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THE MICROSTRUCTURE OF THE HEN'S EGG SHELL - A SHORT REVIEW[1]

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Abstract

The structure of the hen's egg shell can be divided into five separate layers. The innermost layer consists of two distinct membranes known as the inner and outer shell membranes. These membranes are composed of networks of protein/polysaccharide fibres and are ≈70 μm thick. Attached to the outer fibres of the outer membrane are polycrystals of calcite (calcium carbonate) which extend outward in an inverse conical manner until the cones from several sites of crystal initiation fuse together. The fibre/crystal attachment sites, known as basal caps, and the cones form the mammillary knobs layer which is ≈100-110 μm thick. After the cones fuse with each other, continuing calcite deposition produces columnar crystals 10-30 μm in diameter and ≈200 μm in length. These crystals form the palisade layer and are intermingled with a protein/polysaccharide matrix that differs in composition from the shell membranes. Over the columnar crystals is a thin layer (≈5-8 μm thick), known as the vertical crystal layer, of small calcite crystals that are orientated perpendicular to the shell's surface. The cuticle is the outermost layer of the shell; it is ≈10 μm thick and contains predominantly protein. Passing vertically through the palisade layer of the shell from "valleys" between the mammillary knobs to the surface of the vertical crystal layer are funnel-shaped, unbranched pores. These pores are capped by the cuticle which is cracked and thus allows the diffusion of gases between the contents of the egg and its environment. The geometrical configuration of the cones in the mammillary knobs layer is related to the thickness of shell. Specific amino acids in the membrane fibres of the basal caps influence shell strength.

KEY WORDS: Egg shell, microstructure, shell membranes, mammillary knobs, palisade/matrix, cuticle.

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Introduction

The hen's egg shell surrounds the biological material essential to the reproduction of the species (Fig. 1A). Besides providing mechanical

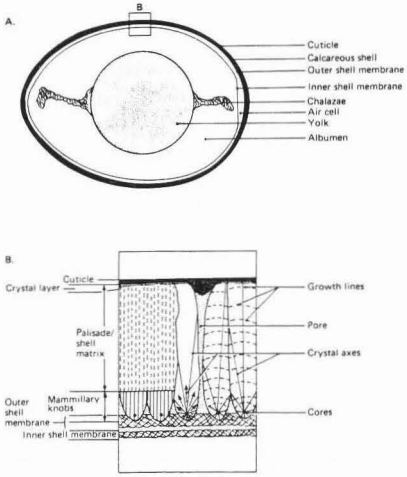

Fig. 1. Schematic diagrams of the principal components of the hen's egg (A) and of the layers that constitute the shell (B). These diagrams are not drawn to scale. Figure B based on diagrams from Schmidt (1962), Simkiss (1968), Tyler (1969a) and Simons (1971b).

protection to the egg's contents or the developing embryo, the shell is a barrier to microorganisms, a reservoir of calcium for the embryo, a permeable membrane that facilitates the exchange of respiratory gases between the egg and its

environment without excessive dehydration and is, for wild birds, a camouflage (Board, 1982).

Interest in the egg shell dates back to at least the time of Aristotle (384-322 BC). According to Tyler (1969a), Purkinje published one of the first detailed studies of the egg shell in 1825. In a series of over 30 "classical" papers from work done between 1821 and 1899 as a hobby, the industrialist von Nathusius demonstrated clearly that the avian egg shell is a complex, highly ordered structure composed of a calcified matrix superimposed on fibrous membranes. Even though von Nathusius had only a simple microscope, his drawings rival in detail photomicrographs obtained with modern light and electron microscopic techniques. The original papers of von Nathusius have been translated into English and edited by Tyler (1964) and include detailed line drawings of the shell structure of eggs from about 130 species, including the domestic hen. More recently, Simons (1971a,b) published the results of a comprehensive study of the hen's egg shell and membranes involving light, and transmission and scanning electron microscopy. This monograph also contains an extensive review of previously published data.

Reviews on the morphological aspects of the hen's egg shells have been published by Stewart (1935), Burmester (1940), Simkiss (1968), Tyler (1969a) and Parsons (1982). Microscopic studies of egg shell formation have been reported by, among others, Simons (1971a), Hoffer (1971), Draper et al. (1972), Creger et al. (1976), Stemberger et al. (1977), Pooley (1979) and Makita (1981). In addition, reviews have been published on shell calcification (Wilbur and Simkiss, 1968; Eastin and Spaziani, 1978a,b), organic components of the shell (Krampitz and Witt, 1978; Krampitz, 1982; Leach, 1982) and the relationship between shell structure and egg shell breakage (Parsons, 1982; Washburn, 1982).

It is now generally accepted that the shell of the hen's egg (Fig. 2) contains at least five distinct layers as indicated schematically in Figure 1B. These layers are 1) inner and outer shell membranes, 2) mammillary knobs, 3) palisade/shell matrix, 4) vertical crystals, and 5) the cuticle (Simons, 1971a). Because this presentation is a tutorial, the discussion will be limited to the major structural and biochemical aspects of these layers. Little evidence will be presented to confirm these aspects since, in some cases, the data is extensive.

Egg and Shell Formation

The following brief description of egg and shell formation is given to indicate the specific area of the hen's reproductive tract and the time required for the synthesis of these layers. The size of the yolk (Fig. 1A) present in the ovary of the hen increases rapidly over a period of 10-14 days before ovulation. During this time its weight increases from about 200 mg to 15-18 g through the incorporation of proteins and lipids that are synthesized mainly in the liver (Redshaw and Follett, 1972). After the yolk or ovum is mature, it is released from the ovary (ovulated) and begins its descent of the hen's reproductive tract where the albumen and shell

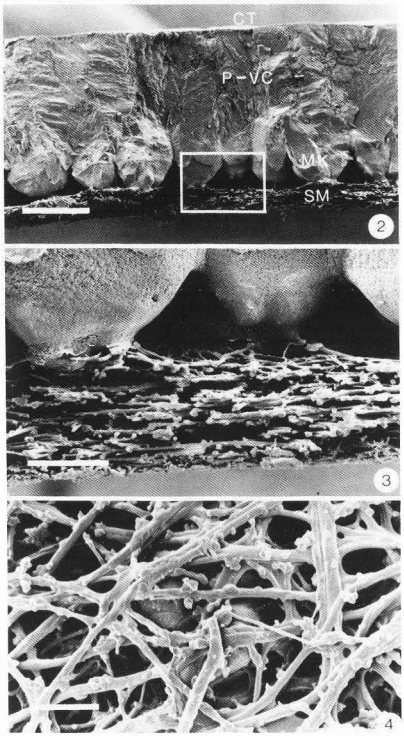

Fig. 2. Radially fractured surface of the shell from a white-shell egg showing the shell membranes (SM), mammillary knobs (MK), palisade and vertical crystals layer (P-VC) and cuticle (CT). Shell section was sputter coated with gold and examined with a scanning electron microscope (EMR 100A; 10 kV). Bar = 100 µm.

Fig. 3. Enlargement of the rectangular section in Fig. 2 showing the shell membranes and mammillary knobs. Bar = 10 µm.

Fig. 4. Outer fibres of the outer shell membrane viewed towards the mammillary knobs in the background. The overlying membrane material was stripped manually. Section was prepared and examined as described in Fig. 2. Bar = 10 µm.

are formed. The average time between successive eggs is 24-25 hours for high producing birds.

The reproductive tract consists of at least five distinct regions, namely: 1) infundibulum, 2) magnum, 3) isthmus, 4) uterus or shell gland, and 5) vagina. The time taken and the component added to the egg during passage through the reproductive tract of the hen are summarized in Table 1. Shell color pigments are incorporated within the last 5 hours of shell deposition (Warren and Conrad, 1942). The brown pigment that colors the eggs from some hens is porphyrin synthesized by the shell gland from δ-amino levulinic acid (Polin, 1957). Further information may be obtained from Robinson (1972) and Gilbert (1971) for albumen synthesis and Simkiss and Taylor (1971), Creger et al. (1976) and Reed and Yurkiewicz (1982) for shell formation.

Table 1. Site of Formation of Egg and Shell Components[1]

Section of Reproductive System	Time Developing Egg Spends in Section (hr)	Components Produced or Added
Ovary	-	Yolk
Infundibulum	0.25-0.5	(Site of fertilization)
Magnum	3	Albumen as a gel
Isthmus	1.25	Water and minerals that dilute the albumen; and shell membranes
Uterus (shell gland)	20-21	Thin albumen and minerals, shell, shell pigments and cuticle.
Vagina		Passage to exterior for the developed egg
Cloaca		

[1]Adapted from Geiger et al., 1974.

Gross Composition and Physical Characteristics of Eggs

The hen's egg contains about 58% albumen, 32% yolk and 10% shell by weight, with the first two components containing approximately 76 and 24% water, respectively (Gilbert, 1971). About 96% of the material in the shell is inorganic and 4% organic (Geiger et al., 1974); the latter is composed of a number of discrete proteins, glycoproteins and glycosaminoglycans. Calcium carbonate constitutes 98% of the inorganic material while magnesium carbonate and calcium phosphate contribute equally to the remainder; trace amounts of other inorganic ions are present.

Numerous factors influence egg size (weight) such as the genotype of the hen, age of hen, position of the egg in a sequence, rate of lay, environmental temperature, diet and disease status (Gilbert, 1971). The data presented in Table 2 show the means and variability in egg and shell characteristics for white- and brown-shell eggs laid by mature hens (Thompson et al., 1981). Characteristics such as shell thickness, compression fracture strength and deformation vary among eggs at a particular site (Table 2) and also among sites on a single egg. For example, the shell is thickest at the equator and thinnest at the narrow pole; thickness at the blunt end is intermediate between the narrow pole and equator (Tyler, 1961; Tyler and Geake, 1965).

Table 2. Means and Standard Deviations of Egg and Shell Characteristics for White- and Brown-Shell Eggs (30 Eggs per Sample)[1]

Characteristics	Mean (Standard Deviation) White-Shell	Brown-Shell
Egg weight, g	65.2 (4.4)	65.0 (5.6)
Length, mm	60.6 (2.2)	59.5 (2.5)
Diameter, mm	43.8 (1.0)	44.1 (1.4)
Shell weight, g	5.91 (0.4)	5.36 (0.5)
Shell thickness[2], μm	334 (21.7)	324 (20.7)
Compression fracture strength[3], N	29.7 (4.2)	29.5 (5.2)
Deformation[4], μm	63.4 (7.4)	75.2 (15.9)

[1]Adapted from Thompson et al., 1981.
[2]Measured at the equator (diameter) of the eggs.
[3]Compression fracture force applied at 20.0 mm/min to the equator of the egg. N = Newtons.
[4]For an applied load of 1.0 kg.

Shell Membranes

Even though the membranes located on the inner surface of the egg shell appear to be a single layer (Fig. 3), they can be divided, by careful manipulation, into two distinct layers of fibrous material. They adhere closely to each other except at the blunt end of the egg where they are separated by the air cell (Fig. 1B). One layer surrounds the albumen while the other is attached to the "tips" of the calcified material of the shell (Fig. 3); these layers are known as the inner and outer shell membranes, respectively.

Microscopic examination of these layers reveals an interwoven meshwork of fibres (Fig. 4) arranged in a random manner (Stewart, 1935; Moran and Hale, 1936; Kaplan and Siegesmund, 1973; Creger et al., 1976; Wong et al., 1984). The fibres of the inner membrane are about one half as thick as those in the outer membrane (22 vs 48 μm; Simons, 1971a). The thickness of the shell membranes vary within an egg and among eggs (Balch and Tyler, 1964). Electron microscopy studies of Masshoff and Stolpmann (1961) showed that each fibre of the membranes had a central core that was fine and fibrillar in structure and this core was surrounded by a sheath. Delicate strands of material spanned the gaps present between the core and the sheath. Fibres from the inner and outer membranes were similar in structure, except the former were

thinner. Draper et al. (1972) described the material in the core as "electron dense" and that in the sheath as "less dense" substance. Simons and Wiertz (1963) and Hoffer (1971) reported similar observations for membranes from hen's eggs and Japanese quail eggs, respectively. Frequently the mantle of adjacent fibres coalesce, giving the appearance of extensive branching of the fibres (Masshoff and Stolpmann, 1961; Fig. 4). Also, evident on these fibres are small protuberances (see Creger et al., 1976; Leach, 1982; Fig. 4), which can be useful when studying the relationship of composition to shell structure such as occurs with dietary copper and manganese deficiencies (Baumgartner et al., 1978; Leach and Gross, 1983; respectively).

Chemical analyses indicate that the shell membranes in their native state contain about 20% water, 75-76% proteinaceous material and 4-5% carbohydrate (Gilbert, 1971). The nature of the proteinaceous material of the shell membranes remains to be resolved. Some researchers have concluded that these membranes contain keratin or ovokeratin (Simkiss and Taylor, 1971) whereas others consider the proteins as collagen-like due to the presence of hydroxyproline (Balch and Cooke, 1970) and hydroxylysine (Candlish and Scougall, 1969). An elastin-like protein has been suggested also to occur in the shell membranes (Simkiss and Tyler, 1957; Harris et al., 1980). The keratinous nature of the membrane proteins has been questioned because of differences in structure, amino acid content, and protein solubility of soft and hard keratins and the proteinaceous material from shell membranes (Wedral et al., 1974; Vadehra et al., 1971). Similarly, results from amino acid analysis, radioimmunoassay and enzymatic hydrolysis led to the conclusion that elastin is not present in the shell membranes (Starcher and King, 1980; Leach et al., 1981; Crombie et al., 1981) even though desmosine and isodesmosine, lysine-derived molecular crosslinks found in elastin, occur in the hydrolysates of the membranes (Baumgartner et al., 1978; Harris et al., 1980; Leach et al., 1981). Wong et al. (1984) demonstrated the presence of both membranes of two forms of collagen-like proteins, similar to collagen I and V, in the ratio of approximately 100:1. Immunofluorescence microscopy indicated that within each membrane, collagen I was associated predominately with the large, coarse fibres (≈2.5 μm diameter) and the collagen V with the delicate, narrow fibres (≈0.6 μm diameter). At the electron microscopic level, the normal 67-nm banding usually seen with type I collagen was not present in either the large, coarse, or the delicate, narrow fibres.

The simple sugars represent 70-80% of the carbohydrate present in the shell membranes (Balch and Cooke, 1970). Glucose, galactose, and mannose were reported by both Balch and Cooke (1970) and Wedral et al. (1974). However, the former also reported the presence of fructose and the latter xylose; the reason for the discrepancy is not evident. Both research groups found sialic acid in small amounts. Balch and Cooke (1970) also found small quantities of glucosamine and galactosamine, but due to the absence of uronic acid they proposed that the galactosamine was not associated with chondroitin sulphate. Abatangelo et al. (1978), however, reported the presence of 0.5% uronic acid in the shell membranes.

Lipids have been isolated in small quantities from the shell membranes by Hasiak et al. (1970a,b) and Britton (1977). These included mono, di and triglycerides, free fatty acids, cholesterol and its esters, lecithin, lysolecithin, cephalin and sphingomyelin (Hasiak et al., 1970a,b). Trace amounts of sodium, potassium, manganese, zinc, copper, boron and aluminum were found to be present in both the inner and outer shell membranes by Wedral et al. (1974). Calcium which was also observed was thought to have a structural role.

Mammillary Knob Layer

In the mammillary knob layer, the calcified portion of the egg shell connects with the fibres of the outer portion of the outer shell membrane (Fig. 1B and 3). The part of the mammillary knob layer that is embedded in the outer membrane fibres is termed the basal caps while the portion above and between these membrane fibres and the palisade layer is known as the cone layer (Tyler, 1965). The membrane fibres penetrate at least 70 μm into the palisade layer (Simons, 1971a). Removal of the membrane by sodium hydroxide (5% w/v) or protease digestion revealed microscopic tracts as evidence of attachment of the mammillary knobs in the fibres (Kaplan and Siegesmund, 1973; Stevenson, 1980; respectively). The appearance of the mammillary knobs after the removal of the membranous fibres is sometimes described as "rose-bud". About one-third of the shell thickness is accounted for by the mammillary knob layer (Burmester, 1940).

The calcified portion of the shell is composed of small individual crystallites (Simons, 1971a) of calcium carbonate in the form of calcite (Terepka, 1963a). These individual crystallites radiate in all directions from the centre of the mammillary core where they attach to the membrane fibre. According to Schmidt (1965), growth of individual crystallites, sometimes known as spherulites (Wilbur and Simkiss, 1968), inwards to the outer membrane is impeded by the resistance of the outer membrane fibres. Their growth is further decreased by a reduced flow of calcifying material from the uterine tissue due to more rapid growth of crystals immediately adjacent to that tissue. As the crystals grow outward from the basal caps, they form irregular polygonal cones and eventually fuse with those from adjacent formations to complete the cones of the mammillary knob layer. The crystallites that grow into membranes are also known as eiosphérites and those that grow outward from the membranes as exopherites (Tyler and Fowler, 1978).

Electron microscopic studies indicate that the structure and spatial configuration of the mammillary knob layer is an important determi-

nant of shell strength. El-Boushy et al. (1968), Robinson and King (1970), King and Robinson (1972) and Bunk and Balloun (1978) observed that the mammillary layer was frequently disorganized in shells of low strength compared to those of high strength. The number of mammillary knobs per square centimeter was higher for shells of low breaking strength than those of high strength, the former shells were thinner than the latter (307 vs 356 μm) (van Toledo et al. 1982). These results support the findings of Tyler and Fowler (1978) that the shell thickness of eggs from wild birds was highly correlated ($r = 0.94$) to the "nearest neighbour" distance between mammillary cones. Leach and Gross (1983), however, found that although the shell impact strength of eggs from manganese deficient hens was lower than for those from non-deficient hens, the former shells had fewer and wider mammillary knobs per unit area than the latter.

Simkiss and Tyler (1957) showed, by histochemical techniques, that the concentration of organic material was higher in the mammillary cones than elsewhere in the mammillary knob layer. Subsequently, Robinson and King (1968) demonstrated that the organic portion of the mammillary cones contained mainly neutral mucopolysaccharides. The results of Cooke and Balch (1970a) confirm the histochemical observations of Robinson and King (1968) and provide quantitative data that about one half of the carbohydrate material present in the mammillary cones was glucosamine.

The chemical composition of the fibres in the mammillary cones also affect shell strength. The amino acid content of the outer portion of the outer membrane influences the shell strength of eggs from young and old hens (Britton and Hale 1977; Blake et al. 1985). Methionine, serine, phenylalanine and aspartic acid along with lysine (Britton and Hale, 1977) and proline (Blake et al., 1985) were found to influence shell deformation and egg specific gravity; deformation and specific gravity are two methods commonly used to estimate egg shell strength (see Hamilton, 1982). On the other hand, Frank et al. (1965) reported that there was little relationship between the ratio of the membranes to total shell and shell breaking strength.

Palisade/Shell Matrix and Vertical Crystal Layers

The palisade layer begins at the point where the individual cones from adjacent formations fuse with each other in the mammillary knob layer, and extends to within about 8 μm of the shell's surface (Fig. 1B; Simons, 1971a). These layers account for about two-thirds of the overall thickness of the egg shell (Stewart, 1935; Burmester, 1940). Interwoven among and through the crystals of the palisade layer is a matrix of organic material (Simons, 1971a,b; Pooley, 1979) which Terepka (1963b) described as having a herring-bone appearance. The vertical crystal, also called the surface crystal, layer is deposited on top of the palisade layer; this layer varies in thickness between 3 and 8 μm. Removal of the shell membranes, by boiling in a 5% (w/v) sodium hydroxide solution, and of the cuticle layer with 5% (w/v) ethylenediaminetetraacetic acid (EDTA, disodium salt at pH 7.5-8), produces the "true" shell as defined by Tyler (1969a). The residue that remains after the "true" shell has been decalcified with 20% EDTA (pH 7.5-8) is known as the "true" shell matrix (Tyler, 1969a).

Detailed studies of the crystalline structure of the egg shell have shown that the palisade layer contains individual columnar crystals of calcium carbonate as rhombohedral calcite (Schmidt, 1962; Terepka, 1963a). Each column of calcite consists of crystallites with reported diameters of 10-15 μm (Terepka, 1963a) to 20-30 μm (Perrott et al., 1981). Both Terepka (1963a) and Schmidt (1964) concluded from microscopic studies with polarized light that the orientation of c-axis of the hexagonal unit cell of the crystallites in the palisade layer was perpendicular to the surface of the egg shell. On the other hand, Cain and Heyn (1964) reported that the c-axis of the calcite crystals in the palisade layer was inclined 28° ± 16° from the perpendicular to the shell surface in X-ray diffraction studies. From a limited X-ray diffraction study, Favejee et al. (1965) observed that the c-axis of calcite in egg shell was randomly arranged and on the average was inclined 45° to the shell's surface. Sharp and Silyn-Roberts (1984) point out that c-axis angles reported by Cain and Heyn (1964) are improbable because of the manner in which they were determined. In contrast, Perrott et al. (1981) found that, while the c-axes of the crystallites were inclined at angles between 12° and 48° from the perpendicular to the surface within a crystal column, there was no evidence from chemically thinned specimens of a preferred orientation among the columns in the palisade layer as a whole. However, when specimens were thinned with an ion beam some preferred orientation within a single crystal was observed, such that the c-axis lay within a range of 30 ± 18° from the perpendicular to the surface. Sharp and Silyn-Roberts (1984) showed by X-ray diffractometry that a preferred orientation developed gradually throughout the shell of hen's eggs, beginning at the shell membranes and reaching a maximum at the exterior surface. Two of 20 shells examined exhibited a single preferred orientation in which the pole of the (001) plane was perpendicular to the shell's surface; whereas, a second preferred orientation, (104) plane, occurred simultaneously with the (001) plane in the remaining eggs. This second orientation occurred only in the outer half of the shell where the (104) pole was perpendicular to the surface. The data reported by Favejee et al. (1965) was interpreted by Sharp and Silyn-Roberts (1984) also to indicate the presence of crystals in two preferred orientations. In another study by Silyn-Roberts and Sharp (1985a) found that the (001) preferred orientation was characteristic of the shells of eggs from ratite and tinamou species of birds and the (104) orientation appeared in the shells of only a small proportion of the species and then only as a sec-

ondary development. This phenomenon was found also to occur in the shells of reptilian eggs by Silyn-Roberts and Sharp (1985b).

Simons (1971a,b) and Pooley (1979) observed that the calcite crystals in the vertical crystal layer were small with their greatest dimension at approximately right angles to the shell's surface. Perrott et al. (1981) also found that the preferred orientation of the crystals in the vertical crystal layer was parallel to the surface.

Holes with diameters of about 0.4 μm are present in the decalcified preparations of the true shell. These holes, known as vesticular holes (Simons, 1971a,b), are apparently more numerous in the mammillary knobs than in the palisade layer. Sometimes minute crystals lie close to the holes (Simons, 1971a; Heyn, 1963) which are air filled spaces (Pooley, 1979).

The organic material that constitutes the matrix of the calcified portion of the shell consists of a protein/polysaccharide complex (Simkiss and Tyler, 1958; Baker and Balch, 1962; Cooke and Balch, 1970a,b; Heaney and Robinson, 1976). This complex contains at least 70% protein and 11% polysaccharide along with a small amount of fat (Baker and Balch, 1962). The matrix protein is characterized by the absence of hydroxyproline (Baker and Balch, 1962; Frank et al., 1965), a low content of aromatic and sulphur amino acids and a ratio of about 2-to-1 for dicarboxylic amino acids to basic amino acids (Baker and Balch, 1962). Frank et al. (1965) reported a moderate correlation (r = 0.88) between the amino acid composition of the shell matrix and that of non-collagenous protein isolated from porcine hyaline cartilage. Chondroitin sulphates A and B, and hyaluronic acid that contained equal molar amounts of glucosamine and galactosamine, were identified in the shell matrix by Baker and Balch (1962), Cooke and Balch (1970b) and Heaney and Robinson (1976), respectively. Small amounts of sialic acid were reported by Frank et al. (1965) and Cooke and Balch (1970b), but when the shell matrix material was analyzed directly, no measurable sialic acid was found in the water soluble residue obtained from the matrix (Abatangelo et al., 1978; and Cortivo et al., 1982). Other carbohydrates reported to be present in the shell matrix include mannose, glucose, fucose and xylose (Cooke and Balch, 1970b).

Cooke and Balch (1970b) demonstrated an uneven distribution of the organic matrix material within and among the crystalline material of the shell. They found its concentration increased to maximum in a region about two-thirds of the distance from the inner membrane and then rapidly decreased towards the shell's surface. These results do not support the postulate of Tyler and Geake (1958) or the conclusion of Carter (1969) that the organic content of the "true shell" or "incremental shell", respectively, is constant.

It is interesting to note the decrease in the acid solubility of the calcified material in the region of the true shell observed by Cooke and Balch (1970b) occurred in approximately the same region Sharp and Silyn-Roberts (1984) found the second preferred orientation in the crystalline structure started to develop in the majority of the shells they examined. Simons (1971b) observed, by electron microscopy, that fractures in the egg shell follow the layers of organic material in the palisade layer; consequently, the presence of both radial and tangential fractures in his photomicrographs may be due to the influence of the organic matrix material on the preferred orientation of crystals in the palisade layer. Both Simkiss and Tyler (1958) and Simons (1971a) postulate that the deposition of organic material precedes that of the inorganic material. Frank et al. (1965) found little evidence that the amino acid content of the shell matrix influences shell breaking strength.

Terepka (1963b) suggested an inverse relationship between the concentration of shell matrix material and mineralization. Simkiss and Tyler (1958) hypothesized that the shell matrix acts as a chelating agent probably due to the mucoitin sulphuric acid, or hyaluronic acid as it is presently known. Abatangelo et al. (1978), however, demonstrated that the egg shell matrix could bind calcium ions only if carboxylic side chain groups were available. Subsequently, Cortivo et al. (1982) isolated a peptide that was resistant to alkaline hydrolysis. About 50% of the amino acid residues in this peptide were aspartic and glutamic acids which were found to be important in the binding of calcium ions. These researchers found no γ-carboxyglutamic acid present in the shell matrix contrary to the results of Krampitz et al. (1980). Blake and Kling (1984) demonstrated that in vivo decarboxylation of γ-carboxyglutamic acid had no significant effect on shell strength when measured by specific gravity.

Histochemical staining of the shell matrix indicates the presence of the enzyme carbonic anhydrase (Robinson, 1970) which has subsequently been isolated (Krampitz, 1982). This enzyme catalyzes the reversible reaction:

$$CO_2 + HOH \rightleftharpoons H^+ + HCO_3^- \qquad (1)$$

(Robinson and King, 1963) and is considered to be involved in shell calcification of the hen's egg (Krampitz and Witt, 1978).

In addition to calcium carbonate as calcite in the true shell, phosphate and magnesium occur in the outer part of the shell (Itoh and Hatano, 1964; Simons, 1971a). Small amounts of potassium, sodium, iron, copper, manganese, sulphur and zinc are present in the shell but their location and purpose is not known (Simons, 1971a). The magnesium content in the shell has been demonstrated to increase from the membranes to the shell's surface (Brooke and Hale, 1955; Simons, 1971a). However, Board and Love (1983) found, from electron probe microanalysis across the radial axis of the egg shell, the occurrence of a narrow band of magnesium-rich shell at the mammillary knobs layer and, after an initial decrease, a progressive increase in magnesium concentration to a maximum at the outer surface of the shell. According to the results of Smith

et al. (1954) most of the phosphate(s) present in the shell was also located in the outer part.

Cuticle

Cuticle is the term used to identify the layer of organic material that is deposited over the vertical crystal layer of the egg shell (Simons, 1971a,b); it is sometimes called the "bloom". The thickness of the cuticle was found by Simons (1971a) to vary between 0.5 and 12.8 μm among eggs and at different sites on the same egg. Many star-shaped crack systems are apparent with scanning electron microscopy (Simons, 1971a,b); these cracks in the cuticle contain vesicles up to about 1 μm. Large crack systems tend to be oval in shape and have clearly defined edges (Simons, 1971a); they are considered to represent the surfaces of oval pore plaques. Small crack systems also occur in the cuticular material on the surface of the egg's shell. The microscopic appearance of the cuticular material changes with time due possibly to drying and oxidation of sulphydryl (-SH) and disulphide (-S-S-) groups present in the protein(s) of the cuticle. At the time the egg is laid, the cuticle may be easily disrupted mechanically, especially if it comes into contact with the wire floor of a cage, or the claws or beak of the hen (Denison, 1967; Tyler and Standen, 1969; Talbot and Tyler, 1974). The areas where the cuticle has been disrupted are translucent in appearance, contain more water and are weaker than the surrounding shell material (Tyler and Geake, 1964). The presence of translucent areas on the egg shell is known as mottling and is more evident in eggs that have been stored possibly because of shrinkage of the cuticle over time.

The cuticle of the shell is considered to be important in the waterproofing of the avian egg (Board and Halls, 1973). However, these researchers found that of 453 brown shelled eggs examined, 3.5% had no demonstrable cuticle and another 8% had no cuticle at either the pointed or broad end of the egg. Belyavin and Boorman (1980) found that removal of the cuticle from the shell with EDTA significantly reduced their thickness, specific gravity and nondestructive deformation which are factors associated indirectly with shell strength.

Chemical analysis indicates that the cuticle contains 85-90% protein (Wedral et al., 1974; Baker and Balch, 1962) most of which is insoluble in water or potassium chloride solution (1% (w/v); Wedral et al., 1974), 4-5% carbohydrate (Wedral et al., 1974; Cooke and Balch, 1970a), 2.5-3.5% lipid (Wedral et al., 1974) and 3-3.5% ash (Wedral et al., 1974; Baker and Balch, 1962). Based on paper or column chromatographic procedures, Baker and Balch (1962) and Wedral et al. (1974), respectively, showed that aspartic and glutamic acids, glycine and arginine were the major amino acids in the proteinaceous material of the cuticle. The major carbohydrate moieties reported present in the shell cuticle include galactose and mannose (Baker and Balch, 1962; Wedral et al. 1974), glucose (Cooke and Balch, 1970a; Wedral et al., 1974), fucose (Baker and Balch, 1962), xylose (Wedral et al., 1974), and sialic acid (Baker and Balch, 1962; Wedral et al., 1974). Although galactosamine has been detected in cuticular material by Cooke and Balch (1970a), the absence of uronic acid was considered to indicate that acid mucopolysaccharides were not present. In addition to the presence of sulphur and phosphorus (≈2 and 0.1 mg/g, respectively), Wedral et al. (1974) reported that the cuticle contains aluminium, boron, copper, iron, magnesium, manganese, potassium, sodium and zinc (0.01-4.0 μg/g) and calcium (≈ 25 μg/g).

Sometimes the cuticles of eggs from young broiler-type hens may be covered with a layer (≈ 45 μm thick) of calcified material especially if oviposition has been delayed due to the presence of two eggs in the shell gland at the same time (Simons, 1971a). The chemical composition of the "cover" material is similar to that in the palisade layer.

Pores

Passing vertically through the palisade layer of the shell from the "valleys" between the mammillary knobs (Board et al., 1977) to the surface of the vertical crystal layer are funnel-shaped pores (Fig. 18; Tyler, 1956). These pores in the shells of eggs from domesticated hens are unbranched and capped by the organic material of the cuticle (Board et al., 1977). Each pore varies in width from 15 to 65 μm at the surface to between 6 to 23 μm at the inner levels of the shell (Tyler, 1956). Scanning electron microscopy indicated that the walls of the pores are rough but without a definite ultrastructure and contain no fibrous or crystalline material in their lumen (Tullett et al., 1975). Simkiss (1961) found that the pores of eggs removed directly from the oviduct were filled with sulphur-containing protein. The opening at the shell surface is plugged with cuticular material that contains cracks which allows the diffusion of respiratory gases.

The distribution of pores in the egg shell of the domestic hen is not random (Tyler, 1965; 1969b). It is estimated that there are between 100 and 300 pores/cm^2 of shell surface (Gilbert, 1971). There is a strong positive linear correlation ($r = 0.92$) between the number of pores/cm^2 and mammillary knobs/0.25 mm^2 which Tullett (1975) speculated may be due to the distribution of cells in the uterus that secrete the sites on which calcification is subsequently initiated. Tyler and Fowler (1978) postulated that when three or more cones having approximately circular cross sections meet at the beginning of the palisade layer a "channel" will be formed between them and this channel might, under certain circumstances, continue through the palisade layer to form a pore. In addition, Tyler (1956) speculates that liquid might pass through these "channels" of the forming shell into the egg, but gradually some of the channels become blocked by the developing crystals until shell deposition is completed.

As a result, only a relatively small proportion of the intitial channels would remain as pores when the egg is laid.

Future Research

Even though the hen's egg shell is less than 400 μm in thickness, it has a complex microstructure that consists of a "composite" material. Further research is needed to establish the interrelationships among the inorganic and organic constituents that form this composite material, the preferred orientation of the crystals of the shell and the strength of an egg's shell, especially at the microstructure level. Composition of the organic matrix material present in the palisade and vertical crystal layers should be reinvestigated with modern analytical techniques. The mechanism involved when the egg shell fractures due to the application of forces slowly or rapidly also requires elucidation. Additional studies are required to increase our understanding of the "biology of egg shell formations" so that it can be explained in terms of biochemical and physiological processes. In order to resolve this problem, the expertise of such groups as electon microscopists, biochemists, material science engineers and poultry scientists are needed.

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References

Abatangelo G, Daga-Gordini D, Castellani I, Cortivo R. (1978). Some observations on the calcium ion binding to the eggshell matrix. Calcif. Tiss. Res. 26, 247-252.

Baker JR, Balch DA. (1962). A study of the organic material of hen's egg shell. Biochem. J. 82, 352-361.

Balch DA, Cooke A. (1970). A study of the composition of hen's eggshell membranes. Ann. Biol. Anim. Biochim. Biophys. 10, 13-25.

Balch DA, Tyler C. (1964). Variation in some shell membrane characteristics over different parts of the same shell. Brit. Poultry Sci. 5, 201-215.

Baumgartner S, Brown DJ, Salevshy Jr E, Leach Jr RM. (1978). Copper deficiency in the laying hen. J. Nutr. 108, 804-811.

Belyavin CG, Boorman KN. (1980). The influence of the cuticle on egg-shell strength. Brit. Poultry Sci. 21, 295-298.

Blake JP, Kling LJ. (1984). Examination of the potential role of γ-carboxyglutamic acid in eggshell quality. Poultry Sci. 63, 981-986.

Blake JP, Kling LJ, Halteman WA. (1985). The relationship of the amino acid composition of a portion of the outer eggshell membrane to eggshell quality. Poultry Sci. 64, 176-182.

Board RG. (1982). Properties of avian egg shells and their adaptive value. Biol. Rev. 57, 1-28.

Board RG, Halls NA. (1973). The cuticle: A barrier to liquid and particle penetration of the shell of the hen's egg. Brit. Poultry Sci. 14, 69-97.

Board RG, Love G. (1983). Magnesium distribution in avian egg shells with particular reference to those of wild fowl (Anatidae). Comp. Biochem. Physiol. 75A, 111-116.

Board, RG, Tullett, SG, Perrott HR. (1977). An arbitrary classification of the pore systems in avian egg shells. J. Zool. (Lond.) 182, 251-265.

Britton WM. (1977). Shell membranes of eggs differing in shell quality from young and old hens. Poultry Sci. 56, 647-653.

Britton WM, Hale Jr KK. (1977). Amino acid analysis of shell membranes of eggs from young and old hens varying in shell quality. Poultry Sci. 56, 865-871.

Brooke J, Hale HP. (1955). Strength of the shell of the hen's egg. Nature (Lond.) 175, 848-849.

Bunk MJ, Balloun SL. (1978). Ultrastructure of the mammillary region of low puncture strength avian eggshells. Poultry Sci. 57, 639-647.

Burmester BR. (1940). A study of the physical and chemical changes of the egg during its passage through the isthmus and uterus of the hen's oviduct. J. Exp. Zool. 84, 445-500.

Cain CJ, Heyn ANJ. (1964). X-ray diffraction studies of the crystalline structure of the avian egg shell. Biophys. J. 4, 23-39.

Candlish JK, Scougall RK. (1969). L-5-hydroxylysine as a constituent of the shell membranes of the hen's egg. Int. J. Protein Res. 1, 299-302.

Carter TC. (1969). The hen's egg: Relationship between shell thickness and the amount of organic matter in the shell. Brit. Poultry Sci. 10, 165-174.

Cooke AS, Balch DA. (1970a). Studies of membrane, mammillary core and cuticle of the hen egg shell. Brit. Poultry Sci. 11, 345-352.

Cooke AS, Balch DA. (1970b). The distribution and carbohydrate composition of the organic matrix in hen egg shell. Brit. Poultry Sci. 11, 353-365.

Cortivo R, Castellani I, Martelli M, Michelotto G, Abatangelo G. (1982). Chemical characterization of the hen eggshell matrix: Isolation of an alkali-resistant peptide. J. Chromatography 237, 127-135.

Creger CR, Phillips H, Scott JT. (1976). Formation of an egg shell. Poultry Sci. 55, 1717-1723.

Crombie G, Snider R, Faris B, Franzblau C. (1981). Lysine-derived cross-links in the egg shell membrane. Biochim. Biophys. Acta 640, 365-367.

Denison JW. (1967). The effect of mechanical disturbance of the egg cuticle in shell mottling. Poultry Sci. 46, 771-772.

Draper MH, Davidson MF, Wyburn GM, Johnston HW. (1972). The fine structure of the isthmus of the oviduct of Gallus domesticus. Quart. J. Exp. Physiol. 57, 297-309.

Eastin Jr WC, Spaziani E. (1978a). On the control of calcium secretion in the avian shell gland (uterus). Biol. Reprod. 19, 493-504.

Eastin Jr WC, Spaziani E. (1978b). On the mechanism of calcium secretion in the avian shell gland (uterus). Biol. Reprod. 19, 505-518.

El-Boushy AR, Simons PCM, Wiertz G. (1968). Structure and ultra-structure of the hen's egg shell as influenced by environmental temperature, humidity and vitamin C additions. Poultry Sci. 47, 456-467.

Favejee JChL, van der Plas L, Schoorl R. (1965). X-ray diffraction of the crystalline structure of the avian egg shell: Some critical remarks. Biophys. J. 5, 359-361.

Frank FR, Burger RE, Swanson MH. (1965). The relationships among shell membrane, selected chemical properties, and the resistance to shell failure of Gallus domesticus eggs. Poultry Sci. 44, 63-69.

Geiger GS, MacLaury DW, Quigley GD, Rollins FD, Schano EA, Talmadge DW. (1974). Science studies in poultry biology. Poultry Sci. 53, 455-503.

Gilbert AB. (1971). The egg: Its physical and chemical aspects. In: "Physiology and Biochemistry of the Fowl" Ed. by DJ Bell, BM Freeman. Academic Press, NY, vol 3, 1379-1399.

Hamilton RMG. (1982). Methods and factors that affect the measurement of egg shell quality. Poultry Sci. 61, 2022-2039.

Harris D, Blount JE, Leach Jr M. (1980). Localization of lysyl oxidase in hen oviduct: Implications in egg shell membrane formation and composition. Science 208, 55-56.

Hasiak RJ, Vadehra DV, Baker RC. (1970a). Fatty acid composition of the egg exterior structures of Gallus gallus. Comp. Biochem. Physiol. 35, 751-755.

Hasiak RJ, Vadehra DV, Baker RC. (1970b). Lipid composition of the egg exteriors of the chick (Gallus gallus). Comp. Biochem. Physiol. 37, 429-435.

Heaney RK, Robinson DS. (1976). The isolation and characterization of hyaluronic acid in egg shell. Biochim. Biophys. Acta 451, 133-142.

Heyn ANJ. (1963). The crystalline structure of calcium carbonate in the avian egg shell. An electron microscope study. J. Ultrastructure Res. 8, 176-188.

Hoffer AP. (1971). The ultrastructure and cytochemistry of the shell membrane-secreting region of the Japanese quail oviduct. Amer. J. Anat. 131, 253-288.

Itoh H, Hatano T. (1964). Variation of magnesium and phosphorus deposition rates during egg shell formation. Poultry Sci. 43, 77-80.

Kaplan S, Siegesmund KA. (1973). The structure of the chicken egg shell and shell membranes as studied with the scanning electron microscope and energy dispersive X-ray microanalysis. Poultry Sci. 52, 1798-1801.

King NR, Robinson DS. (1972). The use of the scanning electron microscope for comparing the structure of weak and strong egg shells. J. Microscopy 95, 437-443.

Krampitz GP. (1982). Structure of the organic matrix in mollusc shells and avian eggshells. In: "Biological Mineralization and Demineralization" Ed. by GH Nancollas. Springer-Verlag, NY, 219-232.

Krampitz G, Witt W. (1978). Biochemical aspects of biomineralization. In: "Topics in Current Chemistry" Ed. by MJS Dewar, K Hafner, E Heilbronner, S Ito, JM Lehn, K Niedenzu, SW Rees, K Schafer, G Wittig. Springer-Verlag, NY, 57-144.

Krampitz G, Meisel H, Witt-Krause W. (1980). Identification of γ-carboxyglutamic acid in ovocalcium. Naturwissenschaften 67, 38-39.

Leach Jr RM. (1982). Biochemistry of the organic matrix of the eggshell. Poultry Sci. 61, 2040-2047.

Leach Jr RM, Gross JR. (1983). The effect of manganese deficiency upon the ultrastructure of the eggshell. Poultry Sci. 62, 499-504.

Leach Jr RM, Rucker RB, Van Dyke GP. (1981). Egg shell membrane protein: A nonelastin desmosine/isodesmosine-containing protein. Arch. Biochem. Biophys. 207, 353-359.

Makita T. (1981). X-ray microanalysis of the avian shell gland and eggshell. Scanning Electron Microscopy 1981; II: 473-480.

Masshoff W, Stolpmann HJ. (1961). Licht-und elektronenmikropische Untersuchungen an der Schalenhaut und Kalkschale des Huhnereies. Z. Zellforsch. mikrosk. Anat. 55, 818-832.

Moran T, Hale HP. (1936). Physics of the hen's egg. 1. Membranes in the egg. J. Exp. Biol. 13, 35-40.

Parsons AH. (1982). Structure of the eggshell. Poultry Sci. 61, 2013-2021.

Perrott HR, Scott VD, Board RG. (1981). Crystal orientation in the shell of the domestic fowl: An electron diffraction study. Calcif. Tissue Int. 33, 119-124.

Polin D. (1957). Formation of porphyrin from delta-amino levulenic acid by uterine and liver tissue from laying hens. Proc. Soc. Exp. Biol. Med. 94, 276-279.

Pooley AS. (1979). Ultrastructural relationships of mineral and organic matter in avian eggshells. Scanning Electron Microscopy 1979; II: 475-482.

Redshaw MR, Follett BK. (1972). The physiology of egg yolk production in the hen. In: "Egg Formation and Production" Ed. by BM Freeman, PE Lake. British Poultry Science Ltd, Edinburgh, 35-49.

Reed JM, Yurkiewicz WJ. (1982). Avian egg shell physiology: A review. Proc. Pens. Acad. Sci. 56, 55-60.

Robinson DS. (1970). The structure of the mammillary layer of the domestic hens' eggshell. Ann. Biol. Anim. Biochim. Biophys. 10(Suppl. 1), 27-35.

Robinson DS. (1972). Egg white glycoproteins and physical properties of egg white. In: "Egg Formation and Production" Ed. by BM Freeman, PE Lake. British Poultry Science Ltd, Edinburgh, 65-86.

Robinson DS, King NR. (1963). Carbonic anhydrase and formation of the hen's egg shell. Nature (Lond.) 199, 497-499.

Robinson DS, King NR. (1968). Mucopolysaccharides of an avian egg shell membrane. J. Roy. Micros. Soc. 88, 13-22.

Robinson DS, King NR. (1970). The structure of the organic mammillary cores in some weak egg shells. Brit. Poultry Sci. 11, 39-44.

Schmidt WJ. (1962). Liegt der Eischalenkalk der Vogel als submikroskopische Kristallite vor? Z. Zellorsch. mikrosk. Anat. 57, 848-880.

Schmidt WJ. (1964). Uber die Struktur einiger abnormer Vogel-Eischalen nebst Bemerkungen zu neueren Auffassungen betreffend Bau und Bildung der kalkschale. Z. Morph. Okal. Tiere 53, 311-361.

Schmidt WJ. (1965). Die Eisospharíten (Basal kalotten) der Schwanen-Eischale. Z. Zellforsch. mikrosk. Anat. 67, 151-164.

Sharp RM, Silyn-Roberts H. (1984). Development of preferred orientation in the eggshell of the domestic fowl. Biophys. J. 56, 175-180.

Silyn-Roberts H, Sharp RM. (1985a). Preferred orientation of calcite in the ratite and timarou eggshells. J. Zool. (Lond.) 205, 39-52.

Silyn-Roberts H, Sharp RM. (1985b). Preferred orientation of calcite and aragonite in the reptilian eggshells. Proc. Roy. Soc. Lond. B 225, 445-455.

Simkiss K. (1961). Calcium metabolism and avian reproduction. Biol. Rev. 36, 321-367.

Simkiss K. (1968). The structure and formation of the shell and shell membranes. In: "Egg Quality: A Study of the Hen's Egg" Ed. by TC Carter. Oliver and Boyd, Edinburgh.

Simkiss K, Taylor TG. (1971). Shell formation. In: "Physiology and Biochemistry of the Domestic Fowl" Ed. by DJ Bell, BM Freeman. Academic Press, NY, vol 3, 1331-1343.

Simkiss K, Tyler C. (1957). A histochemical study of the organic matrix of hen egg-shells. Quart. J. Microscop. Sci. 98, 19-28.

Simkiss K, Tyler C. (1958). Reactions between egg-shell matrix and metallic cations. Quart. J. Microscop. Sci. 99, 5-13.

Simons PCM. (1971a). Ultrastructure of the hen eggshell and its physiological interpretation. Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands. 90pp.

Simons PCM. (1971b). Ultrastructure of the hen eggshell and its physiological interpretation. Figures. Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands. 46 pp.

Simons PCM, Wiertz G. (1963). Notes on the structure of membranes and shell in the hen's egg. An electron microscopical study. Z. Zellforsch. mikrosk. Anat. 59, 555-567.

Smith AH, Winget CM, Blackard JR. (1954). The transfer of phosphorus to the hen's egg, under controlled environment, as traced with radiophosphorus (P^{32}). Poultry Sci. 33, 908-919.

Starcher BC, King GS. (1980). The presence of desmosine and isodesmosine in eggshell membrane protein. Connective Tissue Res. 8, 53-55.

Stemberger H, Mueller J, Leach Jr M. (1977). Microscopic study of the initial stages of egg shell calcification. Poultry Sci. 56, 537-543.

Stevenson IL. (1980). The removal of egg shell membranes by enzymatic treatment to facilitate the study of shell microstructure. Poultry Sci. 59, 1959-1960.

Stewart GF. (1935). The structure of the hen's eggshell. Poultry Sci. 14, 24-32.

Talbot CJ, Tyler C. (1974). A study of the fundamental cause of artificial translucent areas in egg shells. Brit. Poultry Sci. 15, 205-215.

Terepka AR. (1963a). Structure and calcification in avian egg shell. Expt. Cell. Res. 30, 171-182.

Terepka AR. (1963b). Organic-inorganic interrelationships in avian egg shells. Expt. Cell. Res. 30, 183-192.

Thompson BK, Hamilton RMG, Voisey PW. (1981). Relationships among various egg traits related to shell strength among and within five avian species. Poultry Sci. 60, 2388-2394.

Tullett SG. (1975). Regulation of avian eggshell porosity. J. Zool. (Lond.) 177, 339-348.

Tullett SG, Lutz PL, Board RG. (1975). The fine structure of the pores in the shell of the hen's egg. Brit. Poultry Sci. 16, 93-95.

Tyler C. (1956). Studies on egg shells. VII. Some aspects of structure as shown by plastic models. J. Sci. Food Agric. 7, 483-493.

Tyler C. (1961). Studies on egg shells. XVI. Variation in shell thickness over different parts of the same shell. J. Sci. Food Agric. 12, 459-470.

Tyler C. (1964). Wilhelm von Nathusius 1821-1899 on Avian Egg shells. The Berkshire Printing Co., Ltd, Reading. 104 pp.

Tyler C. (1965). A study of the egg shells of the Spheniscifformes. J. Zool. (Lond.) 147, 1-19.

Tyler C. (1969a). Avian egg shells: Their structure and characteristics. Int. Rev. Gen. Exp. Zool. 4, 81-130.

Tyler C. (1969b). The distribution of pores in the eggshells of the domestic fowl: A further study. Brit. Poultry Sci. 10, 375-380.

Tyler C, Fowler S. (1978). The distribution of organic cores, cones, cone junctions and pores in the egg shells of wild birds. J. Zool. (Lond.) 186, 1-14.

Tyler C, Geake FH. (1958). Studies on egg shells. IX. The influence of individuality, breed and season on certain characteristics of egg shells from pullets. J. Sci. Food Agric. 9, 473-483.

Tyler C, Geake FH. (1964). The effect of water of egg shell strength including a study of the translucent areas of the shell. Brit. Poultry Sci. 5, 277-284.

Tyler C, Geake FH. (1965). Egg shell thickness patterns as shown by individual domestic hens. Brit. Poultry Sci. 6, 235-243.

Tyler C, Standen N. (1969). The artificial production of translucent streaks on egg shells and various factors influencing their development. Brit. Poultry Sci. 10, 359-369.

Vadehra DV, Nath KP, Baker RC. (1971). Physicochemical properties of the proteins of egg shell membranes. Poultry Sci. 50, 1638 (Abstract).

van Toledo B, Parsons AH, Combs Jr GF. (1982). Role of ultrastructure in determining eggshell strength. Poultry Sci. 61, 569-572.

Warren DC, Conrad RM. (1942). Time of pigment deposition in brown shelled hen eggs and in turkey eggs. Poultry Sci. 21, 515-520.

Washburn KW. (1982). Incidence, cause and prevention of egg shell breakage in commercial production. Poultry Sci. 61, 2005-2012.

Wedral EM, Vadehra DV, Baker RC. (1974). Chemical composition of the cuticle, and the inner and outer shell membranes from eggs of Gallus gallus. Comp. Biochem. Physiol. 47B, 631-640.

Wilbur KM, Simkiss K. (1968). Calcified shells. In: "Comprehensive Biochemistry" Ed. by M Florkin, EH Stotz. Elsevier Publishing Co., NY, vol 26, pt A, 229-295.

Wong M, Hendrix MJC, von der Mark K, Little C, Stern R. (1984). Collagen in the egg shell membranes of the hen. Develop. Biol. 104, 28-36.

Discussion with Reviewers

A.S. Pooley: Does the structure of the eggshell have any influence on the egg as a food material?

Author: No, eggshell structure has no direct influence on the egg's contents. However, if

thickness, a structural property of the shell, is considered, then shell structure may be considered to have an indirect effect because thin shelled eggs will be more susceptible to moisture loss and/or microbial contamination of their content during storage.

A.S. Pooley: Is anything that has been learned about eggshells in the past 30 years likely to lead to breeding selection that will provide increased resistance to breakage of eggs?

Author: Yes, research on the measurement of eggshell strength has provided techniques that are routinely used in selection programs by the primary breeder to produce the commercial stocks used for egg production. In addition, information from this research, such as cool eggshells are stronger than warm ones, has been discussed with egg producers for use in their operations.