed by chemicals and physical means such as washing, scraping or rubbing of egg[7,8].

B. Shell Membrane

It consists of an outer membrane, an inner membrane and a limiting membrane. Limiting membrane separates the inner 'membrane from' albumen[9]. Electron microscopy has Shown that both inner 'and outer shell membranes are composed of a network of fibres. The concept of shell membranes acting as bacterial filters was introduced for the first time in 1940, 'by Haines and Moran[4]. This rotation has' been repeatedly confirmed in later studies[10]. In conclusion, Studies have proved that shell plus membrane offer greater resistance to bacterial penetration than the shell alone. However, investigations[11,12] done later indicated that shell membranes impede movement of bacteria only temporarily. Once a bacteria crosses the barrier of shell and membranes the viscosity o albumen hinders its attempt to reach the vulnerable part of yolk.

Chemical Defence System

Chemical defence system of the egg is provided by the proteins present in its albumen. Albumen consists of 10% proteins and $0- 90% water. List of some major proteins of egg albumin is given In Table I and II. The properties of these proteins and their role in antimicrobial defence are detailed below: Wurtz[13] was the first person to discover germicidal property of egg white. His conclusion was based upon the fact that typhoid bacillus failed to survive in egg albumen. First detailed study on egg white was carried out by Laschtdchcnko. He observed lysis of vegetative cells and spores of bacillus spp[4].

Lysozyme

In 1922 Fleming[4] suggested that lysis of bacteria is caused by Iysozyme, an enzyme present In egg albumin. As a test organisms, Fleming used Micrococcus Lysodelkticus because it was easily lysed. Later studies showed that lysozymes is not a bacteriocidal agent, but it initiates events of cell death by breaking the cell wall and thus exposing the weak cell membrane to the environment. Although the lytic action of lysozyme in albumin[14] has been demonstrated with lysozyme Sensitive bacteria, there is no direct evidence that it plays an Important role in protecting avian eggs against infection. It Is also possible that lysozyme is involved in the physical rather than chemical defence of egg. Lysozyme combines with ovomucin and forms anetworkwhich confers viscosity to egg white and thus creating a distinct albuminous sac (Figure ib)

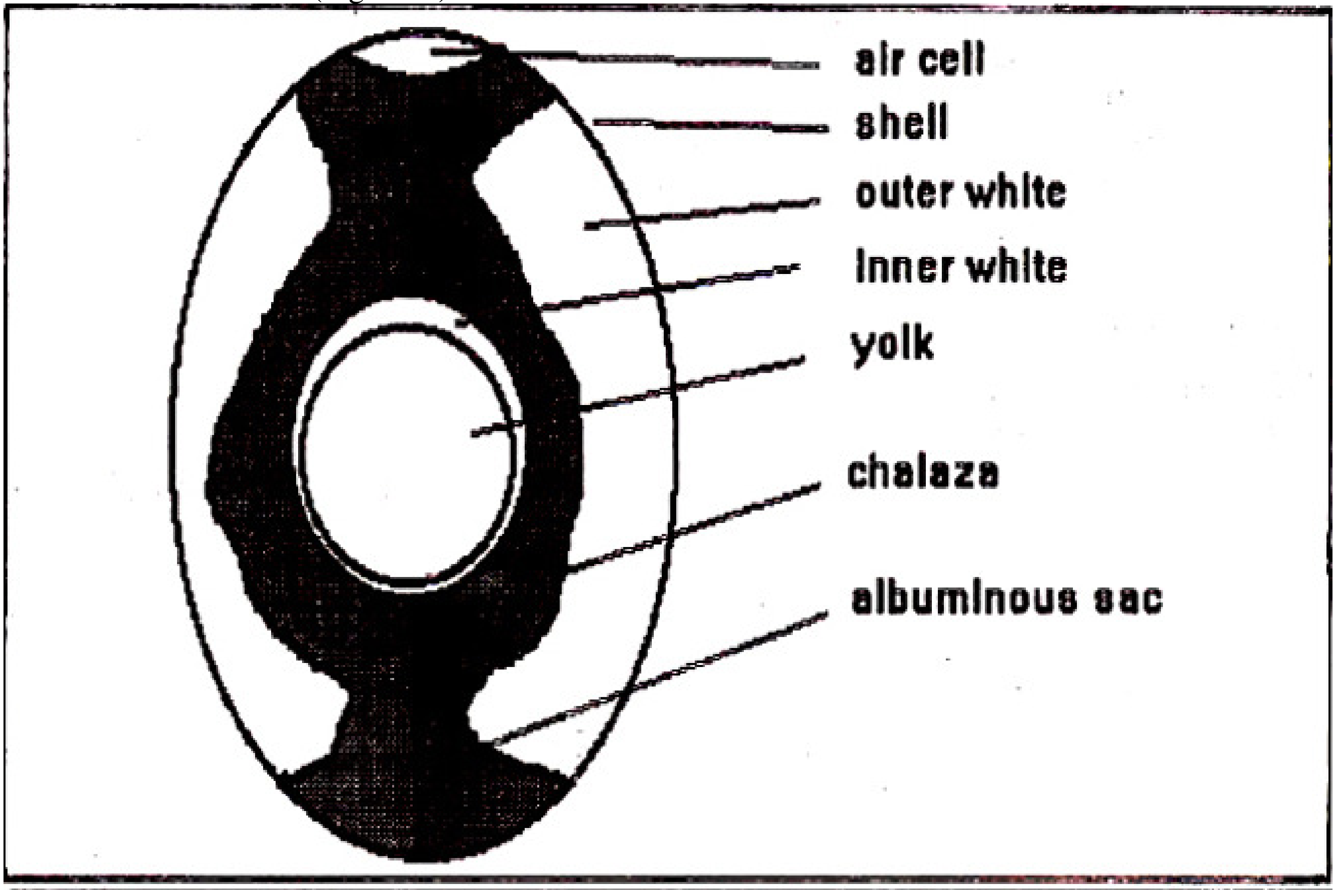

Figure 1 (b). Longitudinal section of hen's egg.

which protects the yolk from any nidus of infection in the shell membrafle[15,16]. It Is therefore, reasonable to assume that lysozyme contributes to antimicrobial systems of egg white via two mechanisms: Lysis of sensitive organisms and more importantly maintenance of the albuminous sac.

Conalbumin (Ovotransferrin)

Albumin contains an iron binding agent conalbumin[4,17]. It Is a glycoprotein and constitutes about 12% of total egg white. It is seen that depriving microorganism of iron causes inhibition of their growth[18]. Extensive bacterial growth does not occur in vitro unless ovotransferrin is quenched with ferric ions[13] or its action is negated by iron transport compounds[19].

Avidin

This forms a non-digestible complex with biotin such that the microorganism cannot utilize It.

Apoprotein

This combines with riboflavin, thereby making it unavailable to microorganism.

Ovomucoid

There are different types of this substance. All of them inhibit trypsin.

Ovoinhibitor

This Inhibits fungal proteases.

Albumin as a Growth Medium

In addition to the action of glycoproteins present in the albumin, the ph of albumin (9.0-10.0) is inimical to many organisms[4] and accentuates ferric ion chelation by ovotransferrin. Majority of researchers agree that ovotransferrin and alkalinity are primarily responsible for the failure of vegetative bacterial cells to grow in albumen[6,11]. It is also seen that effectiveness of enterochelin (a major iron chelating agent of enterobacteriaceae) is diminished by alkaline hydrolysis. In 1984 Tranter and Board studied the influence of pH (alongwith temperature) on antimicrobial properties of egg albumin[16,20]. They proved that bactericidal properties of albumen could be neutralized by changing its pH from 9.0 or above to 7.5 or below. They observed that at 39.5°C, enterochelin allowed the growth of escherichia coli in albumen atpH 7.9 but not at pH 9.4, whereas iron allowed growth at both pH valves. Their study showed that gram positive bacteria (staph. epidermidis, staph. aureus and strep. faecalis including lysozyme resistant strains and yeasts) were killed In egg albumin with or without iron at 30.39.5°C. The albumin was more toxic at 39.5°C for grain negative bacteria (E. coil, salmonella, proteus, enterobacteria), with the exception of pseudomonas fluorescence, acinetobacter and proteus vulgaris. Presence of iron protected these bacteria from being killed in albumin and promoted their growth at 39.5°C. The result of a study by Tranter and Board[17] confirm the observation of Sharp and whitaker[4] that egg albumin was bactericidal at pH 9.0 but bacteriostatic at pH 6.0-6.8. It is now clear that high alkalinity of egg albumin interferes with bacterial iron metabolism by preventing them from obtaining sufficient iron for growth. It has also been established[6] that an abrupt change in temperature (cold shock) or in pH (alkaline shock) of medium causes chemical damage to the bacteria. It should be appreciated that antimicrobial defence system do not cause lysis of contaminates of albumin during embryogenesis[15]. The reason for this is that the breakdown of gram negative bacteria releases pharmacologically active lipopolysaccharide and lipoproteins and such substances are toxic to the cells of the embryo itself. Electrophoretic studies[21] and lysozyme assays[22] support the view that albumin remains an unfavourable medium for microbial growth atleast until it is swallowed by the embryo. Major proteins of egg albumin are shown in Table I.

Table I. Major proteins of egg albumin.

Protein	Amount in albumin (%)
Ovalbumin	54
Ovotransferrin (conalbumin)	12
Ovomucoid	11
Lysozyme	3.4
Ovomucin	3.5
Ovoinhibitor	1.4
Ovoflavoprotein	0.8
Avidin	0.05

The Yolk

Yolk is the principal food reserve of an egg. It is protected from infection by the interaction of physical defence afforded by egg integument and chemical composition of albumin. Egg yolk is a rich source of nutrients and contains a number of substances in it (Table II) comprising mainly of fat, protein and water.

Table II. Composition of an egg.

Constituents	Egg white (%)	Egg yolk (%)
Water	87.6	49.8
Protein	11.1	16.3
Fat	0.2	31.9
Carbohydrate	0.7	0.3
Ash	0.4	1.7

Table III. Mineral composition (ppm).

Minerals	Egg white	Egg yolk
Phosphorus	1000	5900
Sulphur	1960	1570
Potassium	1600	1100
Sodium	1600	700
Chlorine	1500	1000
Calcium	100	1300
Magnesium	100	1300
Iron	1	86
Copper	0.85	4.15
Manganese	-	1.13

Along with these it also has a number of minerals (Table III) and some vitamins like vitamin D, vitamin A, vitamin B1 and Biotin. Riboflavin is the only vitamin present In egg white23. It is because of this highly nutritious property of yolk that It Is a very good medium for growth of microorganisms and vulnerable to Infection.

Conclusion

In conclusion it can be said that the growth of any type of bacteria in albumin is hindered by an interplay of alkaline pH, temperature variation, iron deprivation, lysozyme and lack of adequate amounts of non-protein nitrogenous compounds. On the other hand, egg yolk has highly nutritious properties and is protected very well by integument and albumen.

References

1. Annonymous. Omeletteswithout broken eggs? (editorial). Nature, 1988;336:699-700.
2. Chapman, P.A., Rhodes, P., Rylands, W. Salmonella typhimurium phsge type 141 infection in Sheffield during 1984 and 1985. Communicable Disease Report, 1987;15:3-4.
3. St. Louis, M.E., Morse, D.L., Potter, ME. et sl. The emergence of grade A eggs as s major source of salmonella enteritidis infections. JAMA., 1988;259;2103-7.
4. Bosrd, R.G. The course of microbial infection of the ben's egg, J. AppI. Bacteriol., 1966;29:319-41.
5. Harry, E.G. The effect on embryonic and chick mortality of yolk contamination with bacteria from the hen, vet Rec., 1957;69:1433-39.
6. Reid, W.M., Mscy, T.A., Boyd, F.M., Klechner, AL and Schmittle, S.C Embryo snd babycbick mortalityand morbidityinduced bya strainofE. coil Poult. Sci. 1961;40:1497.
7. Board, R.G. and Halls, N.A. The cuticle, s barriertowaterand partideuptakebytheshell of the hen's egg. British Poult. Sci.,1973;14:69-97.
8. Board, R.G. The avisn shell-s resistance network. J. Appl. Bacteriol., 1980;48:303-13.
9. Tyler, C Studies on egg shell Variation in membrane thickness and in tbe true shell nitrogen overdifferent pan of ssmeshell. 3. Sci. FoodAgric., 1961;12:470-75.
10. Garibaldi, J.A. and Stokes, 3.L. Protective role of shell membranes in bacterial spoilage of eggs. Food Rn., 1958;23:283- 90.
11. Brooks, 3. Mechanisms of multiplicstion of pseudomonss in the hen's egg, 3. Appl. Bacteriol., 1960;23:499-509.
12. Board, R.G. and Ayres, J.C The influence of temperature on bacterial infection of the hen\'s egg. AppL Microbiol, 1965;13:358-64.
13. Tranter, H.S. and Board, ItO. The antimicrobial defences of avian eggs. Biological perspective and chemical basis. 3. AppI. Biochem., 1982;4:295-338
14. Garibaldi, J.A. Factors! in egg white which control growth of bacteria. EL. Res., 1960;25:337-44.
15. Board, R.G. and Fuller, R. Nonspecific antimicrobial defences of tbe avian egg, embryo and neonate. Biol. Rev., 1974;49:15-49.
16. Salton, M.R.J.The properties of lysozyme and its sction on microorganisms. Bact. Rev., 1957;21:82-96.
17. Tranter, H.S. and Board, R.G. The influence of incubation, temperstureand pH on the antimicrobial properties of hen egg albumin. J. Appl. Bscteriol., 1984;56:53 61.
18. Theodore,T.S.and Schade, A.L. Growth of staphylococcus aureus in medisofrestricted and unrestricted inorganic iron availability.). Gen. Microbiol., 1965;39:75-83.
19. Garibaldi, J.A. Role of microbial iron transport compounds in the bacterial spoilage of eggs. Appi Microbiol., 1970;20:558-60.
20. Ayrea, J.C. and Taylor, B. Effects oftemperstureon microbial proliferation in shell eggs. Appi. Microhiol., 1956;4:356-59.,
21. Marshall, M.E. and Deutsche, H.F. Some protein changes in fluid of the developing chicken embryo.). Biol., Chem., 1950;185:155- 61.
22. Greenfield, 3. and Biglsnd, CH. Detection of lyaozyme in developing avian embryos. Pouls. Sci., 1971;50:1748-53.
23. George Borgatrom. Food microbiology and biochemistry. In principles of food science, 1968;2:34-35.