EGGSHELL STRENGTH:
A MECHANICAL / ULTRASTRUCTURAL EVALUATION

By

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DECLARATION

I hereby declare that the work presented in this thesis was carried out by me personally with the exceptions of: Figures 1, 2, 3, 4, 5 and 6, which are modifications of diagrams published by Parsons (1982), Gallagher (1987), Balderes (1987) and Ross (1985), and Figures 56, 66, 68, 69, 73 and 77 which were prepared from scanning electron micrographs taken by Mrs S. Cranston and Mr E. Lowson.

Maureen. M. Bain.

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SUMMARY.

(1). In this dissertation computer graphics have been used in the first instance to facilitate the development of a finite element model of the egg.

(2). In the development of this model, it has been demonstrated that the eggshell should not be regarded as the classic case of a "thin walled shell structure". The components of shear are significant and as a result the egg must be analysed using "thick shell" theory. Finite element analysis unlike existing analytical solutions adopts a "thick shell" formulation in its' method of calculation.

(3). Finite element modelling procedures have permitted the visualisation of eggshell behaviour under load; underlined the importance of eggshape in quality assessment; and have allowed a critical appraisal of eggshape in isolation from other confounding variables.

(4). The stiffness characteristics of the eggshell are resident in the palisade layer ($t_{effective}$). The mammillary layer *per se* does not influence this property.

(5). A method for calculating the elastic modulus of eggshells is given. This not only takes into account the components of shear, but also "true" eggshape and only the effective thickness of the shell. The modulus of eggshells appears to be similar to other calcified tissues such as bone.

(6). The mechanism of shell failure has been studied in detail using both finite element analysis and scanning electron microscopy. Crack initiation precedes the first visible signs of damage. Succeeding this a series of radial cracks propagate from

the load point at first in a stable manner. Ultimately a critical level of stress is reached and at this point one or more of these pre-cracks becomes unstable. The end result is catastrophic failure.

(7). The fracture toughness of eggshells or their resistance to unstable crack growth is dependent upon the nature and magnitude of inherent defects within the shell. Fracture toughness can now be used as a measure of quality, but with reservation.

(8). The concluding chapters of this thesis correlate the mechanical and ultrastructural properties of eggs from two commercial stocks held under identical environmental conditions. Genetic traits have been identified and their role in shell performance under load classified.

GLOSSARY

boundary conditions: Method by which restrictions in movement are imposed at specific points on a finite element model. May also be imposed in symmetric problems where only part of the structure needs to be analysed.

compliance: The relationship between the applied force, the resulting deformation, the geometry, and material properties of finite element models.

crack initiation. The propagation of pre-existing internal cracks through the wall of the eggshell due to the build up of tensional stress just below where the palisade columns fuse. This precedes catastrophic failure.

deformation: The deflection of the eggshell under an applied force.

elastic or Young's modulus: A material property which describes the unique relationship that exists between the stresses and strains induced when a material is initially loaded.

element stiffness equation: The equation that gives the force at an element node point in terms of the sum of the products of element stiffness coefficients and nodal point displacements.

failure: The level of stress or the force necessary to cause unstable crack growth. The egg fails to function in any capacity.

finite element analysis (FE): An analysis procedure in which a structure is imagined to be divided into elements of simpler geometry. Each element is tied together algebraically so as to satisfy conditions of equilibrium and continuity of displacement. The resulting algebraic equations are solved to give predictions of the response of the structure to applied loads.

fracture toughness: A material property which quantifies the relationship between the applied stress necessary to cause failure and the size of any defects that may be present.

non-destructive (ND) deformation: Deformation of an eggshell resulting from the application of a force which is less than that required to break the shell.

node point: Point on an element, and in the finite element representation of a structure, where the element is joined to neighbouring elements or where discrete loads can be applied. In certain cases, node points merely serve as reference points on or within the element.

ND stiffness: Non-destructive deformation test estimate of stiffness.

Poisson's Ratio: The ratio of the lateral strain to the longitudinal strain in a stretched body. A material property.

quasi-static compression test (QSC): The egg is compressed between two flat plates until the shell fractures.

"shell": In engineering terms this defines a solid material enclosed between two closely spaced doubly curved surfaces, the distance between these two surfaces being the thickness of the shell.

shearing force: The force that acts parallel to a plane rather than perpendicularly.

stiffness: The force per unit deformation within the elastic limit.

strain: The change in length per unit length of the shell material under an applied stress. This maybe tension, compression or shear in various combinations depending on the mode of the force application.

stress: The force per unit area developed within the shell material when force is applied to the egg. This may be tension, compression or shear in various combinations depending on the mode of the force application.

stress intensity factor: Geometry, thickness and loading conditions influence the local stress distribution within the immediate vicinity of a crack through this factor.

$t_{effective}$: The effective thickness of eggshells. Corresponds to the palisade, vertical crystal layer and the cuticle.

"thin shell": For $R/t \geq 300$, the shear stresses acting through the thickness of the "shell" can be ignored in calculations.

"thick shell": For $R/t < 300$ both the shear and the normal stress components are significant.

CHAPTER 1

LITERATURE REVIEW AND AIMS OF THESIS.

1.1.1 GENERAL.

The avian eggshell is surely one of natures most remarkable inventions. Not only does it serve as a self contained embryonic chamber for the development of the avian embryo, but it has also provided man with a convenient, compact, readily packaged food stuff.

For the developing embryo, the egg contents provide a fluid medium and all the nourishment necessary for embryonic growth. The eggshell not only adjoins the internal and external environments but has several important roles to play: thus, respiratory gases must be allowed to pass through the shell, whilst water loss and bacterial penetration must be minimised. In addition the shell must protect the embryo from mechanical damage, yet be fragile enough to allow the chick to breakout at the end of the incubation process.

The basic architecture of the avian eggshell is remarkably constant among the many species of the class Aves. In general this thin protective coat is composed approximately of 95% calcium carbonate, primarily in the calcite form, and 5% organic material in the form of the shell membranes, the organic cores and the matrix (Romanoff and Romanoff 1949). Although it is a unified structure various layers have been recognised. Differences however exist in the nomenclature used to define these layers (see Tullett 1985 for details). The principle terminology used in this thesis is summarised in Figure 1 (after Parsons 1982).

1.1.2 THE FORMATION AND STRUCTURAL ORGANISATION OF THE EGGSHELL.

The structural and functional significance of the six regions which comprise the active left oviduct of the domestic chicken have been the subject of many communications (Aitken 1971; Simkiss and Taylor 1969; Draper *et al* 1972; Wyburn *et al* 1973; Gilbert

1971 and 1979; Solomon 1973, 1975,1983 and 1990; Talbot and Tyler 1974, Watt 1989) and the results are herein summarised.

In the domestic fowl the sequential maturation of ova takes place at approximately 24 hour intervals and is achieved through a hierarchical development of follicles (Gilbert 1971). Ovum maturation is achieved through yolk accumulation, the latter being derived from the liver. This is accompanied by a gradual increase in size and change of colour. With the exception of the yolk lipids, all the other components of the egg are produced in, or are transported across, the cells which line the oviduct.

Surges of luteinizing hormone from the pituitary gland are thought to cause ovulation (Gilbert 1971). The released ovum is then engulfed by the fimbriae surrounding the funnel shaped end of the infundibulum. While the prime role of the latter is to direct the ruptured yolk mass into the main part of the oviduct, this region of the oviduct has also been implicated in several other processes: fertilisation (Olsen and Neher 1948); the production of the perivitelline membranes (Bain and Hall 1969); and in the formation and release of enzymes which, at a later date are triggered by changes in the chemical composition of the oviducal milieu to encourage the formation of the twisted albumen strands, the chalazae (Solomon 1990).

The magnum is the longest and most conspicuous part of the oviduct and is readily distinguished from the infundibulum by its 'dead' white colour, its greater diameter and thicker wall (Solomon 1983). This region is responsible for the manufacture and release of the 40 different proteins which comprise the egg white or albumen. The major constituent of albumen at oviposition however is in fact water (80%).

Succeeding its three hours sojourn in the magnum, the egg enters the isthmus. This region is narrower than the magnum and herein are deposited the paired shell membranes. The latter interlace and adhere to each other thus forming a perfuse mat except at the broad pole where an intervening air space is formed.

At the level of the electron microscope both inner and outer membrane fibres are seen to consist of an electron dense inner core which is surrounded by a less dense mantle (Simons 1971). The mantles of adjacent fibres coalesce giving the fibres a branched appearance (Draper *et al* 1972; Simons 1971).

Mashoff and Stolpmann (1961-cited by Parsons 1982) suggested that the membranes consist of approximately 95% protein with a small amount of polysaccharide, with the latter being found primarily in the mantle. While the protein component has since been found to have cross-links similar to those of keratin, collagen and elastin, Leach (1982) concluded that it was nevertheless unique to the egg, but to date it remains unequivocally identified.

According to Robinson and King (1968) the terminal portions of the outer membrane fibres are chemically modified. The interchain disulphide bonds which distinguish these areas from adjacent points along the length of the fibres are thought to act as epitactic centres for calcium salts (Parsons 1982). Cooke and Balch (1970) reported that these organic cores were rich in hexosamine, sialic acid and hexose but could not isolate uronic acid. This lead these authors to the conclusion that chondroitin sulphate, which is involved in the mineralisation of bone, is not involved in shell calcification .

Both calcium and carbonate ions are required for calcification. According to Simkiss (1975) biomineralisation will only occur in regions where the fluids tend towards conditions of supersaturation viz, in sites where calcium and carbonate ions accumulate so as to exceed their solubility constant. Calcium carbonate crystals exists in three polymorphic forms, namely calcite, aragonite and vaterite and each is the end product of quite different physio-chemical conditions (Miller 1975). The avian egg is typically of the calcitic modification (Heyn 1963), while the soft shelled eggs of many reptiles are more typically aragonite (Solomon and Watt 1985).

During the laying period a hen preferentially selects calcium rich sources of limestone from the diet but has the capacity to absorb little more than one gram of calcium per day (Tyler 1940). A normal eggshell contains about two grams of calcium and it is now recognised that the labile medullary bone in the femur supplies the extra calcium required during the laying period (Simkiss 1975). Thus a dynamic equilibrium exists between dietary calcium in the blood, the medullary bone and the calcifying shell.

Calcium homeostasis in the bird is partly controlled by the antagonistic actions of the parathyroid hormone (PTH) and calcitonin. Both hormones are sensitive to changes in the blood (Simkiss 1975) viz, in response to hypocalcaemia, PTH stimulates osteoclast cells to re-model the medullary bone, while calcitonin slows the secretion of PTH when plasma levels of calcium are in excess of that required for shell calcification. Vitamin D (cholecalciferol) has also been implicated in this scheme of things (Simkiss 1975).

Once adsorbed into the blood, dietary calcium either becomes bound to a phosphoprotein or it remains in its ionic form. The unbound or diffusible form is utilised directly by the shell forming regions of the oviduct and this is replenished by the ionisation of some of the non-diffusible fraction (Simkiss and Taylor 1969).

Calcium transfer across the mucosa of the oviduct occurs rapidly and is thought to involve either an active transport mechanism (Ebashi and Lipmann 1962; Helbock *et al* 1966 - cited by Solomon 1983) or a protein carrier (Corradino *et al* 1968 - from Solomon 1983). Details of this transport system however are still being evaluated.

The carbonate moiety of the crystalline shell originates from the hydration of metabolic carbon dioxide to bicarbonate ions within the shell gland mucosa (Hodges and Lorcher 1967). Implicit in this process is the catalytic action of the enzyme carbonic anhydrase. The latter has been located both within the tubular glands and epithelial cells lining the shell gland (Gay and Mueller 1973 - from Sturkie 1986).

The secretion of bicarbonate ions into the lumen of the shell gland appears to be mainly dependent on passive movement down a concentration gradient although active transport mechanisms have also been implicated (Eastin and Spaziani 1978 - from Tullett 1985). In the lumen the bicarbonate ions combine with calcium ions, and this is accompanied by the release of hydrogen ions. Shell formation can therefore pose problems for the acid-base status of the blood (Mongin 1968).

Seeding of calcium salts begins in the tubular shell gland (TSG). Ultrastructurally, this short region of the oviduct resembles the appearance of the isthmus but functionally it is more comparable to the shell gland pouch (SGP) (Solomon 1983). The physical presence of the shell membranes encourages crystal growth upwards and outwards, although some penetration of the fibres does take place (Simkiss 1968; Reid 1984). As a direct result of this, the organic cores subsequently become embedded within that part of the calcified shell known as the basal cap (see Figure 1).

The main phase of calcification takes place in the shell gland pouch (SGP) where the developing egg remains for between 18-20 hours. During the first four to six hours, the plumping fluid is added to the egg white while crystal growth continues slowly outwards and upwards to form the mammillary cones. The latter eventually fuse along their edges to form a continuous upwards growing front. This completes the formation of the mammillary layer and corresponds roughly with the commencement of a more rapid phase of calcification (Solomon 1990).

The palisade layer is formed during the rapid phase of calcification. The latter constitutes the bulk of the shell and is composed of long, fused calcitic columns. In transverse section however, the individual columns cannot be distinguished. X ray diffraction analyses suggests that these columns are not monocrystals (Erben 1970) but no consensus of opinion exists on the diffraction data available (Tullett 1985).

Buss and Stout (1981) indicated that the onset of the rapid phase of calcification occurs sooner in those hybrids which characteristically produce a thicker shell. This then may explain some of the inconsistencies which exist in the literature as to how long the egg remains in the SGP before rapid calcium transfer takes place (see Talbot and Tyler 1974, Creger *et al* 1976, Nys 1986).

In common with other calcified tissues, the inorganic components of the calcified portion of the eggshell are closely associated with an organic matrix. This matrix appears to be unevenly distributed throughout the mineralised portion of the shell, with its concentration increasing to a maximum two-thirds of the way through its' thickness. Thereafter it decreases rapidly towards the outer surface (Cooke and Balch 1970; Simons 1971). The extent to which this distribution has been induced by the decalcifying procedures employed by these authors however is a matter of conjecture and good photographic evidence would still appear to be lacking.

At least 70% of the organic matrix appears to be protein. Of the remainder about 11% is polysaccharide, 35% of which can be accounted for by chondroitin sulphate A and B (Baker and Balch 1962). Like other biological matrix proteins, several components of the eggshell matrix appear to have calcium binding properties. Simkiss and Tyler (1958) were able to demonstrate that the matrix is capable of chelating ions due to its mucopolysaccharide content. More recently Krampitz *et al* (1980) isolated the calcium binding polypeptide, ovocalcin, from the matrix.

Simons (1971) associated the vesicular holes, which give the palisade layer its characteristic honey comb appearance, with the presence of shell matrix. Solomon (1989 - cited by Watt 1989) suggests that these holes are a naturally occurring phenomenon caused by the incomplete fusion during the growth phase of the cubic crystals. As the asynchronous release of shell matrix proteins has never been observed (Wyburn *et al* 1973), it would appear that once crystallisation is initiated, the various calcium binding components of the matrix together with calcium are capable of self-organisation. The role of the shell matrix in this process of mineralisation however is still to be determined.

The continuity of the palisade layer is disrupted only by the presence of funnel shaped pores which run vertically through the entire thickness of the shell. These pores facilitate gaseous exchange and are a consequence of the incomplete fusion of adjacent mammillary columns during the initial stages of calcification (Tullett 1975). According to Tullett (1975) there appears to be a relationship between the numbers of pores and the numbers of mammillae per unit area. The average egg has been estimated to have between 700-1700 pores.

The final calcified portion of the shell, the vertical crystal layer lies directly above the palisade layer and below the cuticle. This layer consists of short narrow crystals aligned roughly perpendicular to the shell surface (Perrott *et al* 1981) and is of variable thickness (Simons 1971). A change in pH and the concentration of phosphate ions in the shell gland fluid towards the end of calcification may be responsible for this change in crystal growth mechanism (Mongin and Sauveur 1970; Perrott *et al* 1981). High levels of phosphate ions have also been implicated in the "cut off" mechanism which terminates calcification (Simkiss 1964).

Unlike the yolk pigments, which are derived from suitable carotenoid precursors in the diet, the pigment associated with the eggshell (protoporphyrin), is synthesised and secreted in the SGP

(Baird *et al* (1975). Both brown and white shelled eggs contain pigment within the calcified matrix, although the amount in white shelled eggs is reduced (Solomon 1985b). According to Lang and Wells (1987) "browness" results from the incorporation of additional pigment into the cuticle just prior to ovulation.

The cuticle forms a waxy organic covering on the outermost surface of the eggshell and is composed of 85-87% protein, 3.5-4.4% carbohydrate and 2.5-3.5% fat with 3.5% ash (Wedral *et al* 1974 - cited by Tullett 1985). This layer not only serves to make the egg waterproof (Board and Halls 1973) but also provides a barrier to the invasion of pathogens by plugging the exposed pore sites. The cuticle however is rarely complete (Solomon 1990) and may vary in thickness from one region of the shell to another (Simons 1971). Reid (1984) found that 5% of birds fed a commercial layers diet showed a defective cuticle, while Board and Halls (1973) found that the cuticle is occasionally completely absent.

At oviposition, the egg leaves the SGP and enters the vagina from where it is rapidly expelled by hormone mediated muscular contraction. While this region does not actively contribute to shell formation, it does contain specialised glands for sperm storage. Sperm can remain viable for a considerable period of time in these glands, before migrating anteriorly towards the infundibulum (Solomon 1983).

1.1.3 EGGSHELL STRENGTH AND QUALITY.

The 1988 "egg crisis" in the UK has focused world wide attention on food spoilage by micro-organisms: thus, eggshell strength and quality is of interest to all participants in the egg industry from the breeder to the consumer. Despite the fact that the egg comes with its own natural defence mechanism, i.e the eggshell, contamination of egg contents can nevertheless occur. There are two possible routes by which this may happen: within the oviduct before the shell is formed, or through penetration of damaged and inferior quality shells. Whilst both routes are possible, the latter is the more probable (Nascimento pers comm).

There are conflicting reports in the literature as to the numbers of eggs that are downgraded due to broken or inferior quality shells. This is perhaps due to the different criteria used by individual packing stations to assess shell quality. Shrimpton and Hann (1967) reported a loss of just 2.7%, but in this study it would appear that those eggs with hairline cracks and gross malformations had previously been removed on the farm before the eggs were graded. Anderson and Carter (1976) in contrast estimated that 6.7% of the eating eggs produced in the UK were downgraded annually due to shell problems. Roland (1977) proposed that in addition to this, 6.1% of the eggs produced on the farm were uncollectable, while a further 7-9% were found to be broken on arrival at the supermarket (Roland 1988). The economic loss to the industry is therefore substantial.

The mature, commercial hen is capable of laying in excess of 300 eggs in one laying year and there is still considerable pressure on the bird to increase output. The latter however is not considered to be the main cause of the increasing incidence of poorer quality shells (Anderson *et al* 1970; Hunton 1982; Washburn 1982). Genetic variation in shell strength and quality is not in dispute, but little is known about the biochemical mechanisms which determine specific shell quality characteristics (Bulfield and McKay 1985).

1.1.4 FACTORS KNOWN TO AFFECT THE QUALITY OF EGGSHELLS.

Various factors are known to affect eggshell quality. These include: the genetic constitution of the hen; the age of the bird; the position and number of eggs laid in a clutch; bird behaviour; the environment (including nutrition, housing system, lighting, temperature and humidity); disease; and the size, type and numbers of insults experienced by the egg en route to the supermarket shelf.

Nevertheless it is the strength of the individual egg, and the evaluation of strength itself, which has posed a more difficult problem in research directed towards improvement.

[i] Strain Differences:

Potts and Washburn (1983) found that the average breaking strength of eggs from white laying stock was consistently higher than that of brown laying stock, the differences however depended on which stock comparisons were made. Buss and Stout (1981) reported that lines genetically selected for thick shells had a greater increase in shell weight and percent shell per unit time in the shell gland than did the thin shell lines, suggesting that the rate of shell deposition may be different between the two.

Solomon (unpublished observation) found ultrastructural differences in the quality of shells both within and between the four main commercial laying stocks in the UK. The incidence of downgrading within and between these flocks however were not included in this study.

[ii] Environmental Effects and Bird Behaviour:

According to Hughes *et al* (1985) the incidence of cracked eggs is greater in the battery system than on range. These authors also suggested that range eggs are thicker than their battery counterparts. The earlier work of Anderson *et al* (1970) indicated that the insults experienced in the battery system were of a greater magnitude *vis à vis* on range.

Recently, Watt (1989) has published findings which suggest that increasing the numbers of birds per cage for 4 hours can cause egg retention and a extra-cuticular coatings to be formed, but more importantly, subsequent eggs from the same birds were also structurally inferior for anything up to 14 days post stress.

Egg production, egg weight and shell thickness are all known to decrease in birds which have been subjected to heat stress (Norstrom 1973), but if the humidity is also high, the depression of egg weight is even more pronounced (Sauveur and Picard 1985). The environment of the hen however is also the environment of the egg at the time of oviposition, and elevated temperatures and a high humidity are also considered to have a direct effect on the inherent strength of eggshells (Lott and Reece 1981; Voisey *et al* 1979).

[iii] Age of Bird:

According to Brooks (1971) during the first month of lay, total breakages on average account for 2.75% of all eggs laid, while during the fifteenth month this may increase to around 13.5%. In a study of two commercial laying flocks, Belyavin and Boorman (1982) provided considerable insight into the complexity and development of this problem. Later Boorman *et al* (1985) conducted a more detailed study on individual birds to assess whether the abrupt breakdown in quality of just a few individuals could be distorting the overall trend for the flock. The results of this study suggested that while there was considerable variation between individuals, the general trend was nevertheless downwards.

A progressive increase in egg size without a concomitant increase in shell deposition, has been associated with the decline in quality with bird age (Peterson 1965, Roland *et al* 1975a). Nevertheless, when old hens are force moulted, the strength of their shells is improved, while egg size remains unchanged (Washburn 1982). Yannakopoulos *et al* (1985) were also unable to find a correlation between decreasing eggshell strength and egg size, while Potts and Washburn (1983) concluded that a genetic variation in egg size was not related to genetic variation in shell strength.

[iv] Time of Oviposition:

The time interval between eggs laid on successive days by most hens ranges from 24 to 28 hours depending on the length of the laying sequence and the position of the egg in the clutch. Several studies have indicated that those eggs laid in the afternoon display better shell characteristics than those eggs laid in the morning (Washburn and Potts 1975; Roland 1981; Arafa *et al* 1982; Belyavin *et al* 1985).

Carter (1971a) suggested that the variation in drop height or stance of the bird at oviposition was another important factor affecting the number of downgraded eggs from cage systems.

[v] Nutrition:

The literature on dietary manipulation with regards to dietary calcium and phosphorus levels is extensive yet there still appears to be no consensus of opinion as to how these improve shell quality. Washburn (1982) reported that a low dietary level of calcium reduces shell thickness while levels in excess of that required by the bird appear to have no beneficial effects on the quality of eggs produced (Hurwitz 1985). In contrast, levels of phosphorus in excess of that required for maximum egg production may form insoluble calcium phosphates within the gastrointestinal tract thus preventing the adsorption of calcium (Taylor 1965). A phosphorus deficiency, however, may also result in poorer egg production (Hurwitz 1985).

The levels of Vitamin D_3, manganese, bicarbonate, chloride, and sodium in the diet can also influence shell quality (Hurwitz 1985).

[vi] Disease:

Diseases implicated in the production of poor quality shells include Newcastle's disease, Infectious Bronchitis, and a haemagglutinating adenovirus Egg Drop Syndrome (EDS-76) (Spackmann 1985). Shell quality in turn has a direct bearing on the ability of bacteria to penetrate into the egg following oviposition. The consequence of such translocation is severe on both hatchibility, and the keeping quality of eggs. Of current interest are the Salmonella species which appear to be able to penetrate even good quality shells (Nascimento pers comm).

[vii] Environmental Insults:

Since any egg will break, if the insult to which it is exposed is great enough (Carter 1970a), a great deal of effort has been directed towards reducing the frequency and magnitude of external insults experienced by an egg from the time at which it is laid to its safe arrival on the supermarket shelf.

Two types of insult can be identified in the field: impact fractures may occur at oviposition, or when one egg collides with another or part of the collecting machinery; while compression fractures are generally associated with packaging and transportation. Attempts have been made to decrease both types: re-designing of cage floors (Anderson and Carter 1972; Overfield 1976); decreasing the slope of rollaway (Elson 1969; Overfield 1976); increasing the frequency of collections (Elson 1969); controlling the speed of the collection belts and minimising the number of right angles (Hamilton *et al* 1979); and the re-designing of egg trays and packaging systems (Nethercote *et al* 1974; Johnston and Ernest 1975). While these approaches have all played a significant role in decreasing the number of down graded eggs, it has nevertheless become increasingly apparent that it maybe an inherent lack of strength in the shell itself which is playing a more significant role in this scheme of events, with the result that insult becomes of secondary importance.

Two important questions must therefore be addressed: a) which features of the shell determine its' strength characteristics? and b) can this information be derived from the type of test currently used to evaluate the strength of eggshells?

1.1.5 ULTRASTRUCTURE AND STRENGTH.

The ultrastructural organisation of eggshells has been studied in detail (Fujii and Tamura 1969, 1970; Fujii *et al* 1970; Simons 1971; Solomon 1985a,1990) but the relationship between this and eggshell strength remains undefined. It is nevertheless clear that the strength of an eggshell is not simply determined by thickness, but is dependent on a balanced shell architecture to which the different components of the shell all contribute. Simons (1971) suggested that the shell membranes, the numbers and size of mammillae per unit area, the distribution of organic matrix, the size and numbers of vesicular holes, the thickness and structural organisation of the palisade columns, and the presence or absence of a cuticle must all play a significant role in this scheme of things.

The shell membranes form the foundation onto which the rest of the calcified shell is laid. Both Tyler and Thomas (1966) and Nelson and Henderson (1974) however showed that the membranes *per se* had no direct effect on shell snapping strength.

Carter (1971b) suggested that only the outer two-thirds of the shell contributed meaningfully to shell strength. The method by which he arrived at this conclusion however is open to debate, particularly since it is the mammillary layer which is most often altered in inherently weak low quality shells. This was first illustrated by King and Robinson (1972) who found that in weak thin shells the mammillae were of irregular shape, porous and frequently fragmented. Bunk and Balloun (1977;1978) subsequently identified and categorized a number of aberrant forms in the mammillary layer. Watt (1985) later described in detail at least seven structural variations which commonly occurred in eggs found cracked on supermarket shelves. The incidence of at least six of these have since been shown to vary with Strain of bird (Solomon, pers comm), age (Watt 1989), and environmental conditions (Mohumed 1986; Watt 1989; Solomon 1990). To date however, it remains unclear if these structural modifications influence the strength characteristics of eggshells in which they are commonly found.

Several authors have also commented on the number and size of mammillae per unit area (Robinson and King 1970; Simons 1971; Bunk and Balloun 1977,1978; Van Toledo *et al* 1980, 1982). These reports however are conflicting and there is still much confusion in the literature as to whether a high or low mammillary density predisposes a shell to break.

The palisade layer appears to be less subject to structural modification although variations in vesicular porosity appear to exist. Simons (1971) distinguished between a spongy palisade layer and a more compact layer in which the vesicular holes were smaller and much more compact, and hypothesised that the thicker this more compact region, the stronger the shell. More recently Solomon (1988) has indicated that a possible link may exist between the structural organisation of the mammillary layer and the porosity of the palisade columns.

The role of the vertical crystal layer (VCL) with respect to shell strength does not appear to have been considered perhaps because this layer is rarely visible in scanning electron micrographs taken at low magnifications. Nevertheless according to Simons (1971), this layer varies in thickness over the surface of the egg, ranging from 3μm to 8μm in places, and has a denser shell matrix than the palisade region.

Belyavin and Boorman (1980) suggested that the cuticle has no significant effect on eggshell strength beyond its contribution to shell thickness.

1.1.6 MEASURING THE STRENGTH OF EGGSHELLS.

A great deal of effort has gone into the design of tests that measure eggshell strength and these have formed the subject matter for several extensive reviews (Tyler 1961a; Wells 1968; Voisey and Hunt 1974; Hamilton 1982; Hunton 1985,1989). In general two types of test can be identified; those which measure eggshell strength directly and those which measure some physical parameter indirectly related to its' strength characteristics.

[i] Direct Measurement of Eggshell Strength.

Many methods have been reported over the years to measure the quasi-static compression fracture strength of eggshells (see Tyler 1961a, and Voisey and Hunt 1974). The method chosen by most researchers is to compress the egg between two flat parallel plates by a steady increasing load. Under these conditions the shell fractures at the upper or the lower point of contact where the tensile stresses are theoretically at a maximum (Voisey and Hunt 1967b). The force and deformation are recorded throughout the test and the strength of the eggshell is then given in terms of the force required to crush the shell.

Many factors influence the behaviour of eggs and the results obtained from quasi-static compression tests (details are given by Voisey and Hunt 1974 and Hamilton 1982). Of prime importance is the control of the speed at which the eggs are compressed (Voisey and Hunt 1969; Reece and Lott 1976). Instruments such as the Instron tensile test machine, which was originally designed for testing industrial materials, have therefore often been employed (Richards and Staley 1967; Tung *et al* 1968,1969; Hunt and Voisey 1966; Voisey and Hunt 1967a, 1967b, 1969). Voisey and MacDonald (1978) developed their own smaller more portable machine designed specifically for use on eggshells. According to Hunton (1989) the latest version of this apparatus is integrated with a personal computer which eliminates the intermediate steps of calculating and listing the results.

Another direct method of measuring eggshell strength is to determine the shells' resistance to impact. This type of test usually involves dropping an object, usually a steel ball, repeatedly onto the surface of the egg from a variable height (Tyler and Geake 1963; Anderson and Carter 1976). Alternatively the ball may be dropped from the same height onto one or more points on the shell. In each case the height or the number of blows required to break the egg is then used as an index of strength (Tyler 1961a). Both techniques however are rather crude (Voisey and Hunt 1974) and it is often difficult to determine which of the multiple blows actually breaks the shell (Tyler and Moore 1965).

Both the quasi-static compression test and the impact tests attempt to simulate the types of stress that an egg is likely to encounter in the field. Several authors have been rather sceptical about this (Hamilton *et al* 1979; Thompson and Hamilton 1986; Hunton 1985,1989) perhaps with justification. While both tests provide a measure of the maximum force or height required to cause an eggshell to break, only one area of the shell, usually the equator, can be tested. Shell strength not only varies from one equatorial region of the shell to another (Voisey and Foster 1970) but also varies from pole to pole (Tyler 1961b).

In puncture tests a punch is applied to the surface of the egg and the force required to penetrate the shell is then used as an index of its' strength. This test has the advantage in that multiple measurements can be made on each egg provided the shell does not suffer catastrophic failure (Voisey and Hunt 1974). The basic requirements for the pucture test however are similar to those of the quasi-static compression test, namely a constant punch speed and a precise method of recording the applied force.

[ii] Indirect Measures Relating to Eggshell Strength:

The simplest and most traditional method of evaluating shell quality has been to measure the thickness of the shell with a screw gauge micrometer. This however requires the shell to be broken and to obtain reliable results several samples from each eggshell must be assessed (Tyler 1961b). Alternative strategies which have been used to estimate the amount of shell material present include measuring the weight of the entire shell, and relating this to the weight of the whole egg or to the surface area of the egg (Hughes 1984).

The non-destructive (ND) deformation test and the specific gravity (SG) test are probably the most widely used of all methods to estimate the strength of eggshells. These tests find favour with industry because they are non-invasive, inexpensive and can be performed rapidly. Multiple measurements are also permitted.

The non-destructive (ND) deformation test, first described by Schoorl and Boersma (1962), represents a compromise between the above mechanical tests which destroy the eggshell and those physical measurements which relate indirectly to eggshell strength. In this test a known load, usually less than that required to break the shell, is applied to the egg and the amount by which the shell deforms is then recorded either using a strain gauge (Schoorl and Boersma 1962) or an electronic transducer (Voisey and Foster 1970).

Theoretically an inverse relationship exists between the stiffness characteristics of the eggshell and its ultimate strength (Voisey and Hamilton 1975). Thus the assumption is that a low deformation value signifies a stronger shell.

An eggs' specific gravity (SG) is assumed to be related to the % shell (Olssen 1934-from Hamilton 1982) which is in turn related to its' thickness. There are two ways in which the specific gravity of an egg can be measured: flotation in saline solutions or by Archimedes' principle. Voisey and Hamilton (1977b) and Hamilton (1982) discuss the potential sources of error associated with this technique.

With the flotation method eggs are immersed sequentially into a series of saline solutions of ascending SG. The SG of any egg is equal to the SG of the solution in which it first floats. Park *et al* (1986) suggest that SG measurements using three rather than five solutions contain enough information to accurately predict sample means.

To assess SG by Archimedes' principle eggs are first weighed in air, submerged in water, then re-weighed. The SG can then be calculated from,

$$S.G = \frac{\text{Egg wt in air}}{[\text{ Egg wt in air - Egg wt in water }]}$$

This method may be more precise, but it is considerably more time consuming.

The problem with all of the above indirect tests is that the parameters measured are often erroneously referred to as being synonymous with eggshell strength but increasing the thickness of an eggshell does not necessarily make it stronger (Hammerle 1969).

1.1.7 STIFFNESS AND STRENGTH OF EGGSHELLS.

When a load is applied to an object, equilibrating internal forces, in terms of stresses and strains are generated. An objects' ability to resist internal stresses and strains is dependent on its' elastic modulus (E) and its' geometry (including thickness). In contrast, strength is the force or stress required to break the bonds between adjacent atoms (ultimate failure stress or UFS) and is distinct from stiffness (Figure 2). It is therefore important to distinguish between material and structural effects when interpreting the performance of an object under load.

In all the methods currently used to measure eggshell strength, the data reported are given in terms of the force required to crush the egg. These data do not describe any physical property of the material from which the eggshell has been constructed, but describe the response of the eggshell as a composite complex structure (Voisey and Hunt 1974).

[i] Direct Measurement of the Material Properties of Eggshells:

Due to its' brittleness and curvature, the evaluation of an eggshells' material properties (E and UFS) by classical engineering methods is difficult (Voisey and Hunt 1974; Hamilton 1982). Such tests usually require specimens to be of a simple shape and size so that the stresses imposed during subsequent testing can be easily derived.

Peterson and Tyler (1967) cut strips of shell from a number of hens eggs and compared their bending strength to that of the Guinea fowl egg. While these tests suggested that the material strength of the latter was greater than that of the hen, no attempt was made to quantify this. Voisey and Hunt (1974) suggested that the simple theory of bending (see Gordon 1976) could have been applied to these data. In order to do so however, the edges of the test specimen must be smooth and parallel as this affects the expected stress distribution along the beam (Voisey and Hunt 1974). Simple beam theory however ignores the fact that the eggshell is curved.

[ii] Indirect Measurement of the Material Properties of Eggshells:

The elastic modulus (E) and ultimate failure stress (UFS) of eggshells can also be derived indirectly from an analysis of stresses induced in the eggshell under some form of loading where the analysis is no longer simple. Until recently however, the mathematics, together with the assumptions and number of unknowns in the relating equations, have essentially discouraged this type of investigation. As a result, there is only a limited amount of data available on the material properties of the eggshell.

Rehkugler (1963) calculated the eggshells' modulus and failure stress from the diametrical deflection of ring and semi-circular portions of eggshell under compression. Only ten specimens were tested, the results were highly variable, and the test apparatus used was extremely crude. Assuming that the eggshell approximates to a prolate spheroid, Voisey and Hunt (1967b) subsequently used Rehkuglers' (1963) values for E and UFS, along with an existing "thin shell" solution (Reissner 1947), to predict the force at failure of eggshells under compression. Their best estimates however were found to be much less than those observed during subsequent laboratory tests.

Tung *et al* (1969) applied existing analytical solutions, as developed for a shallow spherical dome subjected to point or uniformly distributed forces, to the situation in which an egg is loaded at its poles. While both the modulus and the failure stress estimates in this case were consistently higher than those obtained by Rehkugler (1963), the estimated failure stress was also considerably higher than that obtained by Hammerle and Mohsenin (1967) and Sluka *et al* (1967). The latter authors used internal hydrostatic pressure techniques to estimate the ultimate failure stress of the eggshell but it is unclear how this type of stress relates to the type of insult experienced by an egg in the field.

In view of these diverse and conflicting reports, it is still a matter of debate as to whether the geneticist and the nutritionist should concentrate on improving the thickness of the eggshell, or redirect their efforts towards improving its' material properties.

1.1.8 AIMS OF CURRENT WORK.

As a direct result of the increased requirement for high safety standards and quality control, advanced computational methods, such as finite element analysis are now routinely used by engineers to analyse the theoretical levels of stress and strain induced in complex multilayered structures. In this thesis finite element analysis is applied to the eggshell in an attempt to determine the relevant material and structural variables.

The thesis can be divided into three parts. First, the concepts behind the finite element method are examined and the steps involved in the generation of eggshaped finite element models are discussed. Chapter 3 addresses itself to those factors affecting the stiffness characteristics of the eggshell and describes a method for calculating the elastic modulus of eggshells. This is then followed by a detailed investigation into the mechanism of failure in eggs subjected to static loading conditions. Throughout this work, all theoretical considerations are supported by experimental data and attention is directed to the role of shell ultrastructure. Finally, a detailed study of shell quality in two different commercial laying flocks is examined with reference to changes in stiffness, strength and ultrastructural characteristics as the birds age.

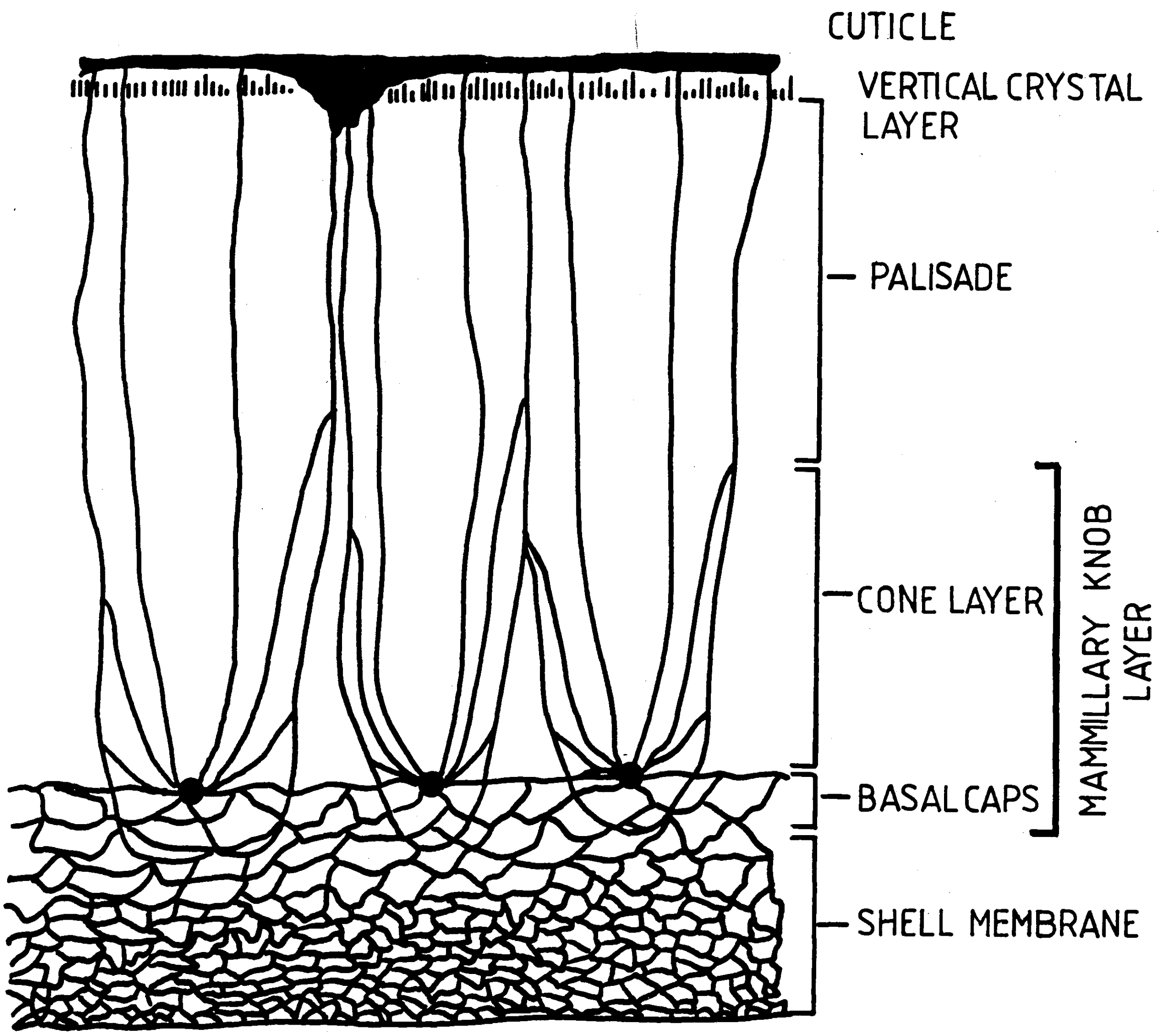


Figure 1 Diagramatic representation of a transverse section through the hens' eggshell (modified from Parsons 1982).

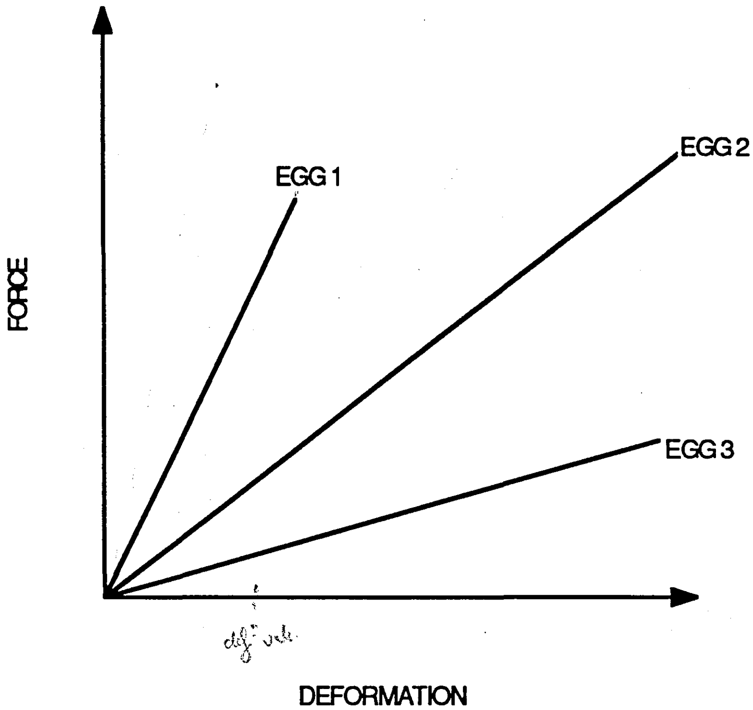


Figure 2 The strength of an eggshell is often independent of its stiffness characteristics. Egg 1 is stiffer but weaker than Egg 2, while Egg 2 is stiffer and stronger than Egg 3.

CHAPTER 2

SECTION 1: AN INTRODUCTION TO FINITE ELEMENT ANALYSIS.

2.1.1 STRUCTURAL ANALYSIS: ANALYTICAL AND NUMERICAL METHODS.

Structural analysis is used by engineers to determine the distribution of forces and displacements in structures under loads. Classical analytical methods describe this type of mechanics problem in terms of partial differential equations which are solved with appropriate boundary conditions. In the majority of cases this requires the use of simplifying assumptions to reduce the problem to one that can be solved. However, in practice most problems are too complicated for a closed-form mathematical solution.

The development of numerical methods for obtaining approximate solutions in structural analysis has largely removed the need for analytical methods. Finite element analysis is possibly the most general and powerful of these methods and is now widely available in commercial computer codes.

The concept of the finite element method (henceforth, referred to as the FE method), is that the structure can be modelled by subdividing it into discrete subregions (the finite elements). Figure 3(a), for example shows a rectangular plate subjected to the action of a series of in-plane forces. A FE analysis of this problem might typically involve the following steps. First, the geometry of the plate is defined and subdivided into a number of finite elements. Figure 3(b) shows the plate divided up into 32 triangular finite elements. Next, the material properties, the loads, and the boundary conditions, are defined. The programme then proceeds to the construction of algebraic equations that describe the behavior of each individual finite element: the element stiffness equations. The element stiffness equations are in turn combined to form the overall stiffness equations for the entire model, which are solved to give the displacements throughout the structure. Finally, by a process of back substitution, the solution for stress and strain within the structure and for the support reactions are obtained.

2.1.2 ELASTIC THEORY, EQUILIBRIUM AND COMPATIBILITY.

FE solutions are often based on static theory (Cook 1981) and must satisfy equilibrium of forces at every point throughout the structure. Similarly the displacements in each element must be continuous (compatibility), in other words, the displacements generated throughout the entire model are such that when the model deforms, no cracks or kinks appear, and no part overlaps another. Elastic FE solutions therefore relate to the stiffness characteristics of a structure in terms of the stresses and strains induced throughout its entirety under the prescribed loading conditions. While this indicates which regions of the structure are most highly stressed or deformed, the strength, or the loading conditions required to induce structural failure cannot be directly obtained from FE analysis. The latter can only be derived experimentally.

As a preliminary to applying FE analysis to the testing of eggshells, the tasks necessary to perform a FE analysis, and some of the above ideas underlying the components of the FE method are now discussed in greater detail.

2.1.3 MODEL GENERATION.

[i] Geometric Considerations:

The first step in any FE analysis is to generate a geometric representation of the structure on which the analysis is to be performed. A number of programmes which employ computer graphics are available to facilitate this. PATRAN and FEMGEN are two commercially available FE mesh generator codes. These codes are capable of creating planes and surfaces from 3 dimensional coordinates. The resulting geometry is then automatically subdivided or meshed once the most appropriate element type and number have been specified.

[ii] Element Formulations and Types of Finite Elements:

Finite elements take many and varied forms depending on the shape of the structure they are to be used to represent. Some of the most commonly used elements are illustrated in Figure 4. The first elements are referred to as thin-membrane elements, and are triangular or quadrilateral in shape. These basic elements are used in a wide range of in-plane loading applications in which the material has no resistance to bending. Extension of this concept to three dimensions results in a family of solid or brick elements of which the tetrahedron and the hexahedron are the most common shapes. The remaining elements depicted in Figure 4 can all be loaded with bending moments and normal loads in addition to in-plane loads. The first of these are used to study the behaviour of flat plates and shells. However in many cases real structures have curved boundaries and so curved-shell elements are usually more suitable (Figure 4d). Finally, axisymmetric or shell-of-revolution elements can be used in a restricted class of symmetric curved shell problems.

Irrespective of type, each element is defined by a series of nodal points. Higher order finite elements have more nodes per element and as a result the internal displacement function is defined using more complex mathematical functions. Thus for a planar triangular element the simplest form is one in which the node points are merely the vertices of the triangle (Figure 5a) and the mathematical function that defines the displacement is linear. These are referred to as first order elements. Two examples of higher order triangles are the six noded element (second order) which is based on a quadratic description of the displacement (Figure 5b), and the ten noded triangle which is based on displacements in the form of cubic polynomials (third order) (Figure 5c)

[iii] Choice of Finite Element:

All FE solution are approximations to the exact solution, and the accuracy of solution can generally be increased by employing more elements. However, the cost of the analysis is often a quadratic or even a cubic function of the number of nodal points in the model. Thus, FE analysis requires a decision on the part of the analyst as to how many elements should be used and how they should be distributed in order to minimise the error and cost.

The optimum choice of element can be established by ensuring that the FE results converge to the correct solution with an increase in mesh refinement (Figure 6a). This can be achieved by first analysing an appropriate but simpler problem for which the results are known analytically. The FE predictions obtained using various types of mesh may then be compared to the correct solution and those combinations which do not show convergence are thus avoided (Figure 6b). The most appropriate FE mesh determined in this way can then be applied with confidence to other related but more complex problems provided the geometry of the latter can be generated in a similar way (Ross 1985). This type of preliminary exercise is often referred to as 'bench marking'.

2.1.4 INPUT DATA.

A typical input file listing is given in Table 1 for the FE code ABAQUS (HKS Inc). Input data include information relating to the node numbers and their coordinates, the element numbers and the nodes which define each individual element, and boundary conditions which are restrictions on movement imposed at specific points on the model. The latter ensure that the model behaves in a manner similar to that observed in the real structure under experimental test conditions. Additional boundary conditions may also be imposed in symmetric problems where only part of the structure needs to be analysed (Cook 1981). The structural properties (characterised by shape and thickness) and the material properties (elastic modulus and Poisson's ratio) are also defined in the input data, followed by the class of problem, for example dynamic or static loading. Lastly, the type of information required from the analysis is specified, for example generalised displacement, maximum and minimum principal stresses.

2.1.5 DATA VERIFICATION AND WAVEFRONT PROCESSING.

The large amounts of data in any FE analysis must be checked, since the consequences of error are costly. Computer graphics, with the use of both display devices and plotters aid this process. 'Wavefront' processing is also carried out. This process determines the magnitude of the problem in terms of disc space and effort required to perform the analysis. In the present code the elements are internally renumbered to optimise the solution time (HKS Inc).

2.1.6 THE ANALYSIS.

In this task the equations describing the FE model are generated and solved. The element stiffness equations represent the basic building block of any FE analysis. These equations relate the forces at the nodes to the displacements at these points. The information needed to formulate the element stiffness equation includes:

1) The elastic constants of the material of which the elements are composed.
2) The relevant strain and the displacement equations for the element.
3) A description of the displaced state of the element.

The elastic constants are defined in the input data. The relationships between stress and displacement are an aspect of elastic theory and are readily defined by reference to a standard text (Timoshenko and Goodier 1970). Generally, the description of the displaced state of an element is an assumption made by the behaviour of the element as it deforms as part of the loaded structure. A very simple form of behaviour is often assumed, typically described by polynomial expansions. In this way the rather complicated behaviour of the total structure is modelled by a large number of simple descriptions of behaviour associated with the component elements. Once the stiffness equations have been constructed for each individual element, the latter are then combined to produce the stiffness equations of the complete model. This is accomplished by enforcing the conditions of equilibrium and continuity at every node at which elements are joined. The calculations are then performed at Gauss points (Ross 1985) within each element and extrapolated to the nodes.

2.1.7 POST PROCESSING.

The output of the analysis must be manipulated and edited in order that it can be presented in "user friendly" formats. This includes conversion of the data to report formats and the plots of internal loads and deflections.

2.1.8 THE SCOPE OF THE FINITE ELEMENT METHOD IN PRACTICAL PROBLEMS.

Advanced FE analysis codes are currently available for solving 3 dimensional solid, plate and "shell" structural problems. The power of the FE method results from a number of features. For example, each and every element can consist of a different thickness, or be assigned different material properties. In addition each element can consist of a number of different layers, each having different material properties. Thus orthotropic and composite materials can be handled with the same ease as isotropic materials as long as the properties of the former are known. Variations in geometry can also be easily handled. Finally, loads can be applied at specific points or can be distributed over the surface of structures thereby acting as applied pressures.

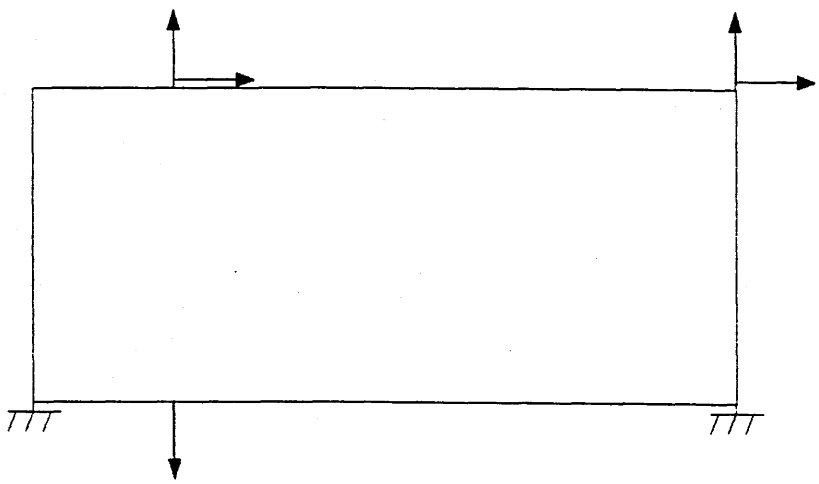


Figure 3a) Rectangular plate subjected to the action of forces in its plane.

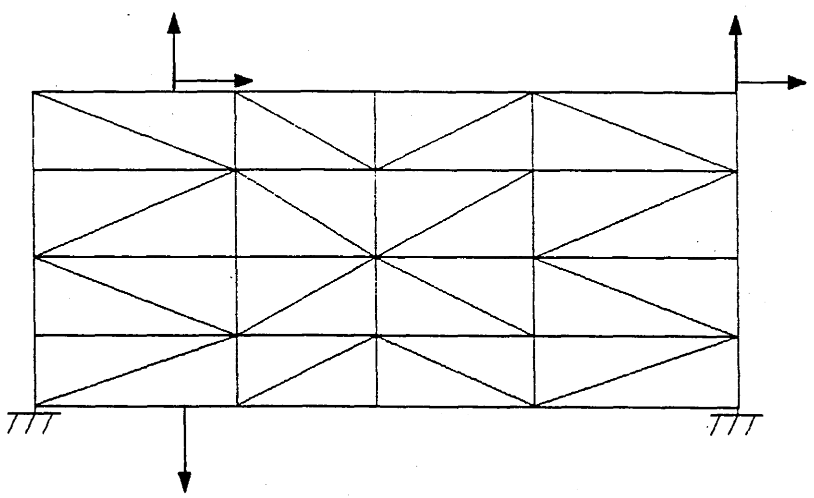


Figure 3b) Finite element representation (modified from Gallagher 1987).

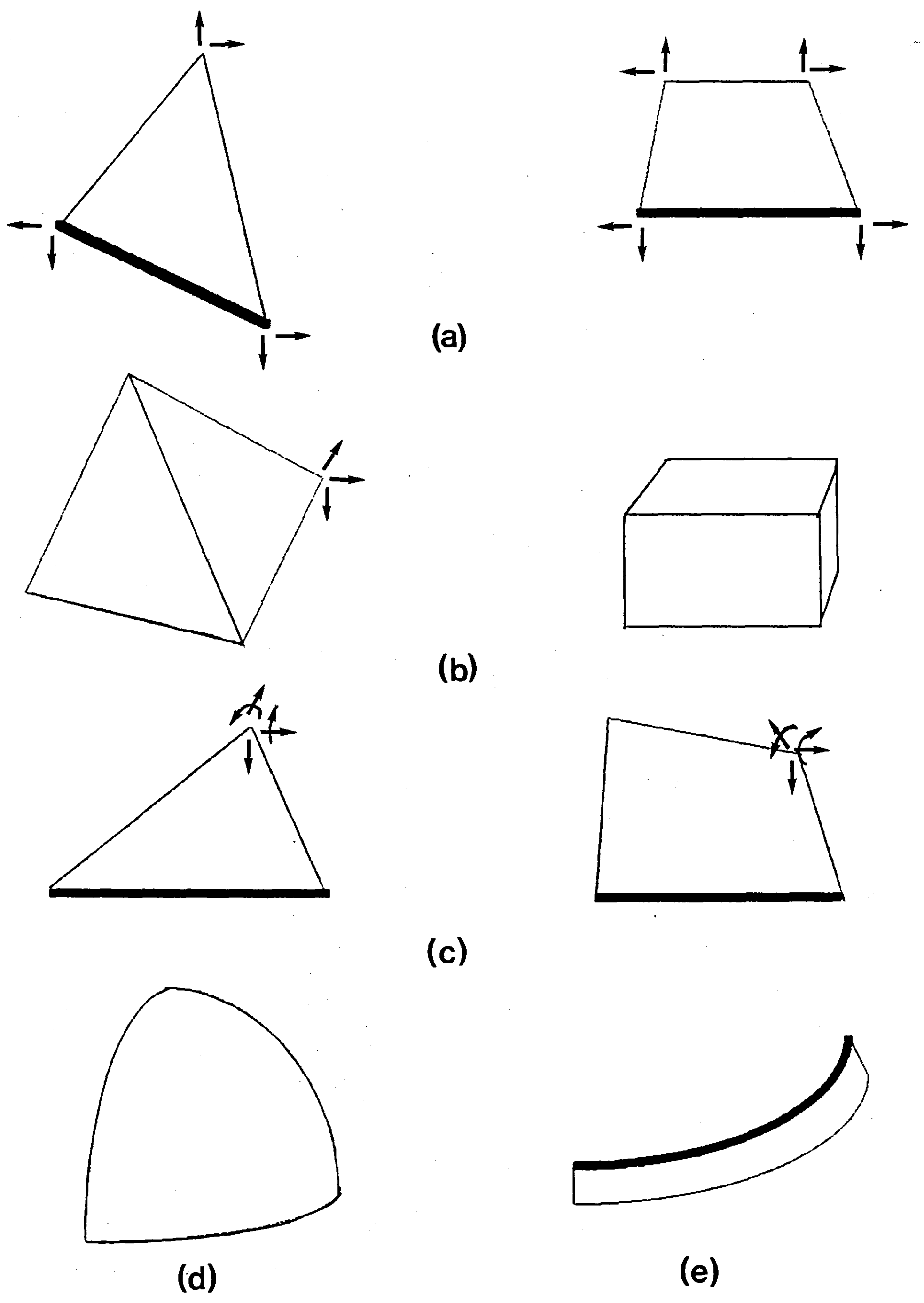
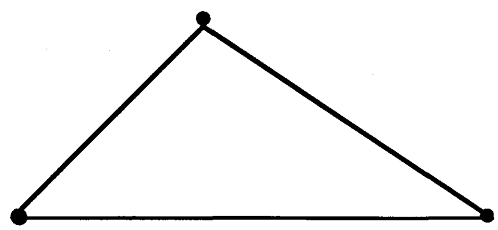
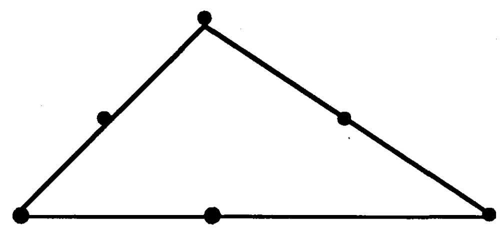


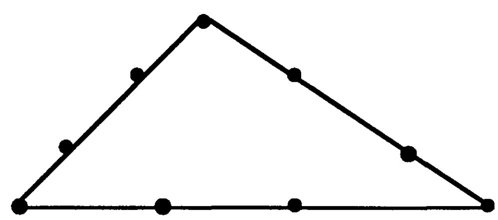
Figure 4 A Selection of different finite element types: a) Membrane elements; b) Solid or brick elements; c) Plate bending general shell elements; d) Curved isoparametric shell elements; e) Axisymmetric shell elements (modified from Balderes 1987).



a) First order triangle.



b) Second order triangle.



c) Third order triangle.

Figure 5 Triangular finite elements: a) Three noded basic or linear triangle; b) Six noded triangle based on quadratic displacements; c) Four noded triangle based on cubic displacement (modified from Gallagher 1987).

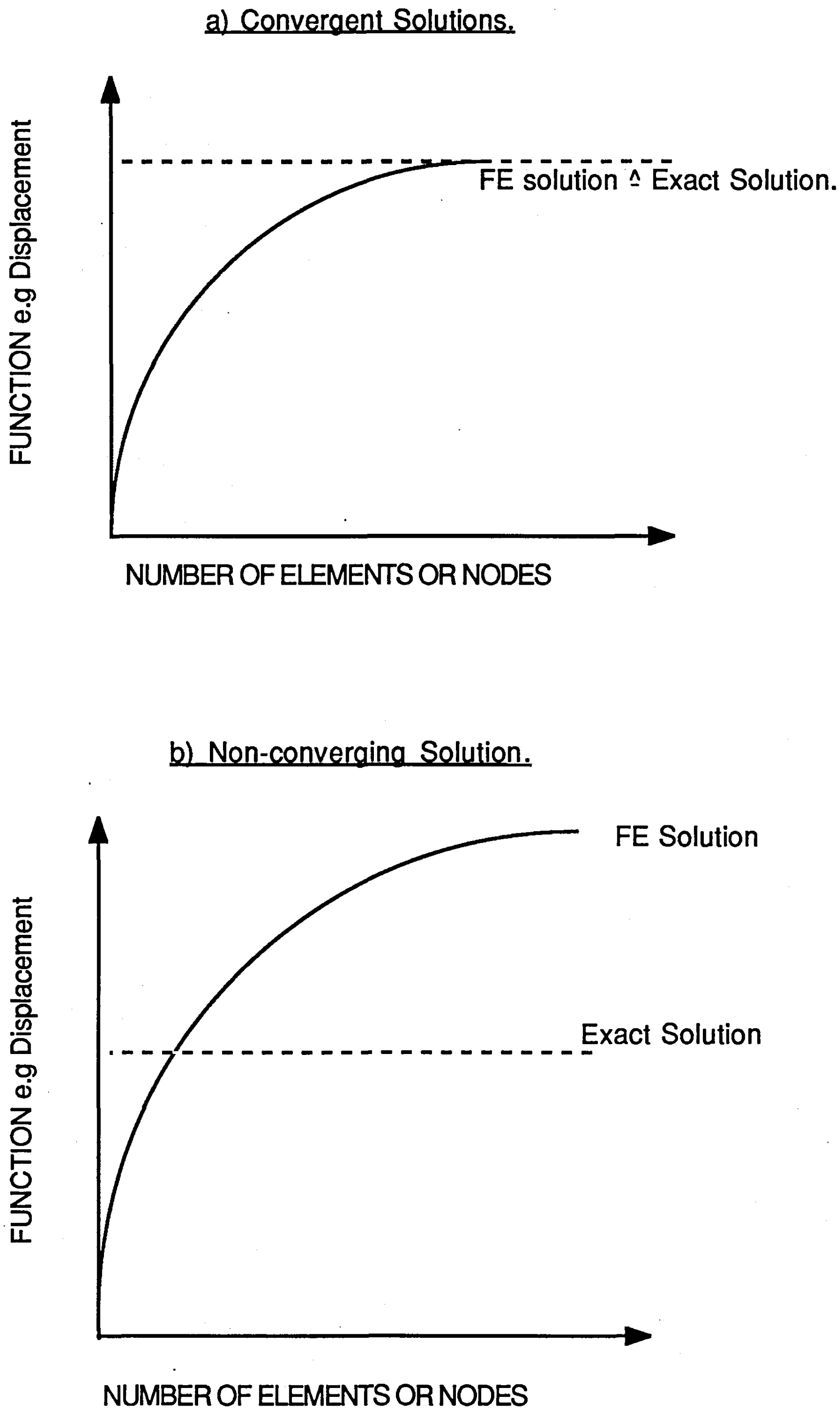


Figure 6 Converging and non-converging FE solutions (modified from Ross 1985).

* HEADING	REFERENCE NAME FOR ANALYSIS
* NODE	GEOMETRY DEFINITION. NODE LIST AND CORRESPONDING CARTESIAN COORDINATES ARE GIVEN.
* ELEMENT	ELEMENT TYPE AND NUMBERS ARE DEFINED AND THE NODE NUMBERS ASSOCIATED WITH EACH ELEMENT ARE DEFINED.
* BOUNDARY	BOUNDARY AND SUPPORT CONDITIONS. DEFINITION OF NODES THAT ARE ALLOWED TO REACT IN A SPECIFIED MANNER e.g MOVEMENT IS PERMITTED IN X,Y,Z PLANES OR ROTATIONS ABOUT THE X,Y,Z AXES. DEFINED IN TERMS OF DEGREES OF FREEDOM.
* MATERIAL	DEFINITION OF MATERIAL PROPERTIES i.e ISOTROPIC, ANISOTROPIC ELASTIC, ELASTIC/PLASTIC etc. THICKNESS , POISSON'S RATIO, ELASTIC MODULUS ARE ALSO REQUIRED.
* PLOT * VIEWPOINT * DRAW	PLOTTING OPTIONS. GRAPHICAL OUTPUT OF MESHED GEOMETRY BEFORE THE ANALYSIS IS CARRIED OUT.
* CLOAD	DEFINITION OF LOADING OPTIONS. TYPE OF TEST IS DEFINED, STATIC OR DYNAMIC; DIRECTION AND MAGNITUDE OF LOAD ; POINT LOAD OR DISTRIBUTED LOAD.
* ELNUM * NODUM	NUMERICAL OUTPUT OPTION. DEFINITION OF ELEMENTS OR NODES WHICH ARE OF PARTICULAR INTEREST AND TYPE OF INFORMATION REQUIRED BY THE ANALYST e.g DISPLACEMENT, MAXIMUM/MINIMUM PRINCIPAL STRESSES.
* PLOT * VIEWPOINT * CONTOUR * DISPLACE	POST ANAYSIS PLOTTING OPTIONS. GRAPHICAL REPRESENTATION OF ANALYSIS FROM A PARTICULAR VIEWPOINT e.g: STRESS CONTOUR MAP IS PLOTTED USING THIS OPTION.. DISPLACED MESH IS PLOTTED USING THIS OPTION.
* END STEP	TERMINATES THE ANALYSIS.

Table 1 A typical Input File Listing for the FE code ABAQUS (HKS Inc).

CHAPTER 2

SECTION 2: MODEL DEVELOPMENT.

2.2.1 INTRODUCTION.

In structural engineering, the term "shell" or "shell structure" is used to define a solid material enclosed between two closely spaced doubly curved surfaces, the distance between these two surfaces being the thickness of the "shell" (Cook and Young 1985). If the thickness (t) is small compared to the overall dimensions of the bounding surfaces (R), shear stresses acting through the thickness of the "shell" can often be ignored and the structure is referred to as a "thin shell".

Analytical solutions exist for many simple "thin shell" structural problems. Koitre (1963) provided an analytical solution for the pinched sphere problem. This problem consists of a hollow sphere with two opposing point loads acting along a diameter of the sphere (Figure 7). The response to loading at each pole is very localised and can be expressed in the following way ;

$$d = \frac{3\sqrt{(1-\nu^2)}}{4} \frac{F R}{E t^2} \left[1 + \frac{2(1+\nu)}{\pi \lambda^2} [\log \lambda + \gamma - 1 + {}^1/_2 \log 2] + \frac{4}{3\pi\lambda^2} + \lambda^{-3} \right] \quad \text{Eq(1)}$$

where, d is the radial displacement, F is the applied force, E is the elastic modulus, ν is Poisson's Ratio, t is the thickness of the "shell", R is the radius, $\lambda = 3(1- \nu^2)$ R/t , and the constant γ is equal to 0.5772.

The trailing term in this expression closely approximates to unity allowing the equation to be rewritten as,

$$\frac{d E t^2}{F R} = C \quad \text{Eq (2)}$$

where C is now defined as the non-dimensionalised compliance.

In many structural problems the shear terms are significant. In such cases the "shell" must be analysed as a "thick shell" in which both the normal stresses and shear components are taken into consideration. Unlike the majority of existing analytical solutions, the present FE method adopts a "thick shell" formulation which reduces to the limiting case of "thin shell" behaviour as the shear components approach zero.

In engineering terms, the eggshell has generally been regarded as a classic case of a thin-walled "shell structure". As a result, analytical solutions, such as that described by Koitre (1963), have been directly applied to the egg (Rehkugler 1963; Voisey and Hunt 1967b; Tung *et al* 1969) in an attempt to determine its material properties. In each case the eggshell was assumed to be spherical, homogeneous and isotropic.

The eggshell is now known to consist of a number of morphologically distinct layers, and as a result it is natural to suggest that each of these will confer upon the egg different properties with respect to both its' stiffness and ultimate strength. Since FE programmes are now available for analysing such complex 3 dimensional multilayered "shell" structures, it was decided to apply this method to the case of the eggshell under load. This section describes the steps involved in the generation of a "standard" egg shaped FE model.

2.2.2 MATERIALS AND METHODS.

Many of the traditional methods of measuring eggshell strength are performed equatorially because the egg is regarded as being weakest at the equator (Voisey and Hunt 1964). In order to standardise the sample site it was decided that in FE models, the load should also be applied at the equator, thus enabling the resulting solutions to be directly compared to experimentally derived data.

Figure 8 summarises the steps involved in the generation of the "standard egg" shaped FE model. Each model was generated with the aid of the commercial FE mesh generating codes PATRAN or FEMGEN, and analysed using the FE code ABAQUS available on a VAX II/750 computer.

[i] Step 1: Development of an Appropriate Bench Mark Solution:

A priori, the eggshell can be expected to behave like a "thin shell", in which the shear components can be ignored. As a result the 'pinched sphere problem' as described by Koitre (1963) would seem to provide a convenient "bench mark" solution on which to base modelling. To check the validity of this assumption, the pinched sphere problem was re-analysed using the FE method for a range of "shell" thicknesses, that is R/t values including those ratios most commonly associated with eggshells.

Due to the symmetry of this problem, the reaction at each loading point can be assumed to be identical (see Figure 7). As a result it was not necessary to model and analyse the entire problem, and so only half of the sphere was generated in each case.

Second order axisymmetric shell elements (henceforth referred to as SAX2) are the most convenient elements for modelling structures with cylindrical or spherical symmetry and so the latter were chosen in this step. To check convergence of the FE solution towards the correct solution, 10, 20, 30 and 100 SAX2 element meshes were generated using PATRAN. After applying the appropriate boundary conditions, each model was then analysed under point load conditions (see Figure 9).

"Shell" problems are widely regarded by mechanical engineers as being insensitive to the choice of Poisson's ratio, and as the solutions are non-dimensionalised, the elastic modulus is removed from the final result (Hancock pers comm). The material constants used in the above models were therefore arbitrarily chosen to equal 150kN/mm^2 (elastic modulus) and 0.3 (Poisson's ratio) respectively.

Following each analysis, the displacement [d] at the load point was recorded and used together with the appropriate values of E, R, F, and t, in Eq(2) to calculate the non-dimensionalised compliance (C) for $25<R/t<1000$. The latter were then compared to that analytically derived by Koitre (1963).

<u>[ii] Step 2: Determination of an Appropriate General Shell Element Mesh for use in Eggshaped FE Models:</u>

Axisymmetric shell elements cannot be used in eggshaped models due to the way in which the load is commonly applied in egg-strength tests. Curved general shell elements must be used to model this type of non-symmetric problem, although it is also possible to use simple flat planar elements for this purpose.

In order to establish the most appropriate element formulation for use in eggshaped models, a bench marking procedure was first carried out using quarter spherical FE models meshed with different types and numbers of the following general shell elements: a) eight noded isoparametric second order shell elements (hereafter referred to as QU8); b) three noded linear strain triangular shell elements (hereafter referred to as STRI3); and c) eight noded isoparametric elements combined with two, three noded linear strain triangular shell elements (hereafter referred to as QU8/STRI3).

QU8 elements are particularly suited to modelling curved shell structures, but due to the complex mathematics associated with this type of element, the number of QU8 elements that can be applied to any problem is limited. In contrast, STRI3 elements are less suited for use in curved shell applications. Indeed it has been suggested that at least three times more planar elements are required within the region of maximum stress to obtain the same degree of accuracy as one isoparametric element in this type of problem (Hancock pers comm). However, since the calculations are performed using linear instead of quadratic functions, there is usually less restriction on the numbers of these simpler elements that can be applied.

The basic geometry used in the bench marking procedure is illustrated in Figure 10. In some cases modifications to this basic model were required before the mesh could be applied. For example, QU8 elements at the time of writing could not be degenerated into triangles, a condition which was necessary at the polar regions when only quarter of the sphere was generated. This problem would not have arisen had the total sphere been modelled, but to do so would have been expensive in terms of the central core processing unit time (CPU) required to perform the analysis. To accommodate the latter type of mesh then, the polar regions of the model had to be flattened by defining additional surfaces (Figure 11). The alternative was to mesh these problematic areas with a triangular element. This concept was applied in the QU8/STRI3 element combination mesh (Figure 12).

Once a satisfactory mesh had been generated in each case, the appropriate boundary conditions were applied, then each model analysed under point loading. The resulting displacement [d] at the load point was recorded in each case and used together with the appropriate values of F, E, R and t in Eq (2) to calculate the non-dimensionalised compliance (C) as above. Each mesh was then assessed in terms of the ease with which it was generated and its accuracy of solution when compared to the "bench mark" solution (see Step 1). The disc space and CPU time required to carry out each analysis was also taken into consideration.

[iii] Step 3: Generation of a Standard Eggshaped Model:

A quarter FE representation of a "standard" shaped egg was generated in a piecewise linear fashion by assuming that the shape of an egg can be mathematically described in terms of a sheared ellipse (Figure 13):

$$\frac{[X + Y \tan ø + a^2]}{a^2} + \frac{Y^2}{b^2} = 1 \qquad \text{Eq (3)}$$

Here a is equal to $^1/_2$ length, b is equal to $^1/_2$ breadth, tan ø is equal to [x-a]/b, x being the distance between the maximum breadth and the midpoint of the egg, and, X and Y are the cartesian coordinates in the X and Y planes respectively.

Measurements of length, breadth and x were obtained for a number of eggs using a shadowgraph machine. The profile of the "standard" shaped FE model was based on their mean values.

The most appropriate general shell element mesh derived from Step 2 was then applied to this model.

2.2.3 RESULTS AND DISCUSSION.

[i] Step 1: The Development of an Appropriate Bench Mark Solution:

According to Koitre's (1963) solution there is only a very localised response when a hollow sphere is subjected to a point load. This was confirmed in current investigations using FE analysis (Figure 14). The radial displacement decays rapidly and is only significant within a 15-20 degree arc of the load point.

The non-dimensionalised compliance values for the pinched sphere problem obtained using 10, 20, 30 and 100 SAX2 elements for a range of "shell" thicknesses are compared to Koitres' (1963) "thin shell" solution in Figure 15. From this figure it is clear that there is no convergence between the FE solutions and Koitres' "thin shell" solution when only 10 or 20 SAX2 elements are used. These results contrast with the findings of Hibbitt *et al* (1988). Comparing the radial displacement derived using various axisymmetric element meshes, the latter authors concluded that for a ratio of R/t = 50, the pinched sphere problem could be accurately modelled using just 10 SAX2 elements. While the 10 SAX2 element solution and Koitres' "thin shell" solution do happen to coincide at R/t=50 in Figure 15, they certainly do not converge, and as a result the findings of Hibbitt *et al* (1988) can now only be considered fortuitous. In contrast, the 30 and 100 SAX2 element solutions not only coincide with each other over the range of R/t values tested but converge with Koitres' "thin shell" solution for all values of R/t>300. Thus it would appear that at least 30 SAX2 elements are required to accurately predict the "thin shell" solution to this problem.

For R/t<300, both the normal and shear components appear to be important in generating the resistance of the sphere to load. This can be deduced from Figure 15 by the fact that the 30 and 100 SAX2 FE solutions diverge from the Koitres' "thin shell" solution within this range of R/t values. These findings are in agreement with Aswell and Gallagher (1976) who found that whilst the shear components could be neglected in the pinched, open-ended, cylinder problem when R/t=320, the shear components must be taken into consideration for cylinders in which the ratio R/t=52.7.

Now, assuming R is equal to half of the breadth dimensions of an eggshell and t is equal to its' total thickness, the ratio R/t most commonly associated with an egg lies within the range 50 < R/t < 100. From Figure 15, it is clear that even within this range the shear components play a significant role, and as a result Koitres' "thin shell" solution becomes inappropriate for use as a "bench mark" solution on which to base egg shaped modelling. The equivalent 30 SAX2 FE solution to this simple problem is now much more appropriate.

[ii] Step 2: Determination of an Appropriate General Shell Element Mesh for use in Eggshaped FE models:

Table 2 summarises the advantages and disadvantages encountered in meshing quarter spherical models with QU8 elements, STRI3 elements and QU8/STRI3 element combinations. The solutions to the pinched sphere problem which were subsequently obtained using these different types of mesh are compared to the appropriate 30 SAX2 element "bench mark" solution in Table 3.

When a 208 QU8 element mesh was applied to the quarter sphere, the non-dimensionalised compliance was underestimated by 23% for R/h=50. This was disappointing but the modifications made to the basic geometry in order to accommodate this mesh made it difficult to concentrate these elements towards the load point.

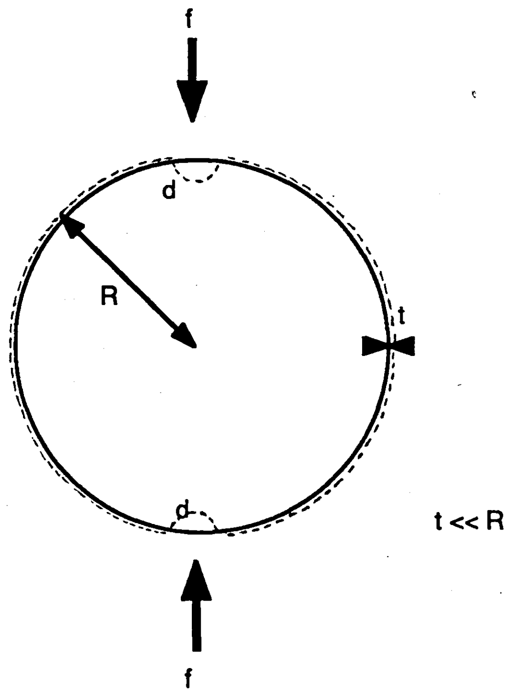
The solution obtained when the model was meshed with 1412 STRI3 elements also underestimated the compliance for R/t=50. However in this case no modifications to the basic geometry were required; there was no restriction to the number of elements that could be applied; and the mesh was easily concentrated towards the load point.

In theory, the combined element mesh should have displayed all of the advantages associated with the use of QU8 elements whilst dispensing with the need for geometric modification and large numbers of STRI3 elements. However when more than one element type is used to mesh a single geometry, it is necessary to define by hand the set of nodes which are common to both element types at their interface thus creating a continuous surface. In the current combined element model, one three noded STRI3 element was constrained to a large number of eight noded QU8 elements at each apical end, and as a result listing the constrained nodes by hand proved tedious and very time consuming. For the effort involved in generating this mesh, the accuracy of the resulting solution was unrewarding (Table 3).

From these tests then it would appear that of the general shell elements that were available at the time of writing, the STRI3 element mesh is the most appropriate to use in the generation of eggshaped FE models. The latter provide a similar accuracy of solution to that obtained using isoparametric curved shell elements, but without the need for geometric modification and restrictions in element numbers. Indeed when the pinched sphere problem was subsequently analysed using 3306 STRI3 elements, the resulting solution converged with the 30 SAX2 bench mark solution for R/t=65 (see Table 3).

[iii] Step 3: A "Standard" Eggshaped FE model:

Figures 16 and 17 show the FE representation of quarter of a "standard" shaped egg which has been meshed using 3306 STRI3 elements.



f = applied load; R = radius; t = thickness; d = resultant displacement; solid lines = original geometry; dashed lines = displaced geometry.

Figure 7 "The Pinched Sphere Problem" (after Koitre 1963).

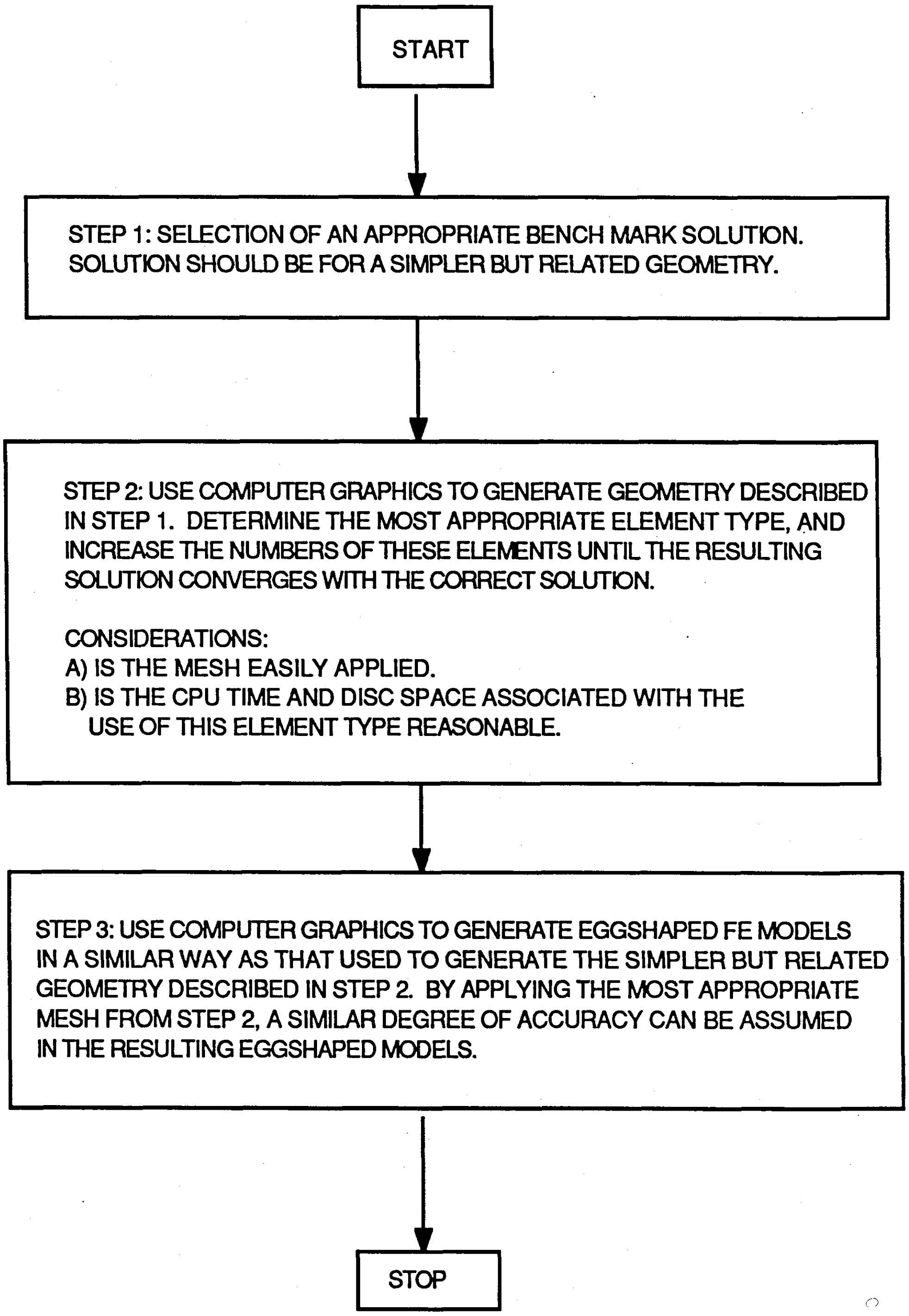


Figure 8 Stepwise generation of eggshaped finite element models.

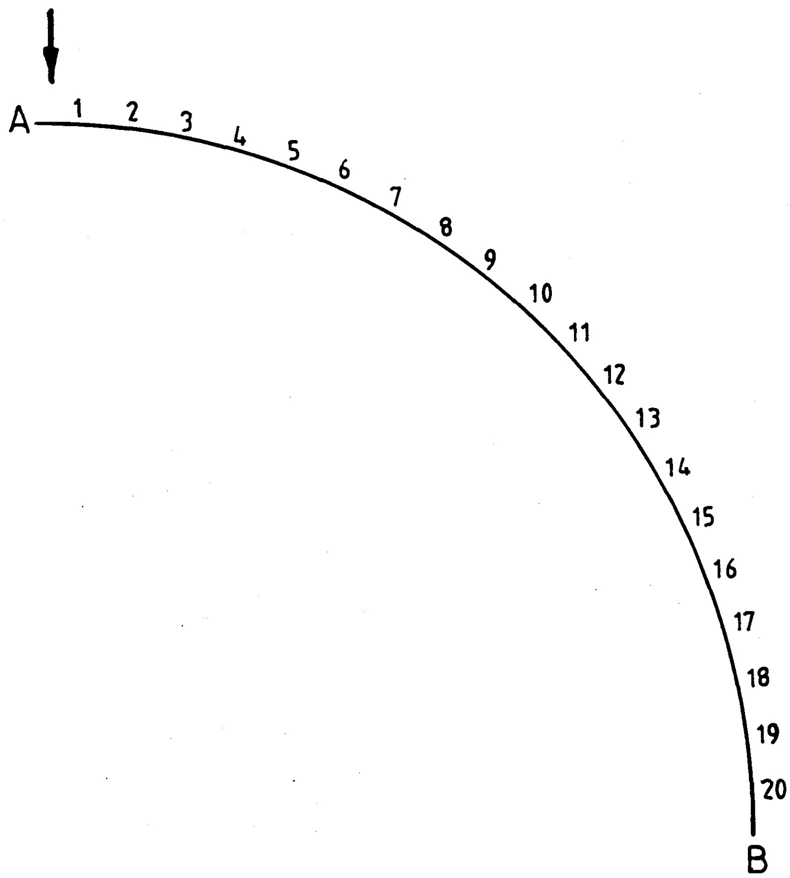


Figure 9 Second order axisymmetric shell element representation of the pinched sphere problem. Only half the geometry is illustrated (Numbers = Elements).

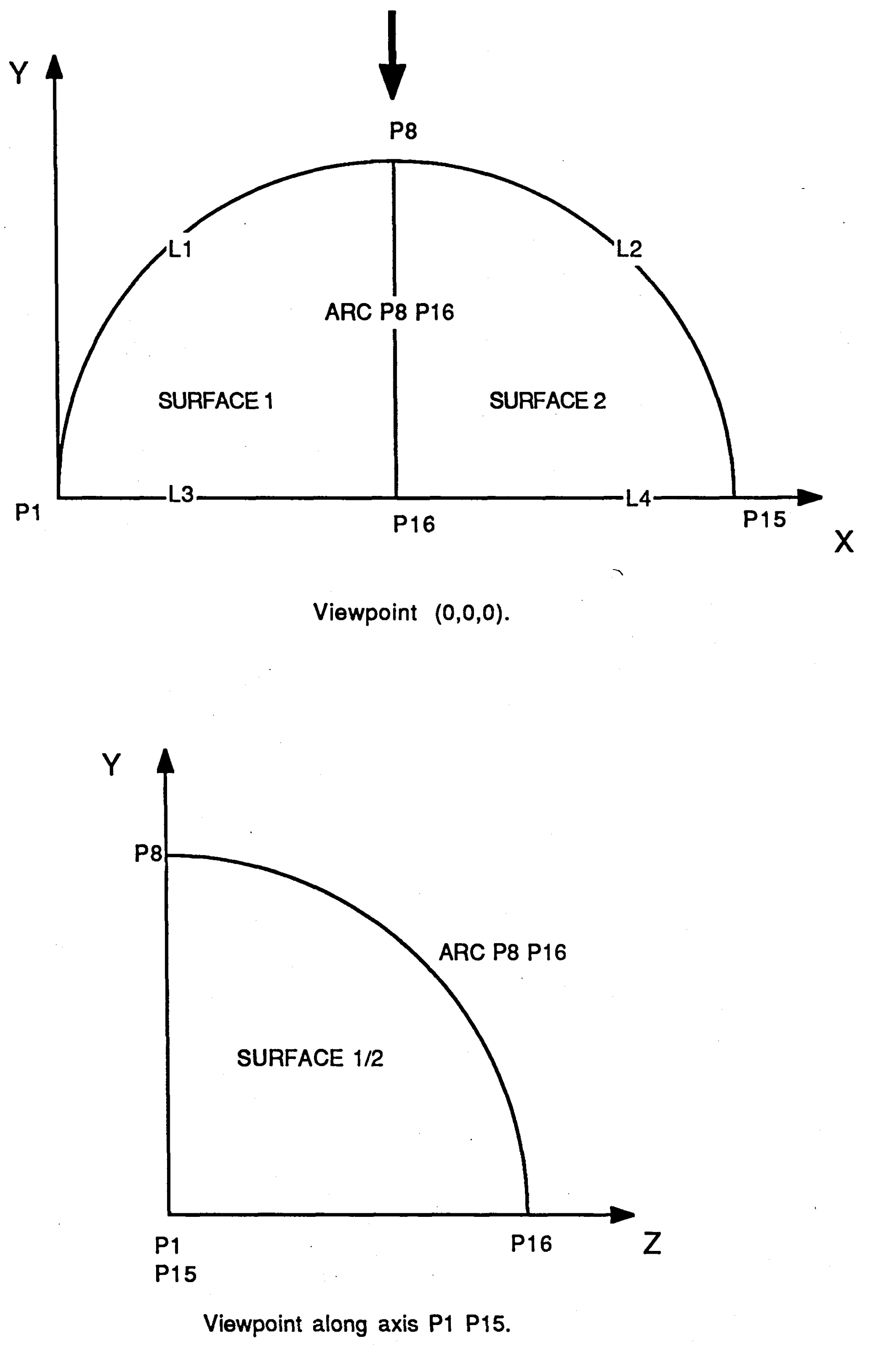
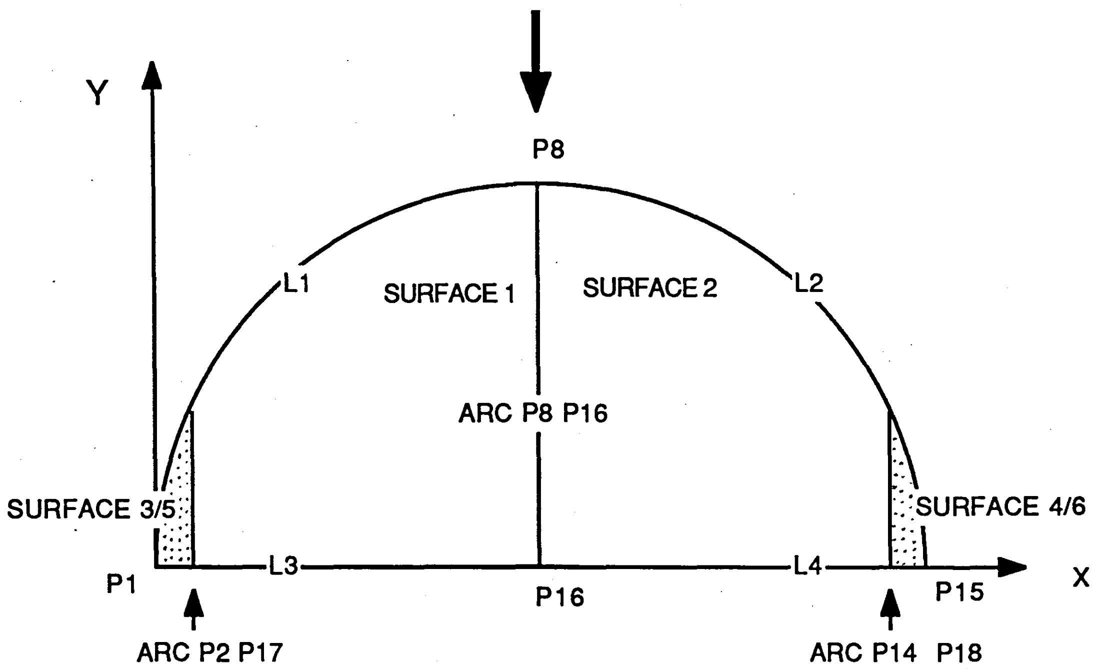
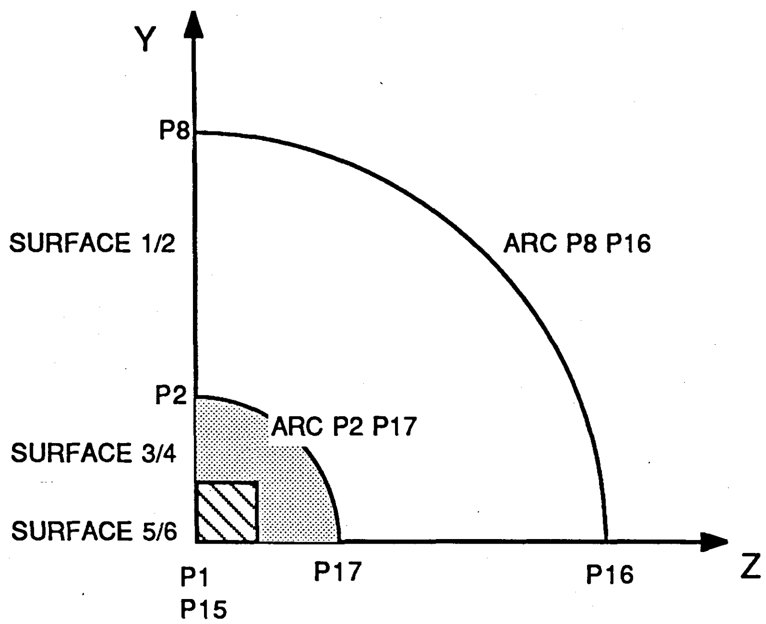


Figure 10 General shell element modelling techniques: generation of a quarter sphere (arrow = load point).



Viewpoint (0,0,0).



Viewpoint along axis P1 P15.

Figure 11 General shell element modelling techniques: modifications required to quarter spherical model to accommodate a QU8 element mesh (arrow=loadpoint).

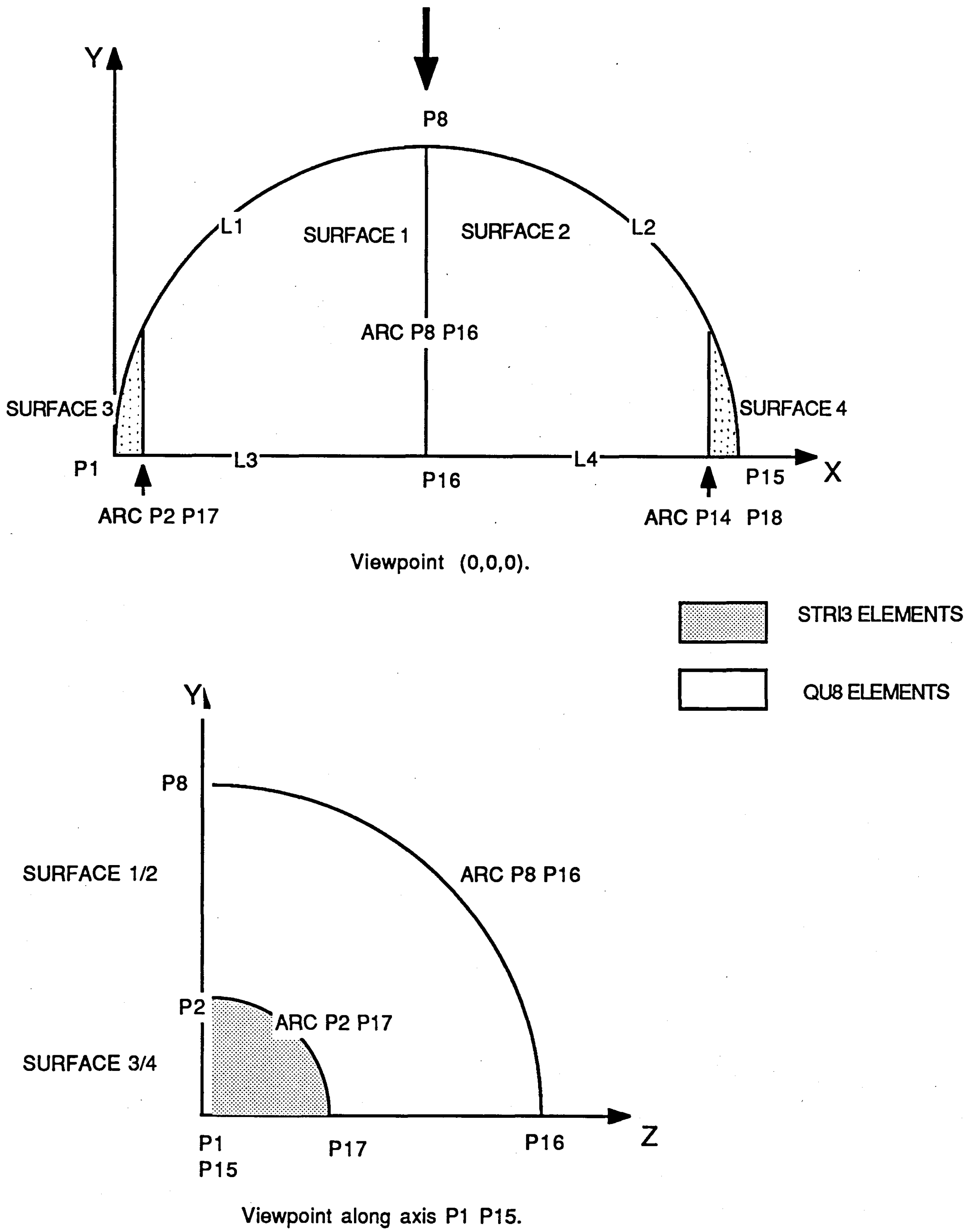


Figure 12 General shell element meshing techniques: modifications to quarter spherical models to accomodate a QU8/STRI3 element combination mesh (arrow=loadpoint).

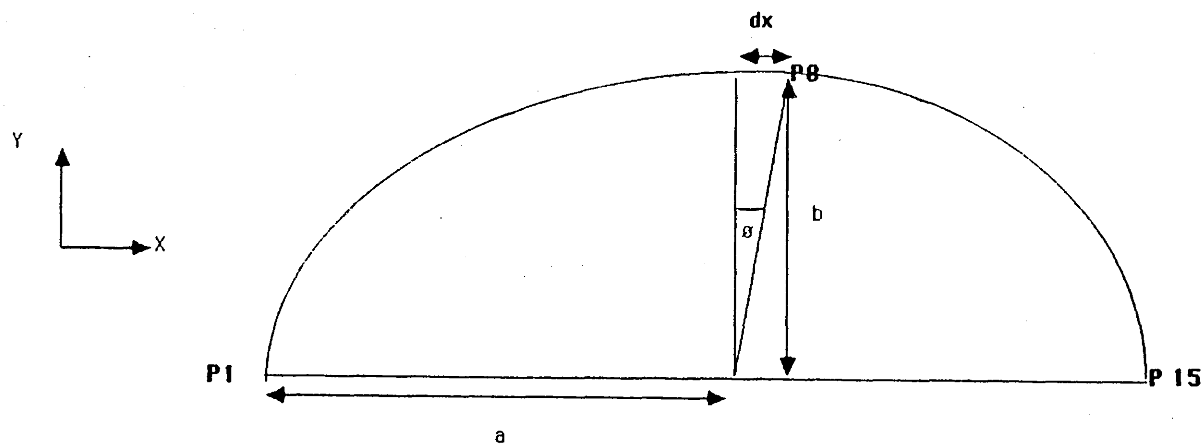


Figure 13 Dimensional data required to generate an eggshaped finite element model (a=length/2 , b=breadth/2, x=distance of the maximum breadth from the midpoint).

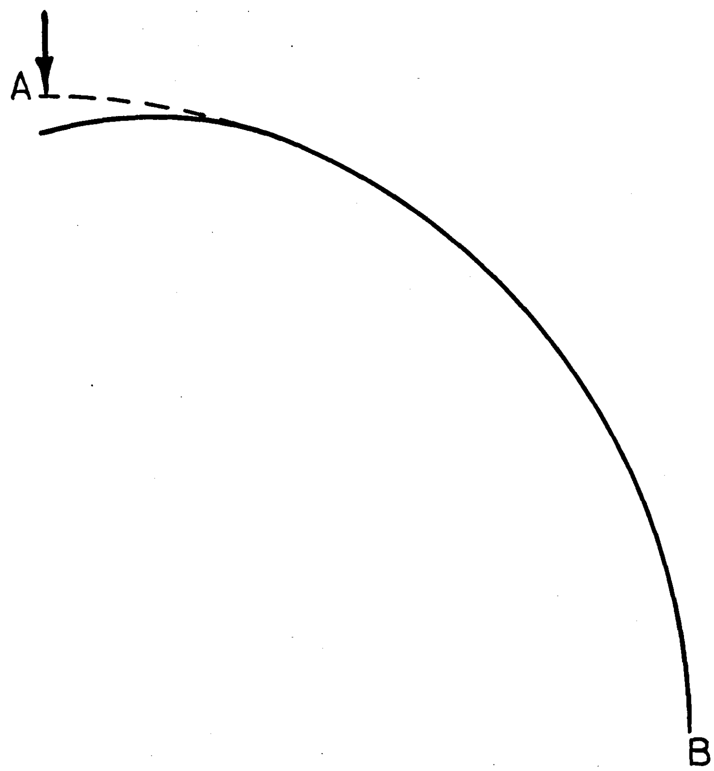


Figure 14 The response is localised when a hollow sphere is subjected to a point load. Only half the geometry is illustrated (solid lines=original geometry, dashed lines=displaced geometry).

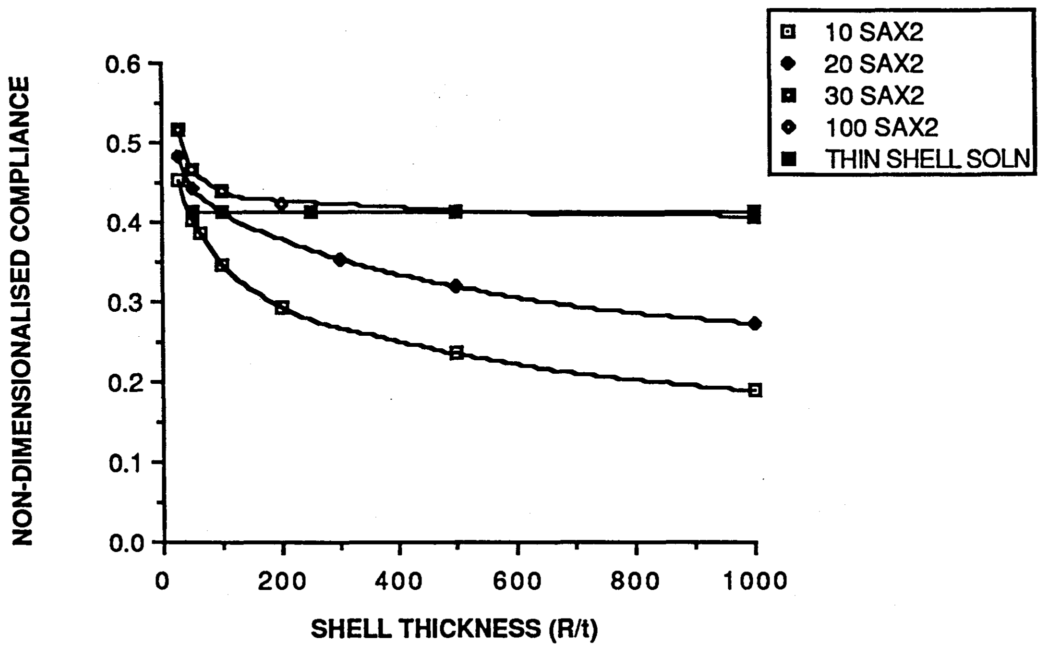


Figure 15 Checking for convergence and the effect of shell thickness (R/t) on the non-dimensionalised compliance.

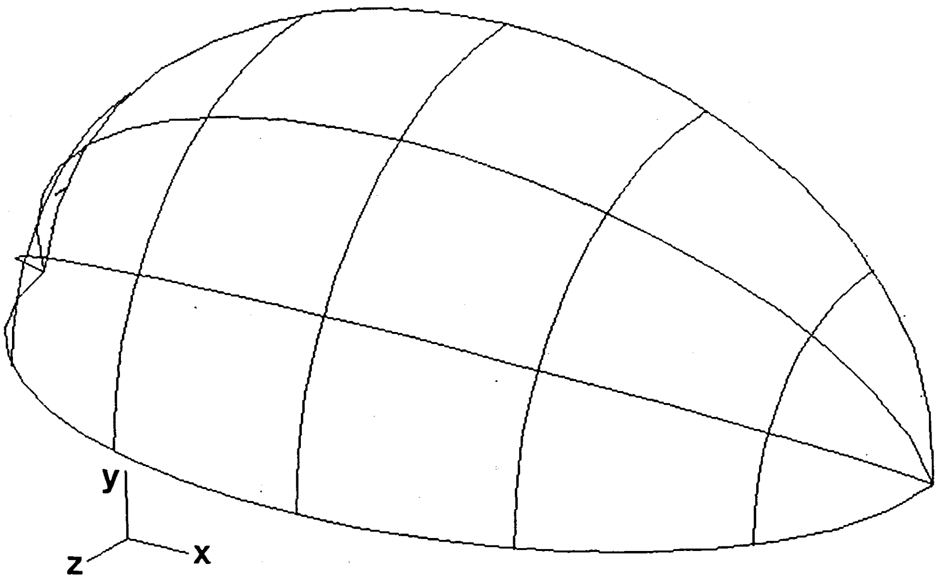


Figure 16 FE representation of the "standard" shaped egg. Only quarter of the total geometry is illustrated.

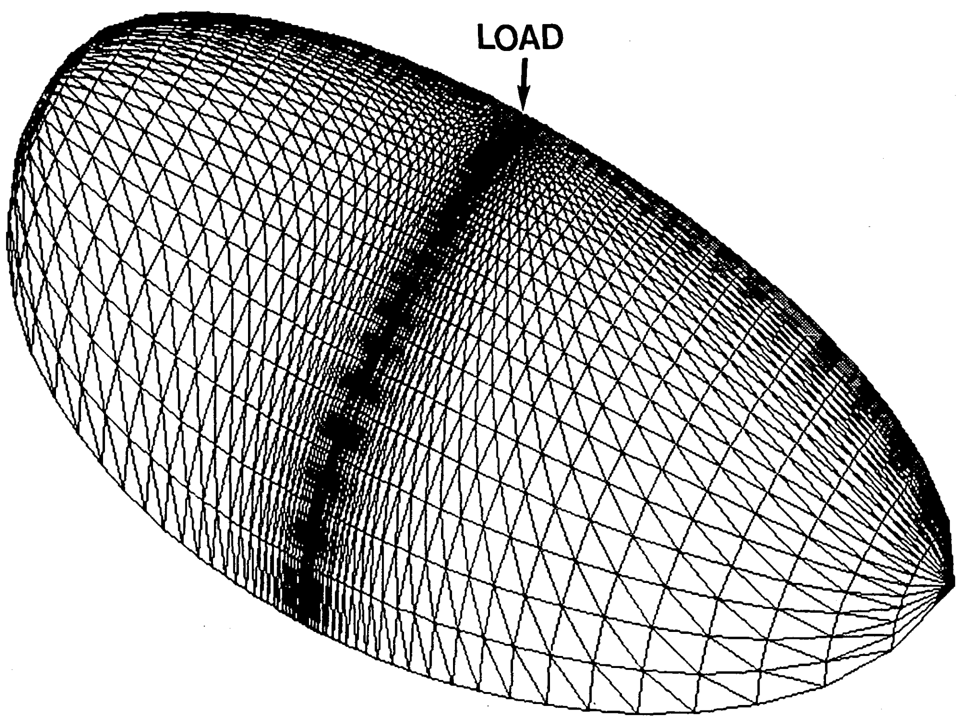


Figure 17 The "standard" egg shaped model meshed with 3306 STRI3 finite elements. In order to obtain accurate solutions the mesh has been weighted towards the loading area (arrowed).

ELEMENT TYPE	MESHING COMMENTS: ADVANTAGES	MESHING COMMENTS: DISADVANTAGES
QU8	Isoparametric therefore particularly suitable for curved shell applications.	Cannot be degenerated into triangles.
	Accuracy normally achieved using few elements.	Limited number of elements can be applied due to complex mathematics.
		Geometric modification required to accommodate mesh.
		Difficulty encountered in concentrating mesh towards load point as a result of above modification.
STRI3	No limit to numbers of elements that can be applied to any one problem due to simpler mathematics involved in calculations.	Large numbers of these elements required in curved shell applications. Planer elements can only approximate to a curved surface.
	Mesh easily applied without need for modification to basic geometry	Large amounts of disc space required for plotting files.
	Mesh easily weighted towards load point.	
QU8/STRI3	Majority of model meshed with QU8 elements.	Use of non-conforming elements requires interface to be defined. Definition of interface tedious.
	No geometric modification required to accommodate mesh.	Interface between elements must occur out with area of maximum interest.

Table 2 The advantages and disadvantages associated with the use of QU8, STRI3 and QU8/STRI3 element combinations in generating a quarter FE model of a sphere.

Element Type	Number of Elements	Number of Nodes	R/t	Compliance (non-dim)	Difference (cf SAX2)	CPU (Hrs:Min:Sec)
QU8	208	685	50	0.358	23%	02:45:34.04
STRI3	1412	751	50	0.364	22%	02:12:30.31
STRI3	3306	1712	65	0.443	~3%	02:10:37.12
QU8/STRI3	242	797	50	0.305	35%	02:54:23.20
SAX2	30	61	50	0.467	-	00:01:47.42
SAX2	30	61	65	0.455	-	00:01:51.52

Table 3 Bench testing: A comparison of QU8, STRI3, and QU8/STRI3 element solutions.

CHAPTER 3

SECTION 1: A GENERAL INTRODUCTION TO THE ELASTIC PROPERTIES OF EGGSHELLS.

3.1.1 GENERAL.

According to Hookes law, the deformation (d) of any linear elastic body is proportional to the applied force (F). The proportionality constant (F/d) is known as the stiffness (S). Alternatively, the reciprocal of the stiffness (S) maybe referred to as a compliance (C). This relationship is illustrated in Figure 18.

Under quasi-static compression between parallel plates, eggshells are considered to obey Hookes law (Brooks and Hale 1955; Voisey and Hunt 1967a; Voisey and Hamilton 1975). Several methods have as a result been developed to measure the deformation of eggs using non-destructive forces (Schoorl and Boersma 1962; Voisey and Foster 1970; Voisey and MacDonald 1978) and it is now widely accepted that the stiffness (S) of the eggshell is an important factor relating to its ultimate strength (Richards and Staley 1967; Tung *et al* 1968; Carter 1970b; Voisey and Hunt 1974; Voisey and Hamilton 1975). In general, a low deformation value is considered to be a characteristic of a stronger shell. That this is not always the case however, suggests that either these non-destructive deformation testers are failing to measure accurately the parameters for which they were designed, or factors other than those which affect the stiffness characteristics of the eggshell determine its' ultimate strength.

Voisey and Hunt (1974) suggested that a difference in the precision of measurement could explain some of this inconsistency. Deformations are very small at non-destructive loads and so the precision of measurement required is exceedingly high. On the other hand, deformation is an elastic reaction dependent on the elastic modulus of eggshell material, whereas fracture is not, and the physical mechanisms governing the two are quite different.

This chapter is devoted to the measurement of, and those factors which influence the elastic properties of eggshells.

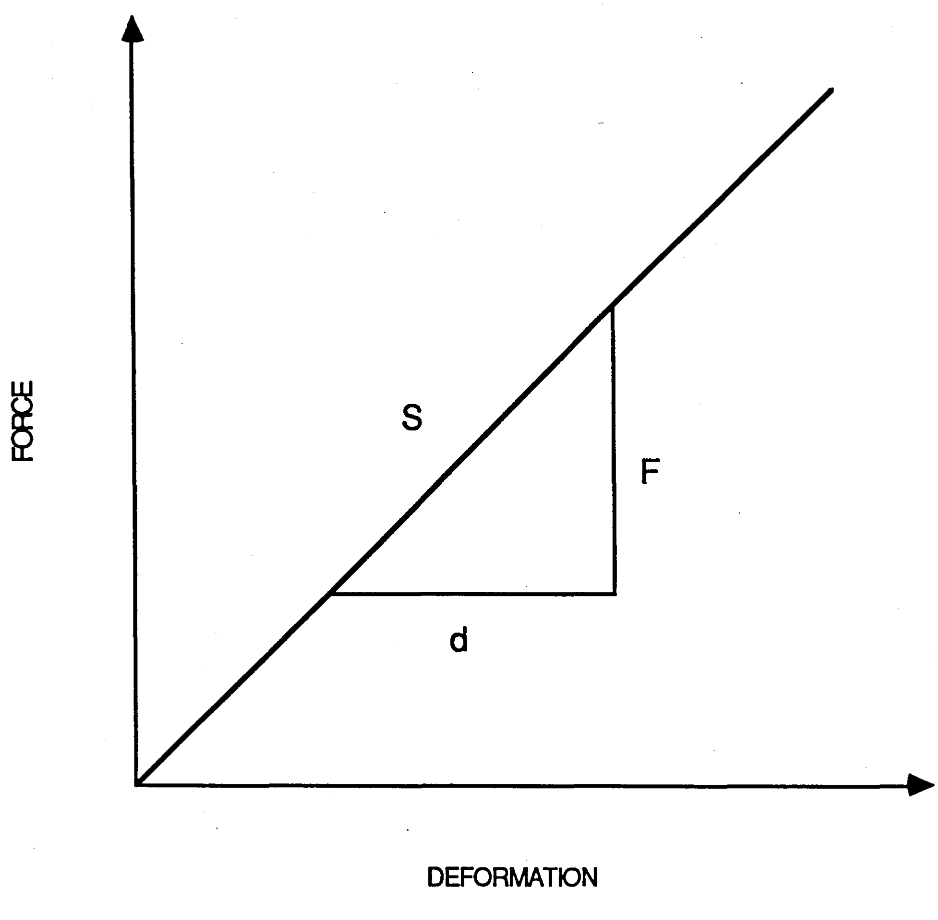


Figure 18 Hookes law states that the deformation (d) of any Hookean body using a non-destructive force (F) is inversely proportional to the slope (S).

CHAPTER 3

SECTION 2: THE COMMERCIAL NON-DESTRUCTIVE DEFORMATION TESTER.

3.2.1 INTRODUCTION.

Schoorl and Boersma (1962) developed a simple apparatus to measure the non-destructive (ND) deformation of eggs under a fixed mass of 500g. This apparatus was later manufactured commercially (Mauris N.V., Hollantleen 18, Utrecht, Netherland), and apart from some basic modifications, remains one of the most widely used tests in the commercial poultry industry today.

To measure the ND deformation of an egg, a rod is lowered until it rests on the shell. A small pressure is then applied to the egg via this rod, and after setting the dial gauge to zero, the 500g weight at the end of the rod opposite the egg, is released. The deflection recovered by the eggshell for a force change of 4.9N is shown as the resultant change of reading on the dial gauge.

When an egg is compressed between two parallel plates, and assuming that the material at the two points of contact is homogeneous, then the deformation at each loadpoint can be expected to be identical. A curved object however does not rest easily on a flat surface without some form of physical restraint. Thus to facilitate measurement on a large scale, many commercial ND deformation testers have, on their lower plate, a depression on which the egg rests. A modification of this type must however result in a different measurement of deformation than would otherwise be obtained using two flat plates; thus Carter (1968) found that the ND deformation at the poles of an egg approximately doubled when a metal plate was placed over this hole. In contrast, Voisey and Hunt (1974) suggested that a difference of 22.6% should be applied when comparing ND deformation data with quasi-static compression test data but did not quote any experimental details to support this statement. This section therefore sets out to determine the magnitude of this discrepancy in more detail thus allowing ND deformation data to be used in future calculations relating to the elastic modulus of eggshells (see 3.5).

3.2.2 MATERIALS AND METHODS.

[i] A Computer Simulation of the Commercial ND Deformation Test:

The difference between compressing an egg between two flat plates and the loading conditions more typical of the commercial ND deformation test can be readily illustrated using the FE method. As the load is non-symmetrically applied in the latter, both upper and lower loadpoints must be included in any FE model simulation. Half of the total geometry therefore needs to be considered, thus requiring twice as many STRI3 elements as that used in the quarter FE model of the egg discussed in Chapter 2.2. For the purpose of this investigation it was therefore computationally simpler to ignore details of egg geometry and analyse spherical models meshed with a small number of axisymmetric shell elements.

Spherical models meshed with axisymmetric shell elements (type SAX2) were generated as before (see 2.2.2 Step 1). In this case however the complete sphere was generated, and the resulting geometry was meshed with 60 rather than 30 elements. The appropriate boundary conditions were then applied (Figures 19a and 19b).

A 5N load was applied to the upper surface of the model in each case. An equal but opposite force was also applied to the models' lower surface in the point load test. In the commercial test, the lower surface of the model was assumed to project into a circular annulus (diameter 1cm), and the opposing load was distributed around the area of contact between these two (Figure 19b). Each model was then analysed using these specifications and the results expressed with the aid of computer graphics.

The total displacement (d) at the top of the sphere, together with the appropriate values of E, R, t and F, were substituted in Eq (2) (see 2.2.1) and from this the corresponding non-dimensionalised compliance (C) values were obtained. This calculation finally yielded a computed correction factor to allow for the difference in deformation that exists between the commercial ND deformation test and the point load test where the egg is compressed between two flat plates.

[ii] Experimental Measurement of an Appropriate Correction Factor for the Commercial ND Deformation Test:

The ND deformation values of a number of eggs were measured using the Mauris ND deformation tester. Replicate measurements were taken at equidistant points along the equator (after Schoorl and Boersma 1962; Carter 1970b). A steel plate was then placed over the hole in the lower plate of the test apparatus, and measurements repeated. On occasion the point load simulation test was carried out first to eliminate bias.

After the completion of each test the mean ND deformation values for each egg were calculated and compared. The difference between these measurements was attributed to the presence or absence of the depression in the lower loading plate.

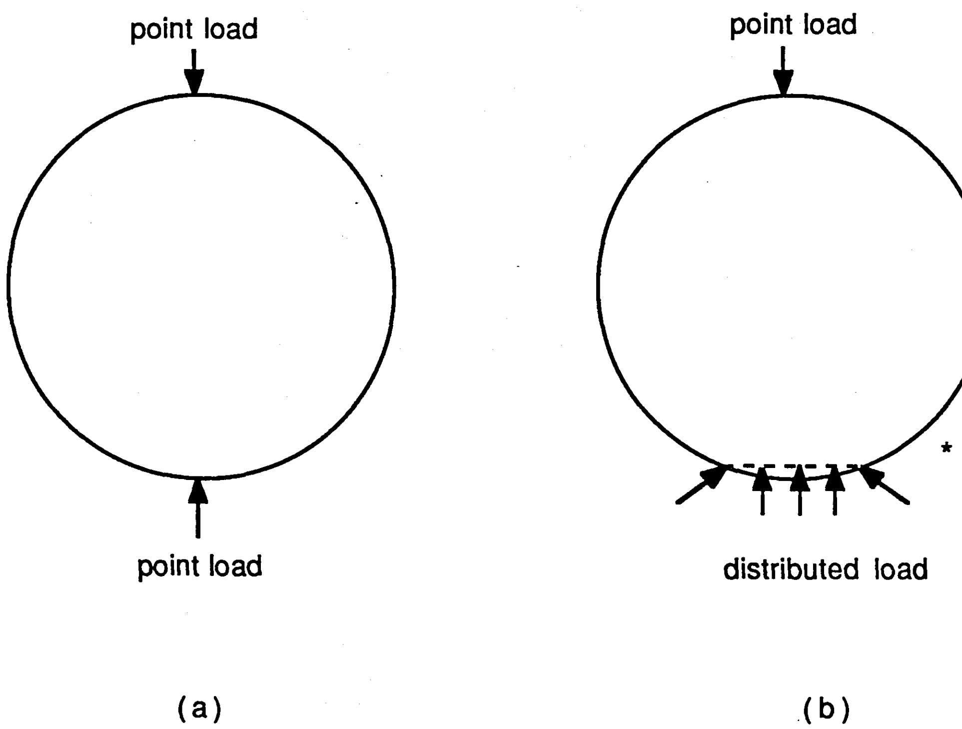
3.2.3 RESULTS AND DISCUSSION.

The displacement induced in a spherical FE model resulting from the application of two point loads is illustrated in Figure 20(a). The displacement at each loadpoint was found to be equal but opposite. Figure 20(b) shows the same spherical model subjected to the loading conditions more commonly associated with the commercial ND deformation test. In this case the lower half of the model was assumed to be supported by a circular annulus. Under these conditions only the top half of the model appreciably deformed.

Table 4 compares the non-dimensionalised compliance values calculated from the displacement at the top of the sphere in each case. These data suggest that the sphere deformed 45% less under the commercial ND test conditions. These data should be compared with the experimentally derived data given in Table 5.

In every case the experimentally derived ND deformation measurements were consistently higher when a flat steel plate was used as the lower loading surface (see Table 5). Indeed, when the average ratio between the two types of measures were calculated it is significant to note that this ratio differed from the FE computed value by approximately 10%. This relatively minor difference can almost certainly be attributed to the effects of eggshape. The latter was not considered in the FE calculations.

Thus it can be concluded that the depression in the lower plate of the commercial ND deformation tester has a very significant effect on the deformation experienced by an egg when a 500g load is applied to its' upper surface. While this will have no effect in terms of ranking eggs for quality control purposes, this discrepancy must be taken into consideration if this non-destructive method is to be used as a measure of stiffness in calculations relating to the elastic modulus of eggshells. For this purpose, a correction factor is therefore required, and so all subsequent ND deformation measures used in this context have been corrected by a factor of 45%.



* load distributed over an area corresponding in size to where the shell is assumed to make contact with the sides of the circular annulus

Figure 19 Boundary conditions applied in FE simulations of a) the point load test, and b) the commercial non-destructive deformation test.

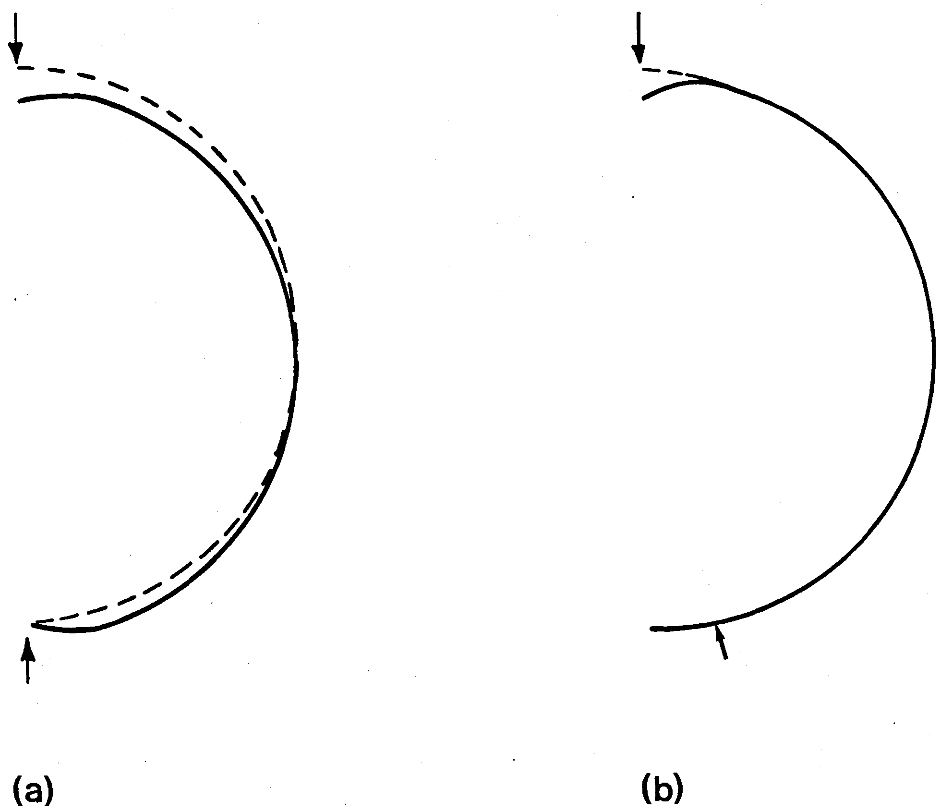


Figure 20 When two point loads are applied to the model, both the upper and lower surfaces deform by an equal but opposite amount. Only the upper surface of the model deforms when the load at the lower surface is nolonger confined to a single point(b).

Model type	R/t	Compliance [Non-dimensionalised]
Commercial ND test	64	0.500
Point Load test	64	0.910

Commercial / point load = 0. 55

Table 4 FE analysis of the ND commercial test and the point load test.

EGG	COMMERCIAL ND DEF TEST		ND DEF + STEEL PLATE		MEAN 1/MEAN 2
	MEAN[1]	S.D.	MEAN[2]	S.D.	
1	19.0	0.6	33.0	1.5	0.58
2	18.2	0.4	30.0	1.3	0.61
3	20.5	0.5	31.2	0.7	0.66
4	23.2	0.7	38.2	0.7	0.61
5	20.9	1.2	34.0	1.1	0.61
6	23.1	0.7	37.1	1.5	0.62
7	29.8	1.2	45.9	1.4	0.65
8	18.5	0.6	31.7	1.3	0.58
9	26.5	1.8	44.7	2.2	0.59
10	16.7	0.6	28.9	1.1	0.58
11	23.4	1.1	37.8	1.6	0.62
12	20.3	0.9	33.0	1.3	0.61
13	30.7	0.8	47.1	1.3	0.65
14	20.1	0.8	32.7	1.7	0.61
15	25.5	1.4	41.7	1.7	0.61
16	17.2	0.5	29.9	0.9	0.57
17	20.8	1.1	33.7	0.8	0.62
18	20.5	0.5	32.7	1.1	0.63
19	14.7	0.4	26.2	1.3	0.56
20	18.8	0.9	31.1	0.8	0.60
21	24.6	1.5	37.8	0.8	0.65
22	24.2	0.9	38.9	0.7	0.62
23	22.7	0.8	33.6	1.0	0.68
24	20.3	0.8	29.9	0.8	0.68
				AVERAGE RATIO	**0. 62 +/- 0.03**

Table 5 The effect of placing a steel plate over the hole in the lower surface of the commercial ND deformation tester.

CHAPTER 3.

SECTION 3: THE CONTRIBUTION MADE BY THE DIFFERENT SHELL LAYERS TO THE STIFFNESS CHARACTERISTICS OF THE EGGSHELL.

3.3.1 INTRODUCTION.

The concept of a relationship between the thickness and stiffness characteristics of the eggshell is generally accepted, although opinions vary as to the correlation coefficient between the two; thus Gruhn and Tschami (1966) quote a correlation coefficient of r=0.8, while El-Boushy *et al* (1968) estimate the value of the coefficient to be more in the region of r = 0.57. More recently, Thompson *et al* (1981) found that the regression of ND deformation on shell thickness was negative within species and positive among species and concluded that this difference could not be explained solely on the basis of egg size or the amount of shell material present.

The stiffness properties of any object are influenced by its composition and organisation, and continued investigations of shell structure in a variety of egg laying species have focused attention on the non-homogeneous nature of the former. Although the bulk of the shell consists of calcium carbonate in its calcite form, the organisation is such that a number of morphologically distinct layers can be resolved at ultrastructural level (Simons 1971; Becking 1975; Parsons 1982).

This section describes the results of a series of tests designed to determine the role played by each of these individual layers on the stiffness characteristics of eggshells.

3.3.2 MATERIALS AND METHODS.

[i]. General:

A selection of pre-packed brown eggs were purchased from a retail outlet as and when required.

Before being selected for use in the following investigations, each egg was candled using a concentrated light source to identify and select against cracked or very translucent shells. Those eggs classified as being structurally sound were subsequently placed into groups according to egg weight.

The stiffness characteristics of each egg were determined before and after the following manipulative procedures using the commercial ND deformation tester as described in Chapter 3.2. In the context of these experiments it was decided not to convert the ND deformation values into units of stiffness. In each case the test was simply used as a means of illustrating the effect of removing differing amounts of shell material on the stiffness characteristics of the shell remaining. For this type of semi-quantitative investigation the ND deformation test was considered particularly suitable since each measure only relates to a small localised area of shell within the close vicinity of the indentor (Schoorl and Boresma 1962).

[ii] Thick and Thin Shells and the Preparation of Samples for Assessing Thickness by Scanning Electron Microscopy (SEM):

The spatial distribution of the various shell layers were classified using a number of thick and thin shelled eggs. Thick shelled eggs characteristically displayed lower than average ND deformations ($\leq 19\mu m$), whilst thin shelled eggs were classified on the basis of having ND deformation values $\geq 26\mu m$.

Following the removal of the egg contents, $1cm^2$ pieces of eggshell were carefully cut out at selected sites around the circumference of each egg using a diamond tipped circular saw. These sites corresponded to the areas at which ND deformation measurements had previously been measured.

After manually removing the loosely attached inner shell membranes, each sample was washed in distilled water to remove traces of albumen, and left to dry at room temperature. Once dry, each piece of shell was snapped into two to obtain a relatively straight edge, affixed edge on using conductive silver paint into grooves cut in aluminium stubs, then coated with gold/palladium for four minutes in an Emscope sputter coater. These transverse sections were subsequently viewed using a Philips 501B scanning electron microscope at 15 kv.

All samples were examined at a magnification of x160, and tilted until neither the mammillary nor cuticular surfaces' were visible. Next, the specimen height was lowered or raised until the working distance was equal to 13 (The working distance is a measure of the distance between the electron beam and the surface of the specimen, and was kept constant throughout these analyses). The thickness measurements required from each sample were obtained either directly from the screen or from photographic negatives.

The total thickness of each specimen was measured as the distance from its' outermost surface to the point where the basal caps inserted into the shell membranes. The thickness of the mammillary layer was also assessed, this being the distance from the basal caps to the point at which the palisade columns first fused. Subtraction of these two values provided a measure of the combined thickness of the palisade, vertical crystal and cuticular layers. Replicate measures were performed in each case and the mean values converted to the nearest 0.001mm using the appropriate scaling factor for magnifications of x160.

[iii] Distribution of Vesicular Holes in Eggshells:

To assess vesicular porosity, transverse sections of shell were subdivided into a number of zones viz; junctional zone; lower palisade, mid palisade, mid/upper palisade, upper palisade, and outer/vertical crystalline layer. High magnification micrographs were taken within each of these zones, and from these the appropriate number and the range in diameter of the holes per zone were established.

Assuming that each hole was spherical, the average area of each vesicle was calculated using the following equation,

$$\text{Area of a circle} = \pi r^2 \qquad \text{Eq (4)}$$

This was in turn used to calculate the porosity associated with each zone,

$$\text{Porosity} = \frac{\text{Total Nos vesicles} \times \text{Average area of vesicles}}{\text{Unit area}} \% \qquad \text{Eq (5)}$$

where the unit area in each case was equal to one field of view at a magnification of x 5000.

[iv] Experimental Removal of the Outer Shell Layers and the Effect of This on the Stiffness Characteristics of the Remaining Shell:

A number of experiments were carried out to determine the most effective way of thinning the shell from the cuticular surface inwards. In every case the eggs used in each experiment were standardised with respect to egg weight and ND deformation before treatment.

Exp 1 A Comparison of Chemical and Mechanical Thinning Techniques.

The outer layers of fourteen eggs were removed by gently moving different grades of sandpaper across the surface of the egg as it was rotated by a lath. A second group of fourteen eggs were immersed in a 50% solution of HCl for varying lengths of time. In each case ND deformation measures were taken before and after treatment, and the thickness of shell remaining was determined using the SEM method described above.

Exp 2 Chemical Removal of the Cuticle Using EDTA.

Fourteen eggs were immersed in a solution of 100g ethylenediaminetetracetic acid (EDTA) per litre (pH 7.5) for between 15 and 20 minutes. Following this, each egg was washed in distilled water and gently rubbed to loosen the cuticle (Board and Halls 1973).

The presence or absence of cuticle was initially determined by dipping each egg into a solution of Edicol supra pea green dye (Board and Halls 1973) for approximately 1 minute. The eggs were then allowed to dry, before repeating ND deformation tests.

To verify that the cuticle had been completely removed, samples from several sites around each egg were subsequently selected for SEM analysis. Each sample was affixed to an aluminum stub outermost surface uppermost, coated then viewed at x1250.

Exp 3 Chemical Removal of a Known Amount of Shell Material From the Outer Surface of Eggs.

The eggs used in this test were dipped into a solution of 50% HCl in such a way as to leave an intermediate strip of unetched shell (Figure 21). ND deformation measurements were taken before and after treatment at both the etched and unetched surfaces. The scanning electron microscope was then used to estimate the amount of shell which had been removed by the acid. This was achieved by comparing the average SEM thickness of the etched surfaces with that of the unetched region. This experimental procedure was carried out twice using two batches of fourteen eggs.

[v] Chemical Removal of the Shell Membranes and the Mammillary Layer of Eggshells:

First, the contents of each egg were removed by blowing. Each eggshell was then re-candled to check for the presence of hairline cracks, then ND deformation tests were repeated. Following this, a 50% solution of HCl was pipetted into the interior of each emptied egg, and the holes at each pole sealed. Each egg was then continuously rotated for between 10 and 40 minutes.

After the designated time interval, the acid inside the egg was neutralised and carefully flushed out. Each sample was then left to dry overnight before again repeating ND deformation measurements.

To establish the degree of etching, four sites from each etched shell were selected for further analysis by scanning electron microscopy.

Twenty eight eggs in all were tested and each classified according to the degree of etching attained as follows; $^1/_2$ cone removed, $^3/_4$ cone removed, total cone removed, or etched into the lower palisade layer.

3.3.3 RESULTS.

[i] Thick and Thin Shells:

Table 6 compares the mean total thickness and mean mammillary layer thickness measurements of seven thick eggshells with seven thin eggshells. The difference between the total thickness and the mammillary layer thickness is equal to the combined thickness of the palisade, vertical crystal and cuticular layers. The ND deformation values of each sample are also given. From these data it is clear that there is a relationship between the thickness of an eggshell and its' stiffness characteristics.

The micrographs shown in Figures 22 and 23 depict transverse sections through the shells of eggs MB1 and MB4. Both micrographs were taken at the same magnification and working distance. The most obvious change associated with an overall increase in thickness appears to take place in the palisade region of the shell, while the thickness of the mammillary layer, vertical crystal and cuticular layers remain approximately constant.

[ii] Distribution of Vesicular Holes in Eggshells:

Table 7 summarises the range in numbers, and the average diameter of vesicular holes found at different levels throughout the eggshell. An estimate of the porosity associated with each zone is also given.

Considerable variation occurred in terms of both the numbers and sizes of the vesicles found in each respective zone, but in each case the transition from higher to lower levels of porosity was a gradual one. The mid to upper palisade region was generally found to be the most porous region of the shell. In some samples this feature manifested itself as an increase in pore diameter, whilst in others the total number of vesicles increased. Figure 24 illustrates the characteristic "spongy" appearance of the mid to upper palisade region of the shell.

Above this "spongy " zone, the numbers of vesicles decreased and as a result the outer boundary of the palisade layer characteristically had a much more compact appearance (Figure 25). Vesicular holes were essentially absent from the vertical crystal layer (Figure 25).

[iii] Experimental Removal of the Outer Shell Layers and the Effect of This on the Stiffness Characteristics of the Remaining Shell:

Exp 1 A Comparison of Chemical and Mechanical Thinning Techniques.

The process of thinning eggs by abrasive methods proved to be very laborious and only eight samples were successfully thinned using this technique. As anticipated sequential removal of successive layers from the outer surface of whole eggs had a significant influence on the ND deformation of the remaining shell (Table 8).

Removal of the outer shell layers by acid etching was considerably less arduous. While those eggs which were dipped into a 50% solution of HCl for only a short period of time did not show any significant change in ND deformation, as the etching time increased, the stiffness characteristics of the remaining shell rapidly changed (Table 9). Prolonged treatment with acid in some cases however caused a significant variation (s.d.≥2μm) in deformation measurements over the surface of the same egg. As this was not accompanied by a concomitant variation in shell thickness, it was assumed that these samples had been infiltrated by the acid (see Tyler 1968). Be that as it may, it was subsequently shown that this effect could be minimised to some extent by shorter repeated exposures to the acid, and by washing the samples between each successive exposure.

Figure 26 compares the effects of mechanically or chemically removing the outer shell layers on the stiffness characteristics (ND deformation) of the remaining shell. Only those chemically thinned eggs which appeared to be equally etched and which were considered not to have been affected by acid penetration are shown. Since both treatments gave similar results, chemical etching was assumed not to cause any weakening effect on the remaining shell as long as the above criteria for removing unequally etched or weakened shells was adopted.

<u>Exp 2</u> Chemical Removal of the Cuticle Using EDTA.

Table 10 summarises the results of experiments in which fourteen eggs were immersed in a solution of EDTA. Most samples displayed a small but significant increase in deformation (≥2μm) after treatment.

Pea green dye confirmed that the cuticle had been removed in each case and scanning electron microscopy revealed that the calcite below the cuticle was now completely exposed (Figures 27 and 28).

Exp 3 Chemical Removal of a Known Amount of Shell Material From the Outer Layers of the Eggshell.

Individual measurements of ND deformation only relate to a small region of the total shell (Schoorl and Boresma 1962; Carter 1970b) and so in these tests the unetched surface was assumed not to affect measurements taken at the etched surfaces after treatment. Indeed when the ND deformation tests were repeated at the unetched sites, they were found in the majority of cases not to differ significantly from the average ND deformation values before treatment (Table 11).

Those eggs which had been thinned the most, again displayed the greatest change in ND deformation. According to Figure 29 there is a curvilinear relationship between the change in stiffness characteristics of the shell, as measured using the ND deformation test, and the amount of shell removed (r=0.97) by chemical etching: the ND deformation value apparently doubled when less than 30% of the shell is etched away. Data however are only available for shells which were thinned to approximately two thirds of their original thickness. Any samples which were thinned more than this failed during subsequent ND deformation tests.

[iv] Removal of Egg contents, and the Chemical Removal of the Shell Membranes, and the Mammillary Layer of Eggshells:

According to Table 12, the ND deformation of eggshells does not significantly change when the egg contents are removed. In addition, it would appear that the stiffness characteristics of the remaining shell are essentially unaffected by the chemical removal of the shell membranes and part or almost complete removal of the mammillary layer. Only once etching had completely removed the whole of the mammillary cone layer, and had began to etch into the palisade layer, was there a significant change ($\geq 2\mu m$) in the ND deformation before and after this treatment.

Eight samples out of the original twenty eight eggs tested were rejected on the basis of damage incurred during the removal of the egg contents. Also, due to the nature of the experiment it was impossible to ensure in every case that the total inner surface of each egg had been etched by the same amount. The degree of etching could only be determined after the experiment had been completed. Despite this difficulty scanning electron microscopy revealed that in the majority of cases etching had been approximately uniform. Those samples marked with † in Table 12 were the exceptions. The latter tended to be more deeply etched in one or more of the four areas observed and characteristically displayed a significant difference in ND deformation from one region of the shell to the next (s.d.$\geq$2μm).

3.3.4 DISCUSSION.

SEM analysis of eggshells which exhibited significantly different ND deformation values confirmed that there must be a relationship between the stiffness characteristics of an eggshell and its thickness. The most obvious change in thickness was observed in the palisade layer, while the mammillary layer, vertical crystal layer and cuticle remained relatively unchanged. The palisade layer is therefore cited as having the greatest influence on the stiffness characteristics of an eggshell.

Brooks and Hale (1955) and Brooks (1961) found a linear gradient of hardness through the thickness of the shell. According to these authors, the shell was apparently hardest near its' outer most surface. In contrast, Tung *et al* (1968,1969) found a curvilinear variation of hardness through the shell wall and related this to a variation in the magnesium content. While the relationship between the hardness of a material and its' elastic properties is unclear, these findings nevertheless suggest that the elastic properties of the shell might also vary through the shell wall (Voisey and Hunt 1974).

Current investigations indicate that a curvilinear relationship exists between the amount of material that is removed from the outer surface of an egg by chemical etching, and the change in stiffness which results from this. A small but significant change in ND deformation was noted when the cuticle was removed using the method described by Board and Halls (1973). While this supports the findings of Belyavin and Boorman (1980) it was difficult to determine how much of the underlying vertical crystal layer had also been removed. In contrast, the non-linearity of the relationship between ND deformation and the amount of shell removed in those shells which were acid etched beyond the cuticle and vertical crystalline layers, suggests that even within the palisade layer the contribution to the shells' stiffness characteristics is non-uniform. This however is not surprising considering the heterogeneous nature of this layer. When viewed at high magnifications with the scanning electron microscope the latter is characterised by the presence of pit-like or vesicular holes (Figures 24 and 25) which vary both in number and size. Peterson and Tyler (1967), and Simons (1971) proposed that the arrangement of these holes was related to the distribution of the organic matrix within the shell. Simons (1971) proposed that within the innermost region of the palisade, the vesicular holes were larger and generally more randomly distributed than in the outer palisade where the holes tended to be smaller and much more compactly arranged. In contrast, Becking (1975) suggested that in the hen's egg the vesicular holes were small and sparsely distributed. Current observations also revealed a much less obvious and variable pattern of arrangement. In general the mid to upper regions of the palisade layer was found to be more porous than the outer palisade/vertical crystal regions but the transition from the former to the latter was gradual. This more gradual change in porosity possibly explains why the above chemically thinned samples generally displayed a moderate rather than a large increase in ND deformation following treatment. Had the shell been clearly divisible into a compact zone and spongy zone (Simons 1971), then a more abrupt change in stiffness might have been anticipated as the more porous material became exposed.

In contrast to the above, the removal of the egg contents, followed by the chemical removal of the shell membranes, mammillary caps and most of the cone layer, had no affect on ND deformation measurements. These results therefore suggest that the mammillary layer *per se* does not significantly contribute to the elastic or stiffness characteristics of the eggshell.

Thus, in conclusion the stiffness characteristics of an egg are influenced by the presence of the cuticle and vertical crystal layers to a limited extent, but more so by the gradual change in porosity and hence elastic properties associated with the palisade layer (see Figure 30). The mammillary layer in contrast would appear to have no direct effect on this property and in consequence must have a very low elastic modulus.

Finally, Tyler (1968) criticised the use of caustic chemicals as a means of thinning eggshells on the grounds that the latter weaken the shell. Both chemical and abrasive methods of removing the outer layers of shell material were compared in the current investigations. While unequal etching or acid penetration did occur in some cases, all of the remaining chemically thinned samples were found to display similar stiffness characteristics to those samples which had been successfully thinned using abrasive techniques. Acid etching was therefore not considered in the above experiments to cause a weakening effect unless the characteristic signs of unequal etching or acid penetration were evident.

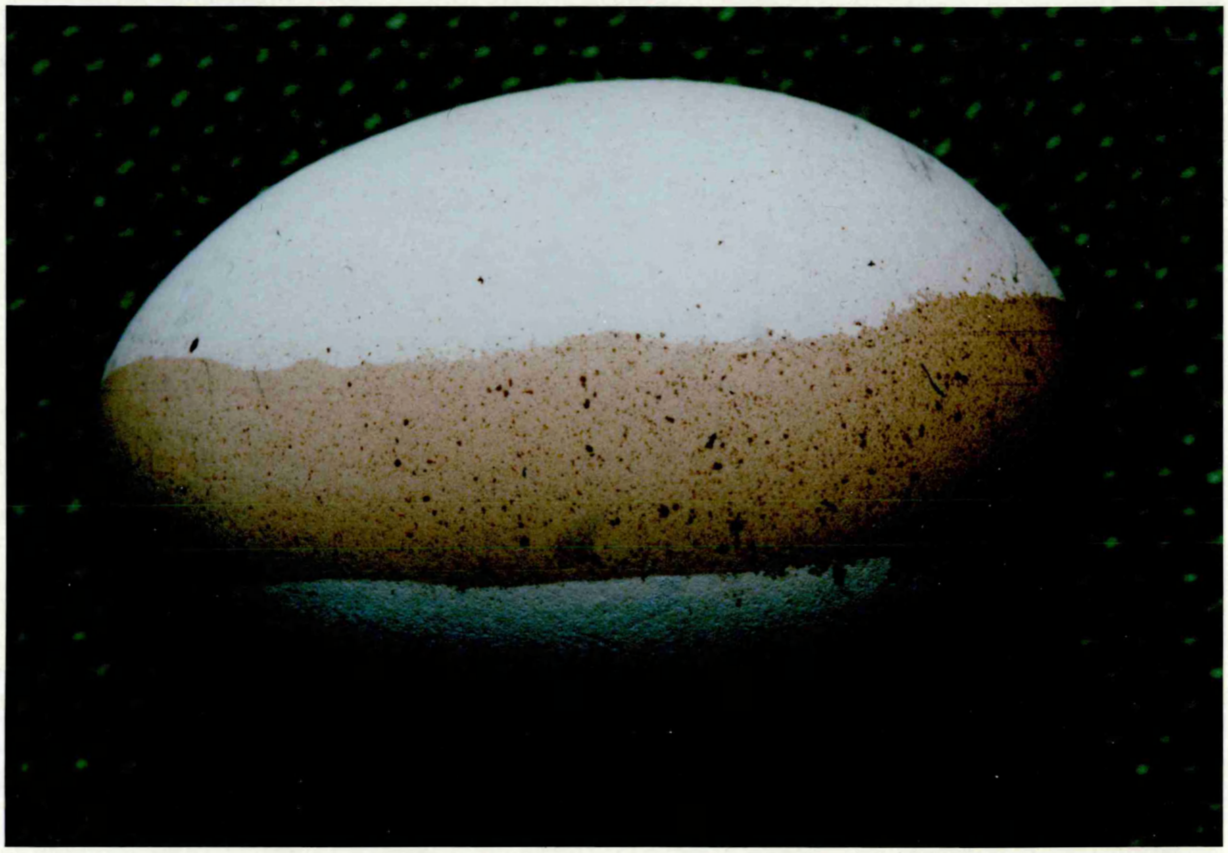


Figure 21 Chemical removal of a known amount of shell material from the outer surface of the eggshell. An intermediate band of unetched material separates the two etched surfaces.

Figure 22 Transverse section of a thick shelled egg x 160. m = mammillary layer; p = palisade; vcl = vertical crystal layer; c = cuticle (see Fig 1).

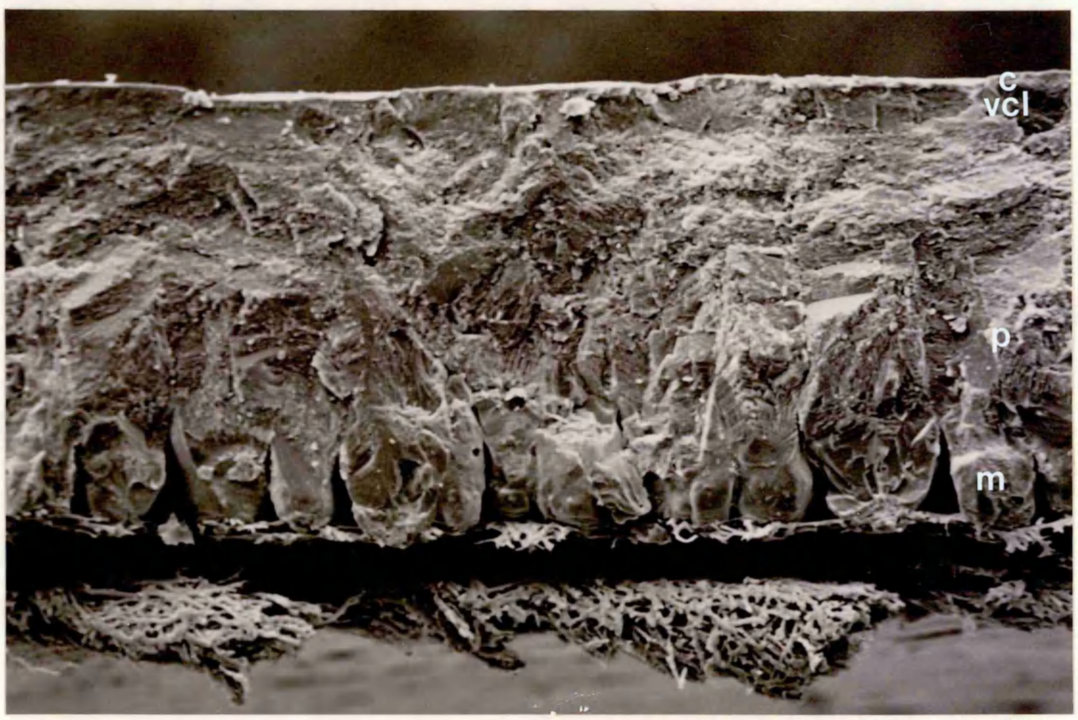


Figure 23 Transverse section of a thin shelled egg x 160. m = mammillary layer; p = palisade, vcl = vertical crystal layer; c = cuticle (see Fig 1).

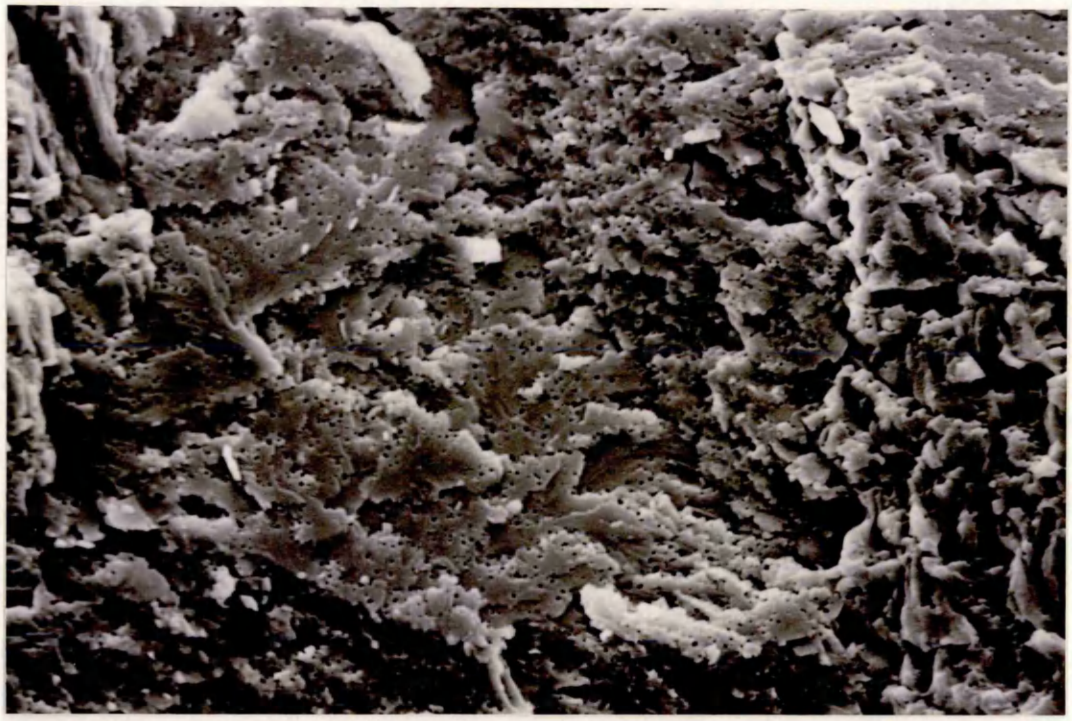


Figure 24 Transverse section through the shell illustrating the characteristic 'spongy' appearance of the mid/upper palisade x 1250.

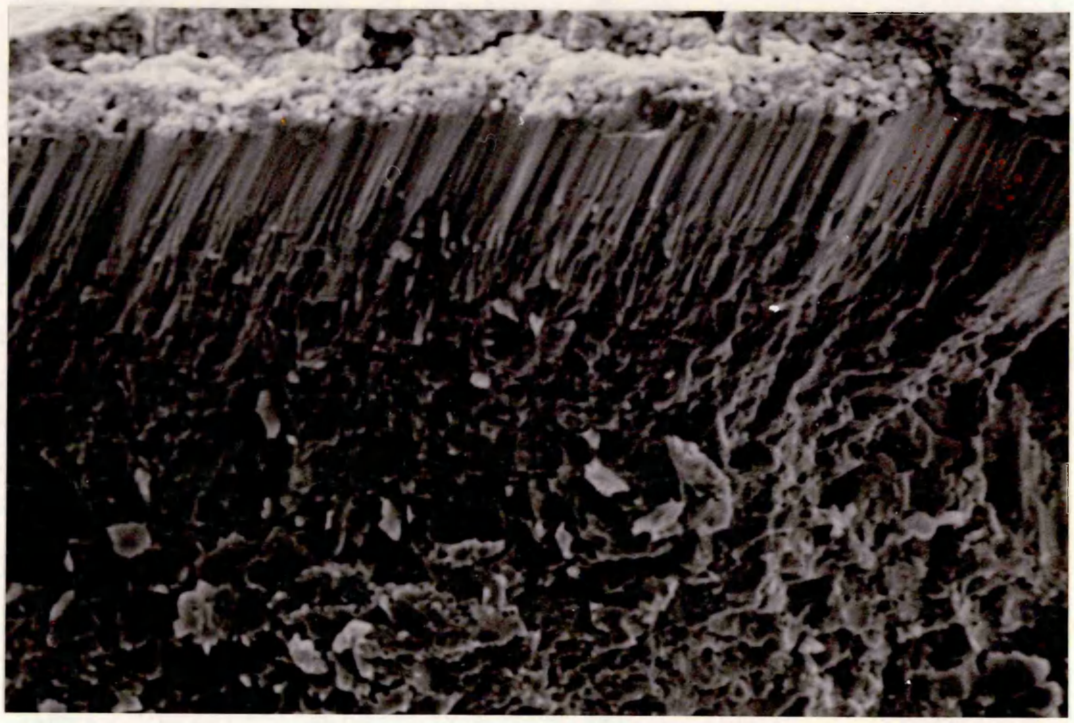


Figure 25 Transverse section through the shell illustrating the more compact appearance of the outer palisade and vertical crystal layer x 1250.

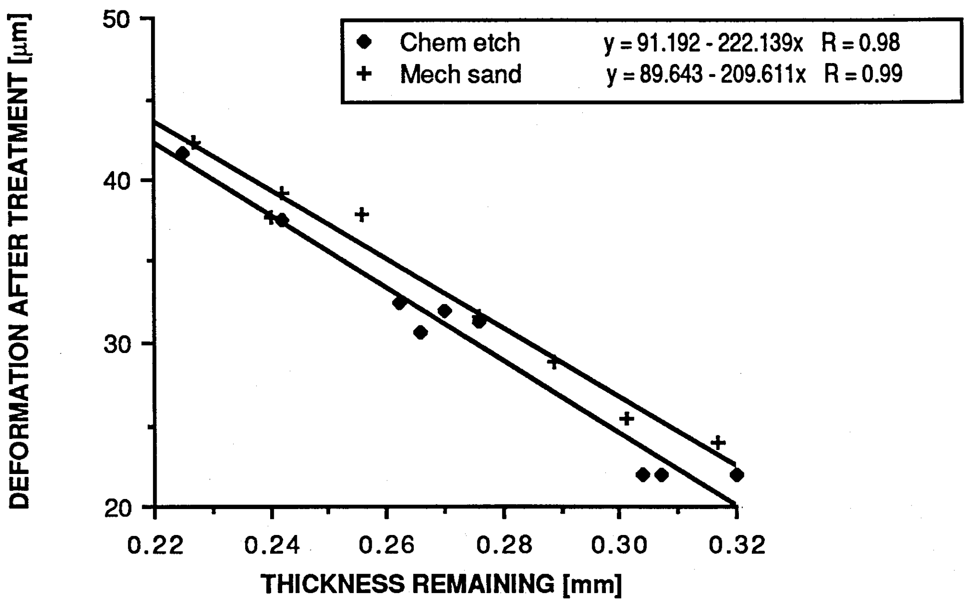


Figure 26 A comparison of mechanical vs chemical thinning techniques. All samples were standardised before treatment with respect to egg weight, and ND deformation.

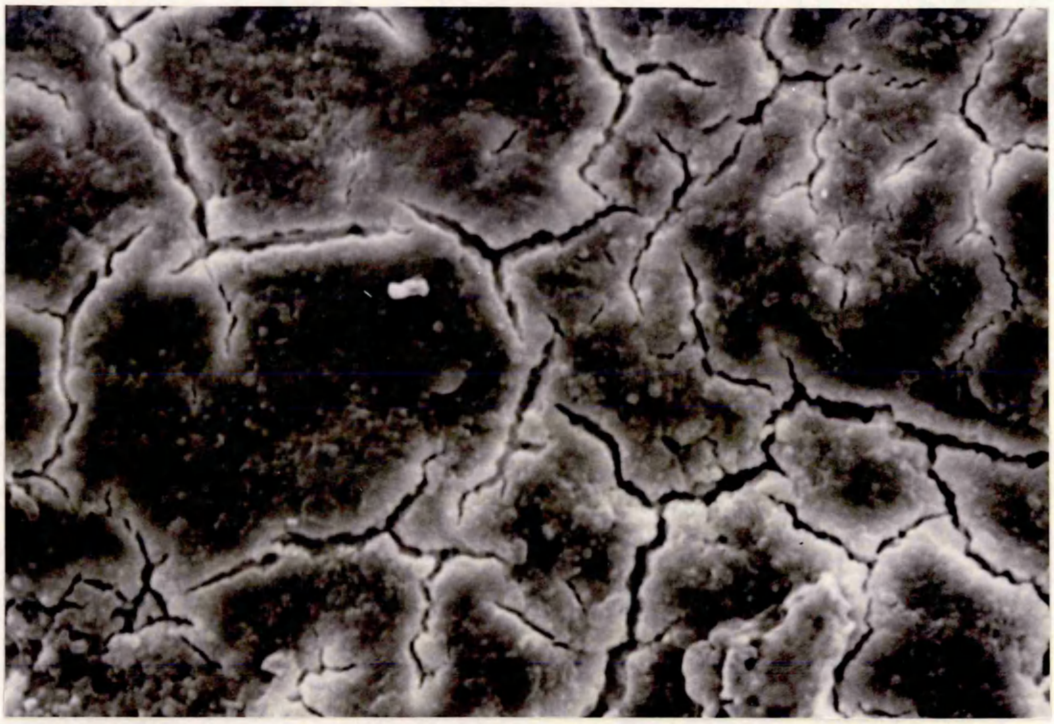


Figure 27 Normal appearance of the cuticle before treatment with EDTA x 1250.

Figure 28 Outer surface of the egg after removal of the cuticle by EDTA. The typical honeycomb appearance of the palisade layer is now visible x 1250.

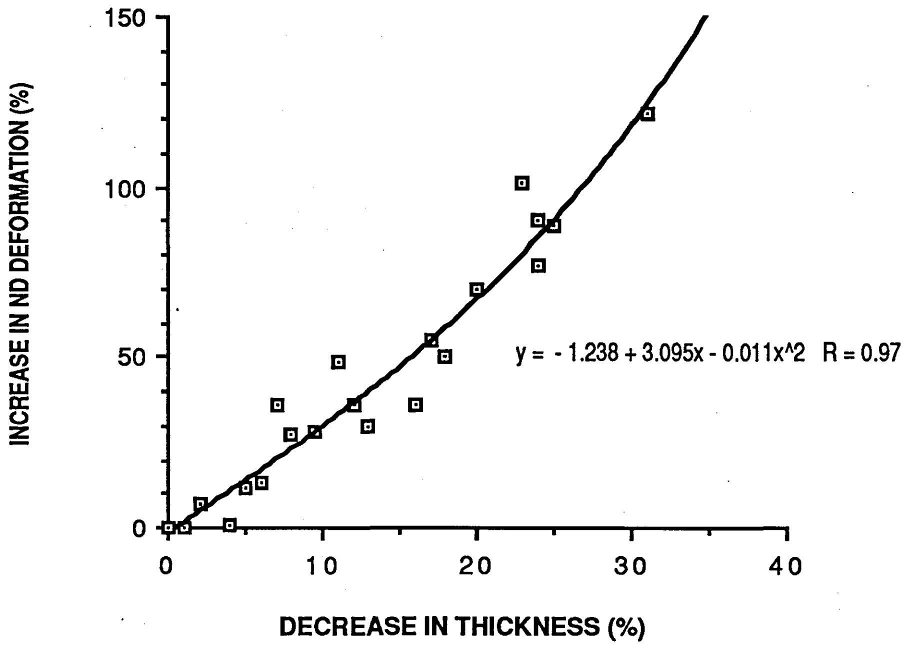


Figure 29 A curvilinear relationship exists between the stiffness characteristics of eggshells, as measured using the non-destructive (ND) deformation test, and the amount of shell removed from their outer surface by chemical etching.

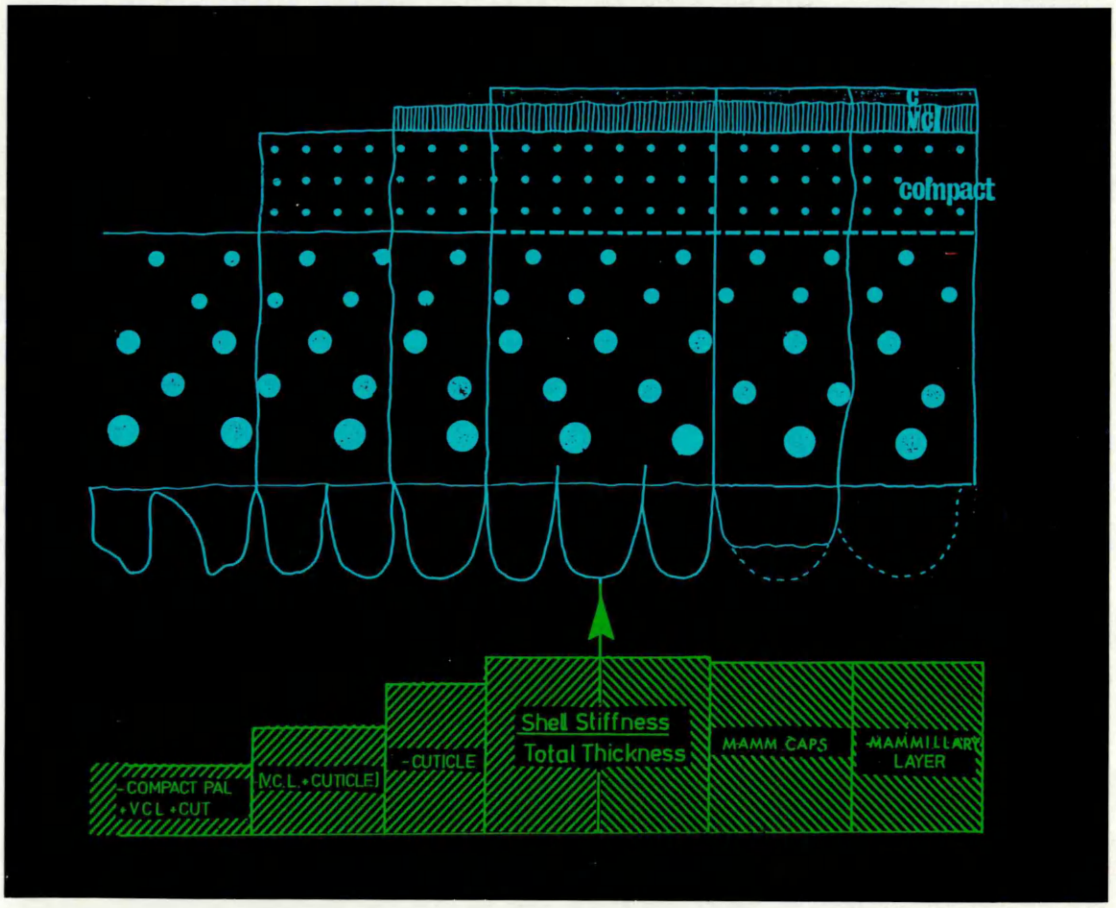


Figure 30 Summary of chemical thinning experiments. The histogram at the bottom of the figure illustrates the effect of removing different layers on the stiffness characteristics of the remaining shell.

EGG NO	ND DEFORMATION [µm]	TOTAL THICKNESS [mm]	MAM THICKNESS [mm]	TOTAL - MAM [mm]
MB4	57.5 +/- 1.90	0.221	0.064	0.157
MB9	33.7 +/- 0.25	0.291	0.072	0.219
MB8	36.5 +/- 0.80	0.242	0.073	0.169
MB3	32.7 +/- 0.50	0.292	0.074	0.218
MB14	41.5 +/- 0.50	0.267	0.065	0.202
MB7	27.2 +/- 0.75	0.283	0.053	0.230
MB6	28.0 +/- 1.82	0.294	0.071	0.223
MB1	19.6 +/- 0.75	0.359	0.075	0.284
MB2	18.8 +/- 1.03	0.354	0.075	0.279
MB5	17.7 +/- 1.26	0.385	0.081	0.304
MB13	15.2 +/- 0.75	0.368	0.071	0.297
MB12	16.2 +/- 0.50	0.358	0.080	0.278
MB11	18.2 +/- 0.75	0.342	0.078	0.264
MB10	19.0 +/- 0.00	0.325	0.064	0.261

Table 6 A comparison of the structural organisation and stiffness characteristics of a number of thick and thin eggshells (TOT-MAM = palisade, VCL and cuticle).

LEVEL	RANGE IN NUMBERS (MAG x 5000)	OVERALL RANGE SIZE (DIAMETER=μm)	SIZE OF MAJORITY IN ZONE (DIAMETER=μm)	ESTIMATED POROSITY (%)
V.C.L / OUTER PALISADE	32-49	0.44-0.08	0.11-0.31	~1%
UPPER PALISADE	128-134	0.46-0.07	0.15-0.33	~3%
MID PALISADE /UPPER PALISADE	98-310	0.44-0.07	0.11-0.22	5-9%
MID PALISADE	58-167	0.69-0.08	0.11-0.69	2-6%
LOWER PALISADE	89-128	0.61-0.07	0.15-0.31	2-3%
JUNCTION PAL/ MAMMILLARY	58-171	0.44-0.07	0.15-0.23	1-3%

Table 7 Distribution of vesicular holes found at different levels within eggshells.

Egg No	Weight [g]	ND Deformation Before Treatment [µm]	ND Deformation After treatment [µm]		SEM Thickness Remaining [mm]
D10	64.16	21.5+/-0.5	25.4+/-1.3	*	0.301+/-0.002
D40	63.91	19.5+/-0.5	24.0+/-0.8	*	0.317+/-0.004
D31	63.10	20.0+/-0.5	31.6+/-1.5	**	0.276+/-0.005
D19	64.62	20.7+/-0.8	39.2+/-0.5	**	0.242+/-0.003
D12	63.03	22.3+/-0.5	37.9+/-0.8	**	0.256+/-0.007
D6	64.09	21.5+/-0.6	28.7+/-1.5	**	0.289+/-0.006
D35	62.89	23.0+/-0.6	42.3+/-0.6	**	0.227+/-0.004
D14	63.09	23.5+/-1.0	37.7+/-2.0	**	0.240+/-0.007

* denotes those samples showing a significant change in ND deformation after treatment.

** denotes samples showing highly significant differences before and after treatment.

Table 8 Results of Mechanical Sanding Experiment.

Egg No	Weight [g]	ND Deformation Before Treatment [μm]	ND Deformation After treatment [μm]	SEM Thickness Remaining [mm]
D1	62.80	22.0 +/- 0.00	21.5 +/- 0.50	0.320+/-0.002
D33	64.32	22.0 +/- 0.50	22.0 +/- 1.00	0.307+/-0.008
D38	62.79	22.0 +/- 0.50	23.0 +/- 1.00	0.302+/-0.005
D24	62.63	22.0 +/- 0.25	31.3 +/- 0.60**	0.270+/-0.006
D3	63.21	22.0 +/- 0.75	32.5 +/- 1.00**	0.262+/-0.003
D15	62.63	22.0 +/- 1.00	31.3 +/- 0.60**	0.276+/-0.009
D39	63.51	22.0 +/- 0.75	30.7 +/- 0.60**	0.266+/-0.012
D20	64.27	20.8 +/- 0.23	37.5 +/- 1.29**	0.242+/-0.002
D29	62.74	21.2 +/- 0.96	41.6 +/- 1.32**	0.225+/-0.007
D13	64.07	23.3 +/- 0.20	36.0 +/- 4.20 †	0.229+/-0.006
D22	63.15	23.0 +/- 0.00	47.6 +/- 3.25 †	0.232+/-0.010
D44	63.46	22.0 +/- 1.00	41.7 +/- 4.40 †	0.224+/-0.006
D32	64.50	22.5 +/- 0.50	46.3 +/- 3.20 †	0.213+/-0.007
D7	63.50	22.0 +/- 1.00	BROKEN	BROKEN

** denotes those samples showing highly significant changes in ND deformation after treatment.

† denotes those samples which were considered to have been affected by acid penetration (s.d ≥ 2.0).

Table 9 Immersion of whole eggs in 50% HCl.

Egg No	Weight [g]	ND Deformation before treatment [μm]	ND Deformation after treatment [μm]
D26	63.27	18.0 +/- 0.0	20.5 +/- 0.5▼
D41	63.59	18.0 +/- 0.2	20.5 +/- 0.5▼
D27	63.85	19.0 +/- 0.0	21.0 +/- 0.0▼
D37	64.01	19.5 +/- 0.5	22.5 +/- 1.5▼
D110	65.63	19.5 +/- 0.5	23.5 +/- 0.5▼
D96	63.97	21.0 +/- 0.8	23.5 +/- 1.7▼
D95	64.09	20.5 +/- 0.5	22.7 +/- 1.0▼
D94	63.47	19.5 +/- 0.5	22.2 +/- 1.7▼
D105	62.63	19.5 +/- 0.5	23.5 +/- 0.5▼
D100	62.30	21.5 +/- 0.5	23.0 +/- 0.8
D102	62.82	20.5 +/- 0.5	21.0 +/- 0.8
D99	63.30	21.5 +/- 0.5	25.0 +/- 0.8▼
D98	62.82	20.5 +/- 0.5	23.0 +/- 0.8▼
D97	64.52	19.2 +/- 0.5	20.7 +/- 1.0

▼denotes those samples showing a significant increase in non-destructive deformation [≥2μm] after treatment.

Table 10 Chemical removal of the Cuticle using EDTA results in a small but nevertheless significant increase in the ND deformation value.

Table 11 ND deformation before and after semi-immersion of eggs in a 50% solution of HCl.

EGG NO	WEIGHT	DEFORMATION	DEFORMATION AFTER ETCHING		SEM THICKNESS AFTER ETCHING	
		BEFORE ETCHING	NORMAL SITE	ETCHED SURFACE	NORMAL SITE	ETCHED SITE
	[g]	[µm]	[µm]	[µm]	[mm]	[mm]
D17	63.69	20.5 +/- 1.5	21.1 +/- 0.9	20.5 +/- 1.0	0.323+/-0.001	0.319+/-0.002
D30	64.23	19.5 +/- 0.5	19.8 +/- 0.4	20.8 +/- 1.3	0.337+/-0.000	0.330+/-0.005
D34	63.10	23.5 +/- 0.5	23.8 +/- 0.7	24.0 +/- 0.6	0.308+/-0.005	0.294+/-0.002
D36	64.84	20.2 +/- 1.0	21.2 +/- 0.4	22.7 +/- 0.5▼	0.334+/-0.005	0.319+/-0.010
D2	62.92	20.5 +/- 0.5	20.5 +/- 0.5	23.2 +/- 0.7▼	0.332+/-0.002	0.312+/-0.005
D28	63.56	20.0 +/- 0.8	20.0 +/- 1.2	27.3 +/- 0.5▼	0.337+/-0.008	0.313+/-0.005
D43	64.52	21.0 +/- 0.8	20.8 +/- 1.2	27.3 +/- 0.5▼	0.339+/-0.004	0.295+/-0.008
D45	63.14	20.0 +/- 0.8	21.8 +/- 0.7	25.5 +/- 1.2▼	0.325+/-0.012	0.299+/-0.004
D9	63.76	23.0 +/- 0.8	23.3 +/- 0.7	31.3 +/- 1.0▼	0.310+/-0.004	0.259+/-0.011
D16	63.48	22.2 +/- 0.5	23.0 +/- 0.9	37.7 +/- 1.6▽	0.293+/-0.010	0.233+/-0.006
D18	64.90	22.5 +/- 0.5	27.2 +/- 1.2▼	39.8 +/- 1.9▽	0.301+/-0.010	0.230+/-0.006
D4	63.21	22.8 +/- 0.3	25.0 +/- 1.0▼	46.7 +/- 1.3▽	0.289+/-0.001	0.222+/-0.007
E2	64.62	18.7 +/- 0.5	19.1 +/- 0.7	25.5 +/- 0.5▼	0.340+/-0.006	0.300+/-0.004
E6	64.58	20.2 +/- 0.5	20.5 +/- 0.5	26.0 +/- 0.5▼	0.347+/-0.005	0.314+/-0.006
E7	63.19	20.6 +/- 0.5	22.3 +/- 1.2	39.0 +/- 0.2▽	0.321+/-0.003	0.245+/-0.011
E9	63.83	20.7 +/- 0.8	22.0 +/- 1.5	31.0 +/- 1.0▼	0.337+/-0.004	0.278+/-0.004
E14	64.10	19.4 +/- 0.5	21.3 +/- 0.2	30.0 +/- 1.0▼	0.346+/-0.005	0.287+/-0.009
E15	63.85	18.0 +/- 0.7	25.5 +/- 2.2▼	40.0 +/- 0.7▽	0.338+/-0.004	0.233+/-0.008
E18	64.89	20.5 +/- 0.5	21.5 +/- 0.5	30.5 +/- 0.5▼	0.343+/-0.006	0.307+/-0.005
E19	63.63	22.5 +/- 0.5	23.3 +/- 0.5	42.5 +/- 0.5▽	0.344+/-0.003	0.257+/-0.006
D42	64.52	19.7 +/- 0.5	UNTREATED		0.337+/-0.002	UNTREATED

▼ denotes those eggs showing significant differences in deformation after treatment.
▽ denotes those eggs showing highly significant changes in deformation after treatment.

DEGREE OF ETCHING ATTAINED	EGG NUMBER	ND DEFORMATION WHOLE EGG [μm]	ND DEFORMATION MINUS CONTENTS [μm]	ND DEFORMATION AFTER ETCHING [μm]	
Etched to midcone	B8	19.7 +/- 1.2	20.7 +/- 1.2	21.9 +/- 0.6	
	B19	22.5 +/- 0.6	22.2 +/- 0.5	23.1 +/- 0.8	
	B24	21.7 +/- 0.5	22.0 +/- 0.8	23.6 +/- 0.5	
	B12	18.6 +/- 1.1	18.9 +/- 1.2	18.8 +/- 1.0	
	B35	21.0 +/- 1.2	21.3 +/- 0.9	20.9 +/- 0.8	
Etched to 3/4 cone	B20	19.5 +/- 1.0	19.2 +/- 1.0	20.0 +/- 0.7	
	B21†	24.4 +/- 0.9	24.2 +/- 0.8	27.4 +/- 2.8	*
	B29	17.5 +/- 0.1	17.5 +/- 0.5	18.5 +/- 1.0	
	B32	21.5 +/- 1.3	20.8 +/- 1.2	21.5 +/- 1.1	
	B36	22.5 +/- 1.3	22.0 +/- 0.7	22.4 +/- 0.9	
Etched to mam/pal junction.	B17	21.0 +/- 1.4	21.2 +/- 1.0	23.6 +/- 0.8	*
	B18	20.7 +/- 1.0	21.5 +/- 1.0	23.2 +/- 1.1	
	B30	17.5 +/- 0.5	17.5 +/- 0.5	20.3 +/- 0.8	*
	B37 †	18.0 +/- 0.0	18.0 +/- 1.0	22.6 +/- 2.4	*
	B39	18.5 +/- 0.5	18.7 +/- 1.0	20.0 +/- 0.8	
	B40	20.8 +/- 0.8	21.0 +/- 0.5	21.5 +/- 0.5	
	B3	17.5 +/- 0.9	17.5 +/- 0.8	20.5 +/- 0.5	*
Etched into Palisade layer	B1 †	22.0 +/- 0.0	22.6 +/- 0.5	27.9 +/- 2.0	**
	B28	26.5 +/- 1.3	27.0 +/- 0.8	37.0 +/- 0.6	**
	B2	21.0 +/- 0.0	21.6 +/- 0.3	32.7 +/- 0.2	**

* denotes those samples which show a significant difference (≥2μm) in ND deformation after treatment.

** denotes those samples showing highly significant difference in ND deformation.

† denotes those samples which were unequally etched (s.d.≥2μm).

Table 12 The effect of removing the egg contents, shell membranes, and the mammillary layer on the stiffness characteristics of the remaining shell as measured by the ND deformation test.

CHAPTER 3

SECTION 4: THE INFLUENCE OF SHAPE ON THE STIFFNESS CHARACTERISTICS OF THE EGGSHELL.

3.4.1 INTRODUCTION.

Diversity, with reference to eggshape, is pronounced within the class Aves and is associated primarily with environmental constraints such as nest site selection; thus the eggs of the guillimot are elongate to facilitate development on narrow ledges. Within domesticated species such as *Gallus domesticus*, eggshape is also variable, and although genetic selection for egg size together with market pressures for uniformity in shape and colour have minimised extremes, elongate and rounded eggs still account for a significant percentage of all eggs laid. The current packaging system fails to accommodate much of this variation and as a result an increase in the incidence of breakages is inevitable.

The influence of an eggs' shape on its' stiffness or strength characteristics has received only limited consideration. The early work of Stewart (1936) discounted the importance of shape. Richards and Swanson (1965) however reported that while thickness accounted for 56% of the observed differences in egg strength, 15-35% of the variability that remained could be associated with a variation in eggshape.

Rehkugler (1963) applied to eggs the theory that had been developed by engineers for use in designing metal "shells" such as pressure vessels, and concluded that the curvature of an eggshell was important in relation to its' stiffness characteristics. This was followed by the theoretical and experimental studies of Sluka *et al* (1965, 1967); Hunt and Voisey (1966); Voisey and Hunt (1967a,1967b); Tung *et al* (1968); and Carter (1970b) which confirmed that shell curvature was relevant. In each case however the egg was assumed to approximate to a prolate spheroid.

Later, Masic *et al* (1972) found a small but significant correlation between eggshape index (breadth/length x100) and shell deformation. In studies of this type however, eggshape cannot be considered in isolation and so physical parameters such as thickness, porosity and chemical composition all have a role to play in the final result. In contrast the FE method now permits a more detailed study of eggshape to be carried out without interference from such confounding variables, and in this section the latter is used to describe the effect of eggshape on the stiffness characteristics of eggshells.

3.4.2 MATERIALS AND METHODS.

[i] Stiffness Characteristics of Standard Shaped Eggs:

The "standard" eggshaped model illustrated in Figure 17 was analysed using the FE method under point load conditions. The displacement directly below the load point (arrowed) was then used together with the appropriate values of E, t, R and F in Eq (2) (see 2.2.1) to calculate this models' non-dimensionalised compliance [C]. The latter was in turn compared to that of a spherical model under similar loading conditions. The values of t, R, E, and F were identical in each case so that any difference in the compliance values could be directly associated with geometry i.e eggshape. For eggshaped modelling, R was assumed to be equivalent to the radius of curvature at the equator and hence was equal to half the maximum breadth dimensions of the "standard" eggshaped model.

[ii] Characterisation of Different Eggshapes:

The length, breadth and distance of the maximum breadth from the midaxis were measured in a number of eggs classified as being 'elongate', 'elliptical', 'rounded' or as having a 'pronounced pole'. These dimensional data were then compared to that which had been used to generate the "standard" eggshaped model described above. The findings of this study were subsequently used with the aid of FE models not only to illustrate the consequences of increasing the length to breadth ratio on an eggshells' stiffness characteristics, but also the effect of moving the maximum breadth away from the midaxis on this property.

To investigate the effect of changing the length to breadth ratio, additional FE models were generated in which this ratio was varied from 1.0 (sphere) to 2.2 (elongated). In each case, the models were generated according to the method described in chapter 2.2.2 (Step.3), and the resulting analyses were specific to the application of an equatorial point load at the midaxis. For comparative purposes, the displacement directly below the load point was recorded after each analysis, then converted into the non-dimensionalised equivalent (C) using the appropriate values of E, t, R and F in Eq (2) (see 2.2.1).

In the second type of analysis, the length to breadth ratio of the FE models was kept constant, while the position of the maximum breadth was moved progressively further away from the midaxis. This was achieved simply by varying the value of x in Eq (3) during the procedure for model generation (see 2.2.2 Step 3). In each case, the load was then applied at this point and the resulting displacement used to calculate the non-dimensionalised compliance values as before.

3.4.3 RESULTS AND DISCUSSION.

The shape of an egg differs from that of a sphere in that its' length usually does not not equal its' breadth, and the maximum breadth often occurs some way from its' midaxis. Eggs in which the length to breadth ratio is greater than 1.4 often appear elongate or torpedo shaped whilst those in which the ratio is less than 1.3 have a much more rounded appearance (Table 13). There is also considerable variation from one egg to another associated with the position of its' maximum breadth. The nearer the latter is to the midaxis, the more elliptical the shape of the egg, whilst those eggs in which the maximum breadth occurs some distance from the midaxis often have a pronounced pole. Some of these extremes in eggshape are illustrated in Figure 31. Throughout this thesis, eggs were classified as being normal or standard within the limits $1.30<L:B<1.38$ and $1.0<x<3.0$ mm.

Voisey and Hunt (1967b) and Carter (1970b) proposed that when an egg is compressed between two flat plates, the deformation occurs almost entirely within the near neighbourhood of the points at which the load is applied. This behaviour is illustrated in Figure 32. The maximum displacement of the "standard eggshaped" FE model occurs directly below the load while areas out with a 15 degree arc of the load point are essentially unaffected.

The non-dimensionalised compliance of this "standard" eggshaped FE model is compared to that of a spherical FE model under similar loading conditions in Table 14 . The dimensional data, and the input data used in each case are given in Appendix 1.

From Table 14, it would appear that the non-dimensionalised compliance of the eggshaped model was 29% greater than that of the spherical model. Since both models were assigned similar material properties and were analysed under identical loading conditions, it is clear that this difference must be due to the dimensional differences associated with being "eggshaped". Thus, all things being equal, the rounder the geometry, the stiffer it is. These results are in agreement with Frank *et al* (1964) and Masic *et al* (1972) who both reported that rounder eggs often had lower ND deformation values.

This hypothesis was tested further using additional FE models in which the L:B ratio was varied from 1.0 (sphere) to 2.2 (elongated). According to Figure 33 an increase in the length to breadth ratio is accompanied by a curvilinear increase in the non-dimensionalised compliance. Most eggs fall within the range of 1.2<L:B<1.6.

As well as a variable L:B ratio, the position of the maximum breadth can also vary in position relative to the midaxis of an egg. Nevertheless, according to Table 15 it would appear that unless the maximum breadth is well "off centre" then this has little or no effect on the non-dimensionalised compliance [C] of FE models. The "rounded" eggshaped model, for example, behaved in a similar way as the sphere despite the fact that the maximum breadth was 'off centre' in the former. The "pronounced pole" model in contrast, had both an abnormally high L:B ratio, and a maximum breadth which occurred approximately 6mm from its' midaxis. In this case, the latter enhanced the effect of having a high L:B ratio. This however must be regarded to be an exceptional case since the maximum breadth most often occurs within 3mm of the midaxis (Table 13).

In conclusion, the shape of the egg does have a significant effect on its' stiffness characteristics, and it is the length to breadth ratio (and the effect of this on the curvature of the shell) rather than the position of the maximum breadth, which is mainly responsible for this.

Figure 31 Within domesticated species, genetic selection for egg size, together with market pressures for uniformity in shape and colour have to some extent served to minimise extremes in egg shape. Nevertheless elongate and rounded eggs still account for a significant percentage of all eggs laid.

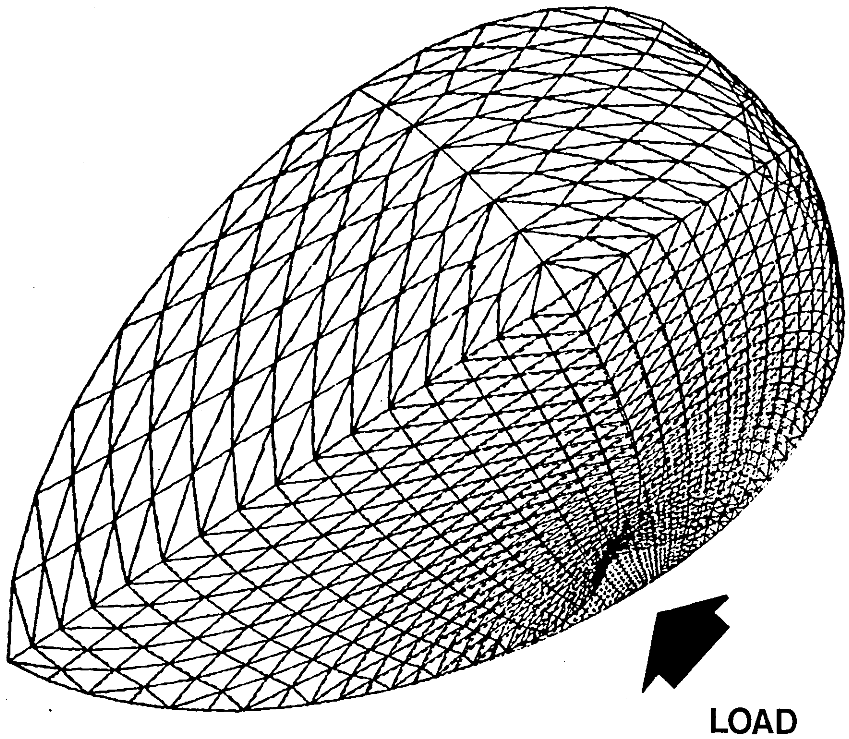
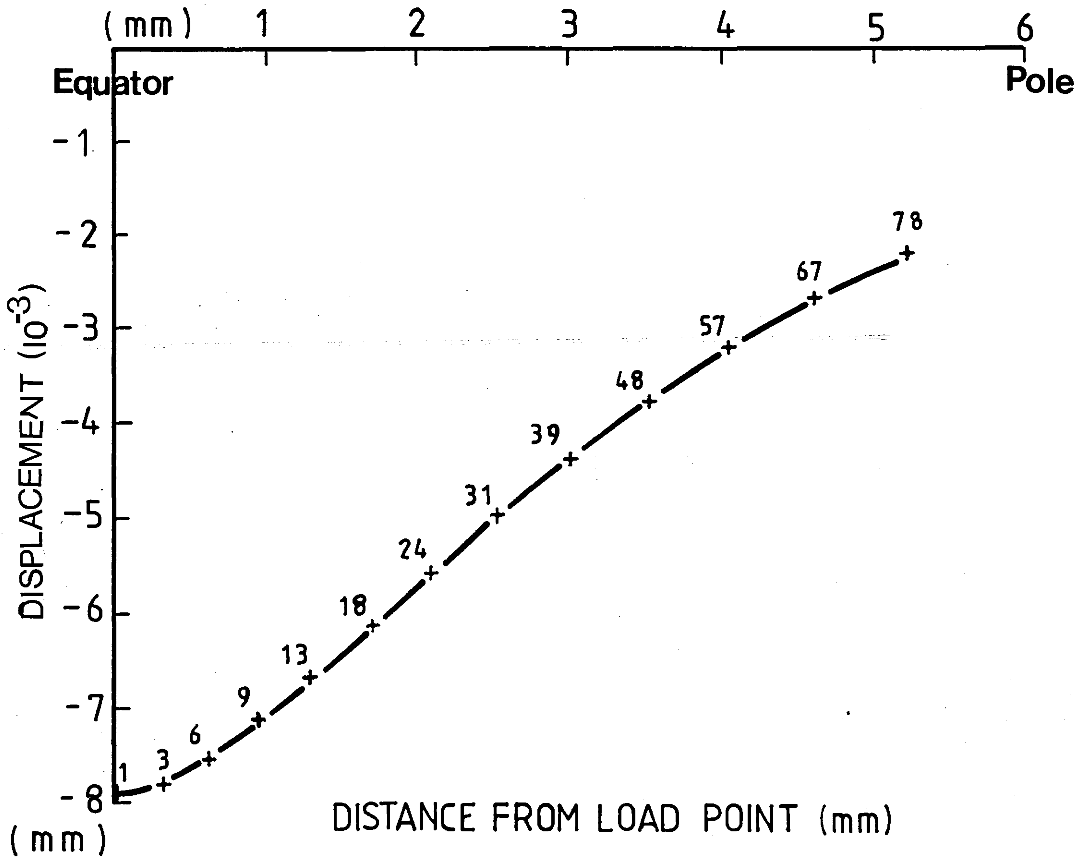


Figure 32 The displacement is localised when a point load is applied to the "standard" eggshaped FE model.



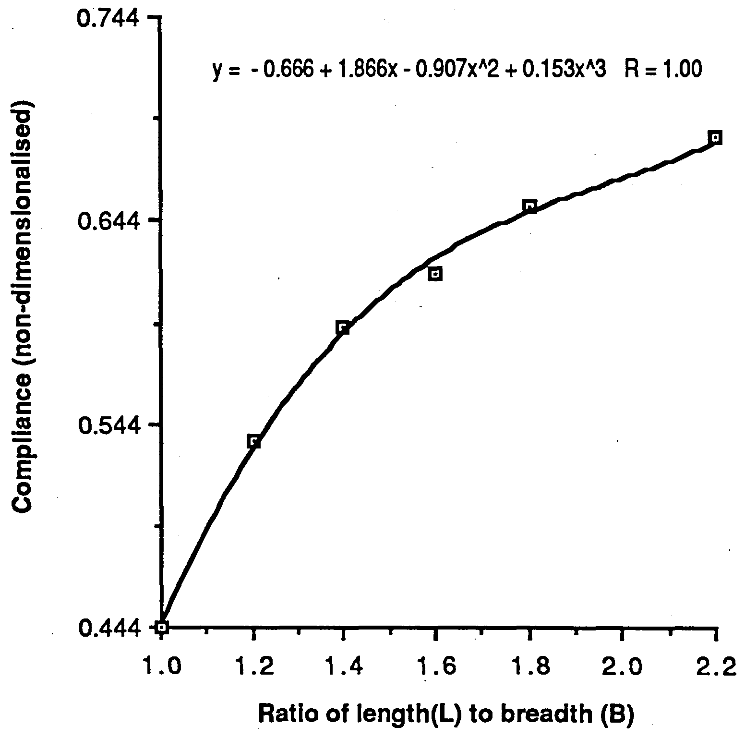


Figure 33 The effect of increasing the length to breadth ratio on the non-dimensionalised compliance (sphere L:B=1; eggshape 1.0<L:B≤2.2).

	Length	Breadth	x	L:B ratio	Visual Assessment
1	61.23	42.96	3.89	1.42	Pron.Pole
2	55.86	43.45	1.33	1.29	S.Standard
3	61.30	43.45	0.20	1.41	Elongated
4	58.23	43.50	2.29	1.34	Standard
5	58.82	44.99	0.16	1.31	Rounded
6	58.44	43.44	3.06	1.34	Pron.Pole
7	59.37	43.98	2.40	1.35	Standard
8	61.11	44.53	3.00	1.37	Pron.Pole
9	61.98	45.22	1.66	1.37	Standard
10	60.68	44.58	1.72	1.36	Standard
11	61.30	43.55	0.49	1.41	Elongated
12	56.11	43.60	1.70	1.29	Pron.Pole
13	55.79	43.22	0.18	1.29	Rounded
14	62.61	45.68	0.26	1.37	Elliptical
15	62.56	44.02	2.61	1.42	L.Standard
16	56.75	43.20	2.36	1.31	Standard
17	60.36	45.03	0.61	1.34	Elliptical
18	56.80	43.51	3.61	1.30	Pron.Pole
19	58.27	42.21	1.36	1.38	Standard
20	59.54	43.95	1.07	1.35	Standard
21	59.36	43.94	1.55	1.35	Standard
22	56.56	42.50	1.64	1.33	Standard
23	58.47	44.79	3.65	1.30	Pron.Pole
24	59.77	44.70	1.47	1.34	Standard
25	56.71	43.74	1.28	1.30	S.Standard
26	58.01	43.97	2.41	1.32	Standard
27	59.85	43.42	2.34	1.38	Standard
28	58.36	44.03	1.19	1.32	Elliptical
29	58.74	44.09	2.82	1.33	Standard
30	69.50	42.10	5.90	1.65	V.Pron.Pole

Table 13 Categorisation of different eggshapes.

MODEL	COMPLIANCE (Non-dimensionalised)	DIFFERENCE (%)
Sphere	0.445	-
Standard Eggshape	0.573	29 %

Table 14 A comparison of the non-dimensionalised compliance of spherical and "standard" eggshaped FE models.

MODEL	L:B	DISTANCE MAX BREADTH TO MIDAXIS x (mm)	COMPLIANCE (Non-dim)	DIFFERENCE (%)
Sphere	1.00	0.00	0.444	-
Rounded	1.00	1.78	0.443	<1%
Elongated	1.65	0.00	0.669	-
Pronounced Pole	1.65	5.90	0.701	~5%

Table 15 The effect of increasing the distance of the maximum breadth to the mid axis on non-dimensionalised compliance values.

CHAPTER 3

SECTION 5: CALCULATION OF THE ELASTIC MODULUS OF EGGSHELL MATERIAL.

3.5.1 INTRODUCTION.

In tests such as the non-destructive deformation test, the elastic properties or stiffness characteristics of an egg are given in terms of the deformation per unit load. While this type of measurement relates to the characteristics of the material from which the shell is made, it also includes the response of the egg as a load bearing structure. Thus, it is possible for two eggs of different shape, thickness or porosity to have a similar deformation or stiffness value. In contrast, if the physical characteristics of the shell material itself could be determined in terms of fundamental properties, then the effect of geometry (including thickness) would be eliminated from this type of investigation.

The elastic modulus (E) of any material describes the unique relationship that exists between the stresses and strains induced when that material is initially loaded. For many materials, determination of this modulus is relatively simple and involves the use of an extensometer mounted on a standard specimen of defined shape and size. The specimen is then loaded by stretching, compressing, or twisting under specified conditions, and a record of the force and deformation during the test provides a direct measure of the elastic constants and ultimate stresses (Sandor 1978). This type of test however cannot be used to measure the modulus of the eggshell due to its' curved nature and the variability in thickness from one specimen to the next (Voisey and Hunt 1974).

Several workers have attempted to determine the elastic modulus of the eggshell by various indirect means, and in most cases this has involved the application of existing engineering theories in which the modulus can be derived from an analysis of the stresses and strains induced under some form of loading (Rehkugler 1963; Sluka *et al* 1967; Hammerle and Mohsenin 1967; Tung *et al* 1969; Nelson and Henderson 1974). However, implicit in the use of such theories are the assumptions that "thin shell" theory applies, that the egg is spherical, and that the material from which the egg is made is uniformly isotropic and elastic.

The results of Chapter 2.2.3 (Step 1) now suggest that the eggshell should in fact be analysed using "thick shell" theory in which both the normal and shear components of stress are taken into account. In addition it is now clear that the shape of an egg has a considerable effect on its' deformation behaviour. The present work has also demonstrated the non-homogeneous nature of the eggshell. While it was not possible to separate the relative contributions made by the palisade, vertical crystal and cuticular layers to the stiffness characteristics of the intact shell, the mammillary layer was shown to have no effect on this property and so must have a very low modulus in comparison to those adjacent layers.

In this section the elastic modulus of the eggshell is determined with the aid of FE solutions which take account of both the shear and normal components of stress, the shape of the egg, and the fact that the mammillary layer has only a minimal role to play in the deformation behaviour of eggs.

3.5.2 THEORETICAL CONSIDERATIONS.

According to Koitres'(1963) solution for a spherical shell subjected to two point loads, the elastic modulus (E) can be determined from measurements of force (F), displacement (d), shell thickness (t), the radius of curvature (R), and the compliance (C).

$$E = C \frac{F \ R}{d \ t^2} \qquad \text{Eq (6)}$$

The elastic modulus of the eggshell (E_{shell}) can now be calculated using a similar equation provided C is taken as the FE calculation of the non-dimensionalised compliance such that,

$$C = C_{sphere} \times A \qquad \text{Eq (7)}$$

Here C_{sphere} is the "thick shell" equivalent to Koitres' (1963) "thin shell" solution (see Figure 15), and A is a factor, the value of which is dependent on the shape of the egg as defined by the length to breadth ratio (see Figure 33). The derivation of this equation is discussed more fully in Appendix 2.

The modulus in Eq (6) must also be considered as a weighted average equal to the combined elastic properties of the mammillary layer, palisade layer, verticle crystal layer (VCL) and cuticle. However, as the stiffness of the shell is unaffected when its' total thickness (t) is reduced by an amount corresponding to that of the mammillary layer (t_m), then the contribution to E_{shell} by the latter can be assumed to be negligible. A more realistic measure of E_{shell} is therefore given by,

$$E_{shell} = C \frac{F\ R}{d\ t^2_{effective}} \qquad \text{Eq (8)}$$

where $t_{effective}$ is equal to the combined thickness of the palisade, VCL and cuticle.

Earlier in this thesis attention was drawn to, and criticism levelled at, those who failed to recognise the heterogeneous nature of the egg. This is not in dispute, but if one considers the egg in engineering terms, then implicit in this consideration is the fact that it will contain flaws. On a macroscopic level the basic assumption of homogeneity must therefore be considered as valid, as without this there is no basis for analysis (Hammerle 1969).

3.5.3 MATERIALS AND METHODS.

ND deformation measurements were carried out on 40 eggs. As point load conditions are assumed in Eq (8), the average ND deformation values for each egg were subsequently corrected for a systematic error of 45% (see 3.2.3) before being converted into units of stiffness (F/d). The length and breadth of each egg was also measured, in this case with hand calipers to the nearest 0.01mm.

After removal of the egg contents, the mean total thickness (t), and mean thickness of the mammillary layer (t_m) of each egg were determined using the SEM method described earlier (see 3.3.2[ii]). From these data the combined thickness ($t_{effective}$) of the palisade layer, VCL and cuticle were obtained.

A simple computer programme was then written in which the elastic modulus (E_{shell}) for each egg was calculated from the appropriate values of F/d, L, B, and $t_{effective}$ using Eq (8). For comparative purposes the modulus values were also derived using Koitres' (1963) "thin shell" solution (Eq (6)), and the FE analysis "thick shell" equivalent to this (C_{sphere}) as given in Appendix 2.

To confirm that the contribution made by the mammillary layer to the elastic modulus of the shell is negligible, an interactive method was used in which a "standard" eggshaped FE model was defined in terms of one and then two layers. The material properties of the one layered model were defined in terms of $t_{effective}$ and E_{shell}, as determined from the above analysis. In the two layered model, the material properties of layer 1 were similarly defined in terms of $t_{effective}$ and E_{shell}, while layer 2, was defined in terms of thickness t_m and the unknown modulus E_{mam}.

In a previous section (3.3.3[iv]) it was shown that the mammillary layer has no effect on the deforming behaviour of the remaining shell. Thus, the value of E_{mam} was estimated by substituting different values for this constant into the input file of the two layered model until the displacement of the latter equalled that obtained when the otherwise identical one layered model was subjected to the same loading conditions.

3.5.4 RESULTS AND DISCUSSION.

The mean values for the elastic modulus of eggshells derived using Eq (8) are compared to those obtained using Koitres' (1963) "thin shell" solution, and the FE "thick shell" equivalent of this (C_{sphere}), in Table 16. Deviations from the mean values are also given. From this table, it is clear that the method used to calculate the modulus has a considerable effect on the values obtained.

The values obtained using Koitres' (1963) "thin shell" solution are similar to those reported by Rehkugler (1963) and Manceau and Henderson (1970), but are considerably lower than those cited by Tung *et al* (1969) and Nelson and Henderson (1974). In each case however the modulus was derived using classical "thin shell" theory in which the shear components of stress are ignored.

When the shear components are taken into consideration there is a small but significant (Student's t-test; $p<0.01$) increase in the mean modulus value (Table 16). The greatest difference however takes place, when in addition to this, eggshape and, only those layers of the shell which influence its' stiffness characteristics ($t_{effective}$), are used in the calculations (Eq (8)). The latter however assumes that the contribution made by the mammillary layer to the shells' elastic properties is negligible in comparison to those adjacent layers. This was confirmed using one and two layered FE models of the egg (see Table 17).

From Table 17 it would appear that in order to deform by the same amount as an otherwise identical one layered FE model, the value of E_{mam} in the two layered FE models must be ≤1% of that ascribed to layer 1 (E_{shell}). The assumption that the mammillary layer does not significantly contribute to the elastic properties of the eggshell is therefore valid.

The full range of modulus values for the forty eggs tested are given in Table 18 along with the dimensional data, stiffness, and thickness values used in the calculations. The modulus of approximately 90% of the eggs occurs within the range 25,000-35,000 Nmm^{-2}. This is considerably lower than the average published values for limestone (59,000 Nmm^{-2}) and marble (56,000 Nmm^{-2}) both of which are similar in chemical composition to eggshells. Tung *et al* (1968) suggested that the presence of the organic matrix may lower the modulus of eggshells. In this respect it is interesting to note that the values of E_{shell} in Table 18 are similar to that of bone (30,000 Nmm^{-2} from Gordon 1976).

The presence of vesicular holes throughout the effective thickness of the eggshell must also have an effect on its' elastic modulus. Had these vesicular holes been equally distributed throughout the entire thickness of the shell then perhaps,

$$E_{shell} \sim E_{Limestone}(1-V) \qquad \text{Eq (9)}$$

where V is the volume fraction of vesicular holes at any level through the shell wall. In real terms however both the size and number of these vesicular holes is highly variable (see 3.3.3).

In conclusion, Eq (8) now permits the elastic properties of eggshells to be described and compared in terms of a single fundamental parameter: E_{shell}. By calculating this property differences in thickness ($t_{effective}$) or stiffness (F/d) can be eliminated from comparative type investigations, but more importantly, the effects of eggshape are also eliminated.

Theory	Considerations	E_{shell} (mean +/- s.d.)
Koitres' thin shell	Spherical; R/t>300; L:B=1; t_{total}	13900 +/- 1700 Nmm^{-2}
FE thick shell	Spherical; R/t<300; L:B=1; t_{total}	15400 +/- 1800 Nmm^{-2}
FE (Eq(8))	Eggshaped; R/t<300; L:B>1; $t_{effective}$	30200 +/- 3900 Nmm^{-2}

Table 16 Comparison of methods used to calculate the elastic modulus of eggshells.

Model type	$t_{effective}$ [mm]	t_{mam} [mm]	E_{shell} [Nmm^{-2}]	E_{mam} [Nmm^{-2}]	Displacement [mm]
i) I LAYERED MODEL	0.251	-	36400	-	0.028
ii) 2 LAYERED MODEL	0.251	0.079	36400	740	0.023
iii) 2 LAYERED MODEL	0.251	0.079	36400	400	0.028

Table 17 Derivation of the elastic modulus of the mammillary layer (E_{mam}) from FE analysis of one and two layered models. The displacement of the two layered model is approximately equal to the one layered model when E_{mam} is 400 Nmm^{-2}.

	Length (mm)	Breadth (mm)	L:B	F/d (Nmm^{-1})	t_{total} (mm)	t_{mam} (mm)	$t_{effective}$ (mm)	Eshell (Nmm^{-2})
1	53.6	40.2	1.33	179.000	0.332	0.066	0.266	2.94e+4
2	55.2	41.8	1.32	200.000	0.316	0.062	0.254	3.58e+4
3	54.2	41.7	1.30	179.000	0.355	0.072	0.283	2.71e+4
4	57.7	44.7	1.29	189.000	0.351	0.070	0.281	3.04e+4
5	56.6	42.5	1.33	155.000	0.320	0.073	0.247	3.08e+4
6	54.3	40.5	1.34	175.000	0.332	0.067	0.265	2.91e+4
7	54.4	42.8	1.27	181.000	0.347	0.067	0.280	2.70e+4
8	54.3	41.0	1.32	192.000	0.360	0.066	0.294	2.65e+4
9	54.4	42.9	1.27	192.000	0.364	0.086	0.278	2.95e+4
10	56.3	42.2	1.33	203.000	0.327	0.064	0.263	3.55e+4
11	54.5	41.9	1.30	181.000	0.343	0.064	0.279	2.81e+4
12	53.4	42.4	1.26	172.000	0.327	0.068	0.259	3.00e+4
13	60.0	44.0	1.36	162.000	0.326	0.060	0.266	2.94e+4
14	58.0	44.9	1.29	188.000	0.359	0.082	0.278	3.08e+4
15	57.7	42.0	1.37	199.000	0.325	0.067	0.258	3.66e+4
16	61.5	44.7	1.38	162.000	0.337	0.082	0.254	3.30e+4
17	58.4	43.8	1.33	126.000	0.280	0.062	0.218	3.28e+4
18	62.4	42.7	1.46	110.000	0.347	0.064	0.283	1.81e+4
19	58.8	46.5	1.26	188.000	0.326	0.081	0.245	3.97e+4
20	61.5	45.0	1.37	152.000	0.298	0.069	0.229	3.70e+4
21	59.0	45.7	1.29	188.000	0.354	0.062	0.292	2.87e+4
22	56.9	46.0	1.24	179.000	0.345	0.064	0.281	2.84e+4
23	58.3	45.4	1.28	162.000	0.343	0.069	0.275	2.69e+4
24	56.3	43.9	1.28	212.000	0.365	0.079	0.286	3.15e+4
25	61.0	45.6	1.34	157.000	0.292	0.060	0.232	3.83e+4
26	59.3	43.8	1.35	212.000	0.385	0.066	0.319	2.70e+4
27	58.6	45.5	1.29	131.000	0.297	0.056	0.241	2.81e+4
28	59.8	43.6	1.37	188.000	0.377	0.081	0.296	2.75e+4
29	57.2	44.7	1.28	272.000	0.429	0.070	0.359	2.67e+4
30	61.9	44.4	1.39	199.000	0.370	0.065	0.304	2.82e+4
31	54.9	41.6	1.32	188.000	0.354	0.069	0.285	2.76e+4
32	56.8	43.8	1.30	167.000	0.326	0.071	0.255	3.17e+4
33	59.3	43.6	1.36	192.000	0.358	0.070	0.288	2.97e+4
34	64.6	45.0	1.44	172.000	0.355	0.075	0.280	2.92e+4
35	58.9	43.4	1.36	159.000	0.311	0.065	0.246	3.26e+4
36	58.3	43.3	1.35	164.000	0.326	0.062	0.264	2.91e+4
37	61.1	43.5	1.40	108.000	0.263	0.053	0.210	3.06e+4
38	61.0	46.3	1.32	220.000	0.375	0.053	0.322	2.84e+4
39	57.6	46.2	1.25	241.000	0.373	0.051	0.322	2.99e+4
40	59.1	46.0	1.28	126.000	0.286	0.068	0.218	3.31e+4

Table 18 Comparison of individaul egg Eshell values . E_{shell} in each case has been computed by substituting the appropriate values of F/d, R, $t_{effective}$, and C into Eq (8).

CHAPTER 4

SECTION 1: AN INTRODUCTION TO FRACTURE MECHANICS.

4.1.1 GENERAL.

Eggshells can support large forces provided the forces are distributed uniformly over its' surface (Rehkugler 1963), but since contact is usually with a non-matching surface, then the initial contact can be assumed to be confined to a single point.

A complete understanding of the reaction of the eggshell to external forces requires a knowledge of the stress distribution, and the effect of this on naturally occurring flaws in the ultrastructure. Classical engineering theories indicate that failure occurs when the maximum principal stress in any structure attains a critical level. Using this approach, Voisey and Hunt (1967b) concluded that the tensile stresses were at maximum at the inner surface of the eggshell directly beneath the load, and predicted that failure initiated at this site when this stress reached a critical value. Nevertheless, it is obvious that even with the best available estimates of the eggshells' ultimate failure stress (Rehkugler 1963; Hammerle and Mohsenin 1967; Hammerle 1969; Tung et al 1969), classical engineering theories fail to accurately predict the force required to break the shell.

It is possible that the eggshell flattens immediately following the first initial contact, and as a result the load is not confined to a single point; thus the level of stress at the inner surface will be less than predicted for any given load. However, "the worst sign in an engineering material is not a lack of strength or lack of stiffness, desirable as these properties are, but a lack of toughness, that is to say a lack of resistance to the propagation of cracks" Gordon (1976).

Arguably, the most important failure criteria in modern engineering are those which take account of a materials resistance to crack growth since it is now generally accepted that most structures contain cracks or flaws, introduced during manufacture or initiated early in their life. Likewise the eggshell can be considered as the end product of a biological process controlled by an inherent blue print, but which is subject to modification depending upon an individual birds harmony with its' environment. The greatest variation in the ultrastructure of the eggshell takes place at the mammillary layer (Bunk and Balloun 1977,1978; Watt 1985; Solomon 1985a,1990), which is characterised by the presence of many naturally occurring fissures. These "intermammillary spaces" can easily be regarded as defects since they vary both in depth, size and distribution. Nowadays, fracture mechanics problems deal largely with the effects of such defects or cracks, on the ability of a structure to resist load.

The basis of fracture mechanics is the premise that for crack growth to occur two conditions are necessary and sufficient. Firstly, sufficient stress must be available at the crack tip to operate some mechanism of crack growth and, secondly sufficient energy must flow to the crack tip to supply the work done in the creation of new surfaces. Initially it was believed that only the first condition was required. According to Inglis (1913-cited by Gordon 1976) the maximum stress ahead of an elliptical hole with semi-axes a and b is

$$\sigma_{max} = \sigma(1 + 2\sqrt{a/b}) \qquad \text{Eq (10)}$$

This solution is insufficient in that it reduces to the problem of a crack when b=0 (Figure 34). As the crack tip is approached the elastic stress tends to infinity, thus leading to the paradoxical conclusion that a cracked body cannot support a load.

This paradox was resolved by A.A Griffith during the period 1893-1963 (from Hayes 1978a). Griffith used an energy balance approach based on surface energy to explain the fracture behaviour of glass. His basic premise was that unstable propagation of a crack takes place only if an increment of crack growth results in more stored energy being released than is absorbed by the creation of the new crack surface (Figure 35). This leads to the energetic criterion for crack growth, of the form,

$$\sigma = \sqrt{(4E'\gamma / \pi a)} \qquad \text{Eq (11)}$$

where γ is the specific surface energy required to break a critical area of material.

Irwin (1957-from Hayes 1978b) introduced an alternative interpretation of fracture phenomena generally referred to as the stress intensity factor approach. The underlying philosophy of this approach is that the stress distribution in the immediate vicinity of a crack tip has unique characteristics defined by a parameter known as the stress intensity factor (K). Geometry, thickness, and loading conditions influence the local stress distribution through this parameter.

$$K = \alpha\sigma\sqrt{(\pi a)} \qquad \text{Eq (12)}$$

Here σ is the applied stress, α is a function of geometry and a is the crack length. For a central crack in an infinite plane, the Griffith relation can be rewritten as,

$$\sigma\sqrt{(\pi a)} = \sqrt{4E'\gamma} \qquad \text{Eq (13)}$$

where γ is the specific surface energy required to break a critical area of material. Using Irwins' (1957) notation Eq(13) can now be written in the form

$$K_c = \sqrt{(E'G_c)} \qquad \text{Eq (14)}$$

where,

$$K_c = \sigma_{max}\sqrt{(\pi a)} \qquad \text{Eq (15)}$$

and,

$$4\gamma = G_c \qquad \text{Eq (16)}$$

G is strain energy release rate per unit thickness associated with the cracks' advancement, and the suffix c denotes a critical value associated with failure.

Thus from Eq (14), fracture is characterised by the attainment of a critical value of the stress intensity factor (K_c) at which point crack propagation is energetically favourable.

The importance of the stress intensity approach is that it enables quantitative relationships to be obtained between the applied stress necessary to cause failure (σ_{max}), and the size of any defects that may be present; thus a specimen with a large flaw will fail at a smaller load than an otherwise identical specimen with shallower defects. The material property which links these two parameters is the fracture toughness. A comparison of fracture toughness values can therefore provide some indication of the severity of defects in two otherwise identical specimens and hence aid the interpretation of fracture test data.

In the next section (4.2.) the mechanism of failure in eggshells is examined in detail. Information from this study is then used together with FE analysis to develop a method for quantifying the fracture toughness of eggshells (see 4.3.). In section 4.4. the results of experiments designed to monitor shell quality in two commercial laying stocks throughout one laying year, are subsequently interpreted both in terms of structural and material property differences. The concluding section (4.5.) attempts to correlate the fracture toughness of eggshells with the type of ultrastructural defects that occur in intrinsically weak and low quality shells.

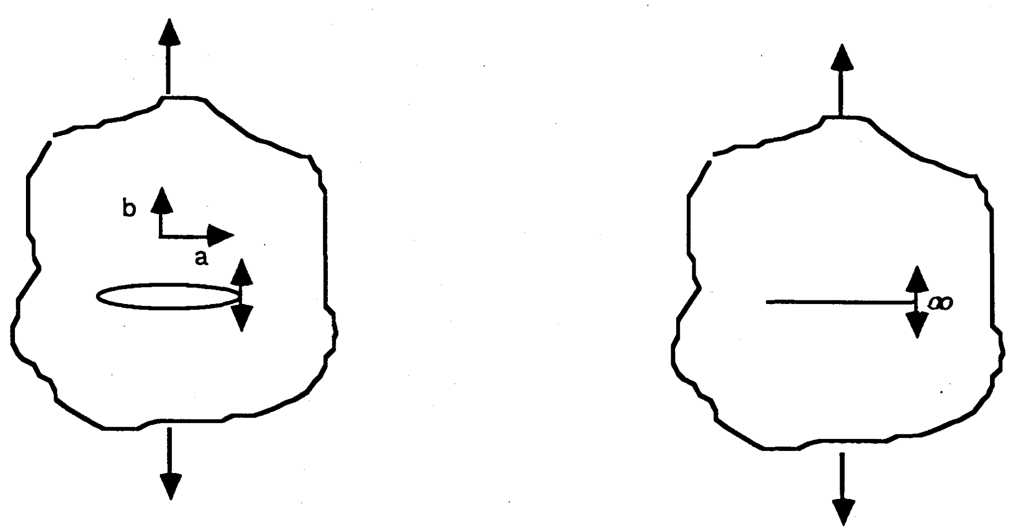


Figure 34 The solution for an elliptical hole is significant in that it reduces to the problem of a crack when b=0. This gives the paradoxical solution that the stress at the tip of a sharp crack is infinite.

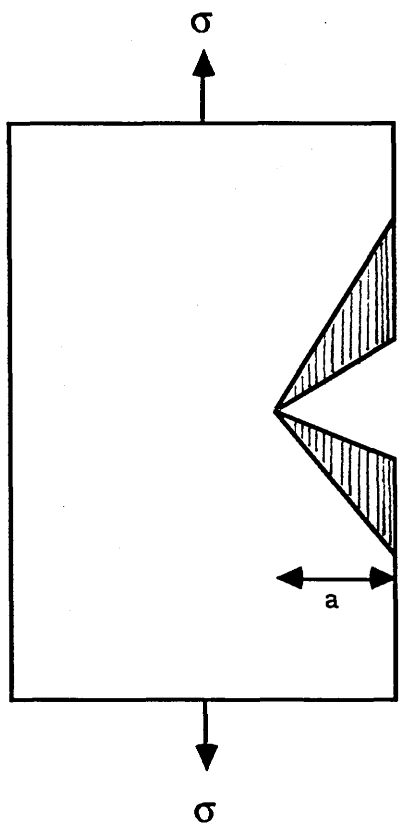


Figure 35 Griffith criterion for crack propagation. As the crack extends the material in the shaded area is relaxed and releases its strain energy. This released energy then become available to propagate the crack.

CHAPTER 4.

SECTION 2: STRESS ANALYSIS AND FRACTURE CHARACTERISTICS OF EGGSHELLS.

4.2.1 INTRODUCTION.

In this section, the distribution of stress within the eggshell is examined by finite element analysis. This information is then used to interpret the behaviour of an egg when it is compressed between two flat plates with particular reference to pre and post fracture behaviour.

4.2.2 MATERIALS AND METHODS.

[i] Finite Element Modelling:

Stress analysis was carried out using the "standard" eggshaped FE model defined in terms of $t_{effective}$ and E_{shell}. The maximum principal stress resulting from the application of a 50N equatorial point load was determined at three points through the thickness of this model, and the results displayed using computer graphics.

[ii] Quasi-static Compression Tests [QSC]:

Each egg was candled to eliminate those which were pre-cracked, holed or checked. Badly misshapen eggs or eggs with abnormalities in texture were also discarded. Of the remaining eggs the position of translucent patches, spots or lines were clearly outlined in pencil. Each egg was then compressed at a constant displacement rate between two flat ground steel surfaces by means of a J.J. Lloyd screw driven testing machine. In every case the egg was placed with its major axis perpendicular to the compression surfaces, in such a way that the force was applied equatorially. The force exerted on the shell and the resulting deformation were recorded and displayed throughout the experiment by means of an X Y chart recorder connected to the output terminal of the test machine.

In the majority of cases the egg was compressed until failure, as characterised by a sharp drop in the force deformation curve and a clearly audible cracking noise. At this point, the crosshead of the machine was immediately reversed, then the area of shell which had been in contact with the upper moving plate was clearly marked in pencil.

On a few occasions, the pressure on the egg and the recordings of the force vs displacement were continued beyond the point of failure in an attempt to characterise post fracture behaviour. Alternatively, the crosshead was reversed before visible damage occurred. The latter non-destructive tests were used to investigate the possibility of crack initiation preceding catastrophic failure.

After each experiment, the eggs were left for several hours to permit diffusion of moisture from the egg contents into the crack space, and then they were re-candled. In each case, photographs were taken of the visible damage within the 'crack initiation zone' and the eggs classified according to crack type, namely multiple, or mainline. The direction of the longest mainline crack was then recorded using a numerical rating procedure similar to that described by Hunt and Voisey (1966). Evidence of damage induced at the opposite loading point, was also noted.

[iii] Examination of Cracksites by Scanning Electron Microscopy [SEM]:

'Crack initiation sites', and damaged areas from the opposite load site were carefully removed using a diamond tipped circular drill piece. Due to the brittle nature of eggshell, a large binding surface was removed along with these areas in an attempt to preserve the experimentally induced crack lines. Samples were also selected for SEM analysis at positions around the egg at, and away from the major crack line. Each sample was then prepared for scanning electron microscopy (SEM).

The bulk of the shell membranes were carefully removed by first soaking in water. The loosely adhering membranes were then gently peeled from the edge of the sample inwards. To remove any remaining membrane fibres, each sample was then subjected to the non-destructive technique of plasma etching for four hours (Reid 1983).

The Nanotech Plasmaprep 100 plasma Chemistry Unit uses a low temperature activated plasma to remove the tightly bound outer membrane fibres from the under surface of the calcified shell. Each specimen is placed innerside uppermost in an atmosphere of oxygen gas at 133.3 Pascals. This is then made reactive by applying a radio frequency of 100 ohms. The organic component of the membrane fibres is removed during this process by volatilisation. Any residual ash is blown off after treatment by lightly applying a "dust off" jet pressure duster leaving the crystalline part of the shell intact.

Samples from both load sites, which had remained intact following these preparative treatments, were mounted innerside uppermost on aluminium stubs, coated with gold/palladium and examined using the SEM for evidence of internal damage. External damage was also assessed by mounting unplasmolysed samples on stubs with their cuticular surface outermost.

Samples selected at points along the mainline cracks tended to separate into two halves following plasma treatment. However, the mainline crack could be reconstructed by careful mounting procedures. The latter were examined to determine if the crack direction was influenced by the structural organisation of the mammillary layer.

4.2.3 RESULTS AND DISCUSSION.

Under point load conditions, the principal stresses at the outer surface of the "standard" eggshaped FE model were found to be compressive in nature, while the inner surface of the model was found to be in tension. The distribution of tensional stress at the inner surface of the model is illustrated in Figure 36. In both cases the stresses were found to be at a maximum directly beneath the point at which the load was applied. These results therefore support the contention that failure will be initiated at this site (see Voisey and Hunt 1967b).

A typical compression test force vs deformation plot is given in Figure 37. The eggshell fails in a typical brittle elastic manner in which the force and deformation increase linearly up to the point at which failure occurs. Most brittle materials characteristically fail in tension (Gordon 1976); thus, the hypothesis is that failure is initiated in the eggshell just below where the palisade columns fuse due to the development of tensile stress concentrations (see Figure 50).

Post fracture behaviour of eggshells is illustrated in Figure 38. After the ultimate failure point has been reached, a degree of resistance is re-established (d). That the shell does not collapse after the first relaxation adds weight to the hypothesis that the mechanism of failure in eggshells is dictated by the resistance of the shell to crack growth rather than by the attainment of the ultimate tensile stress.

Continual loading of the shell was characterised by a second audible crack and a second relaxation period. This cycle of resistance and failure was repeated several times (see Figure 38), until the eggshell eventually imploded.

Figure 39 illustrates the typical type of shell damage produced as a result of compressive loading. Similar types of shell damage have been recorded under dynamic forces (Tyler and Moore 1965) and have also been observed in the field (Carter 1970a; Belyavin and Boorman 1982). Only the major crack lines shown in these figures were visible to the naked eye. The small radiating cracks within the 'crack initiation zone' were only visible when the fractured shells were subsequently candled. Candling also revealed the presence of a translucent patch at the opposite load site (Figure 40) when the eggs were left to stand for several hours after testing. These translucent areas had not previously existed.

Similar translucent patches were found at each load site in the eggs which were tested using non-destructive QSC loads ($15N \leq Force \leq 25N$). When these sites were analysed using the scanning electron microscope, microscopic radiating cracks on the inner and outer surfaces of the shell were revealed (Figures 41 and 42). This type of crack growth pattern is typically found in structures at stress intensities below those normally associated with catastrophic failure but is quickly superseded by a more rapid phase of growth if the interactions between the structure and its' loading environment continue. Photographic evidence suggests that for the eggshell, this critical level of stress must occur when one or more of these minor cracks attains a length of between 6+/-1mm. Thereafter they give rise to mainline cracking.

From Table 19, it would appear that it is the radial cracks which run along the major axis of the egg which most frequently gave rise to the unstable form of crack growth. The reason for this is unclear. Hunt and Voisey (1966) suggested that the direction of the mainline crack maybe influenced by the curvature of the shell and variations in shell thickness, but could find no evidence to support this hypothesis. Current SEM observations suggest that the structural organisation of the mammillae within the 'crack initiation zone' may have a role to play in this scheme of events. Thus, where individual mammillae are aligned the cracks tend to move more easily between the columns (Figures 43 and 44) while randomly arranged mammillae apparently check the rate of crack movement. In contrast, out with the 'crack initiation zone' the mainline cracks were found to be essentially unaffected by the structural arrangement of mammillae. Nevertheless, there was some evidence to suggest that confluent areas were avoided (Figure 45), while pitted or poorly structured areas tended to be favoured by the advancing crack (Figures 46, 47 and 48).

On several occasions circular cracks similar to those described by Tyler and Moore (1965) were found in conjunction with the radial cracks within the 'crack initiation zone' (Figure 49). This phenomenon however was more pronounced in those eggs tested beyond the ultimate failure point (see Figure 38). The fact that the major crack lines passed through these circular cracks, suggests that the latter were formed sometime after the ultimate point of failure. Carter (1970a) proposed that these circular cracks might arise due to the formation of bending moments which are set up as the shell flattens to conform with the loading plate. In other words the load site is bi-axially stressed.

Thus, it can be hypothesised that the mechanism of eggshell failure under compressive forces, and presumably under dynamic forces, consists of a number of phases: First, stress concentrations arise at the inter-mammillary spaces and as a result a crack quickly propagates through the shell wall towards the outer surface (Figure 50). A series of radial cracks then form as the interactions between the shell and its' loading environment continue to increase in magnitude (Figures 41,42 and 51). At a critical level of stress one or more of these radiating cracks becomes unstable and this is accompanied by the characteristic relaxation in the force deformation curve normally associated with shell failure (Figure 37) Post fracture behaviour of the eggshell is characterised by the re-establishment of resistance to crack growth. Thereafter circular cracking (Figure 49) is induced as the shell conforms to the loading plate, and this is accompanied by a series of secondary failures until the shell completely collapses.

While it was impossible to distinguish between 'crack initiation' and the formation of the first radial cracks in these current investigations, it is nevertheless clear that both occur in advance of the first obvious signs of failure. As a result it is now much more meaningful to interpret the strength of eggshells in terms of those factors which affect its ability to resist unstable crack growth (fracture toughness), rather than in terms of its ultimate tensile strength.

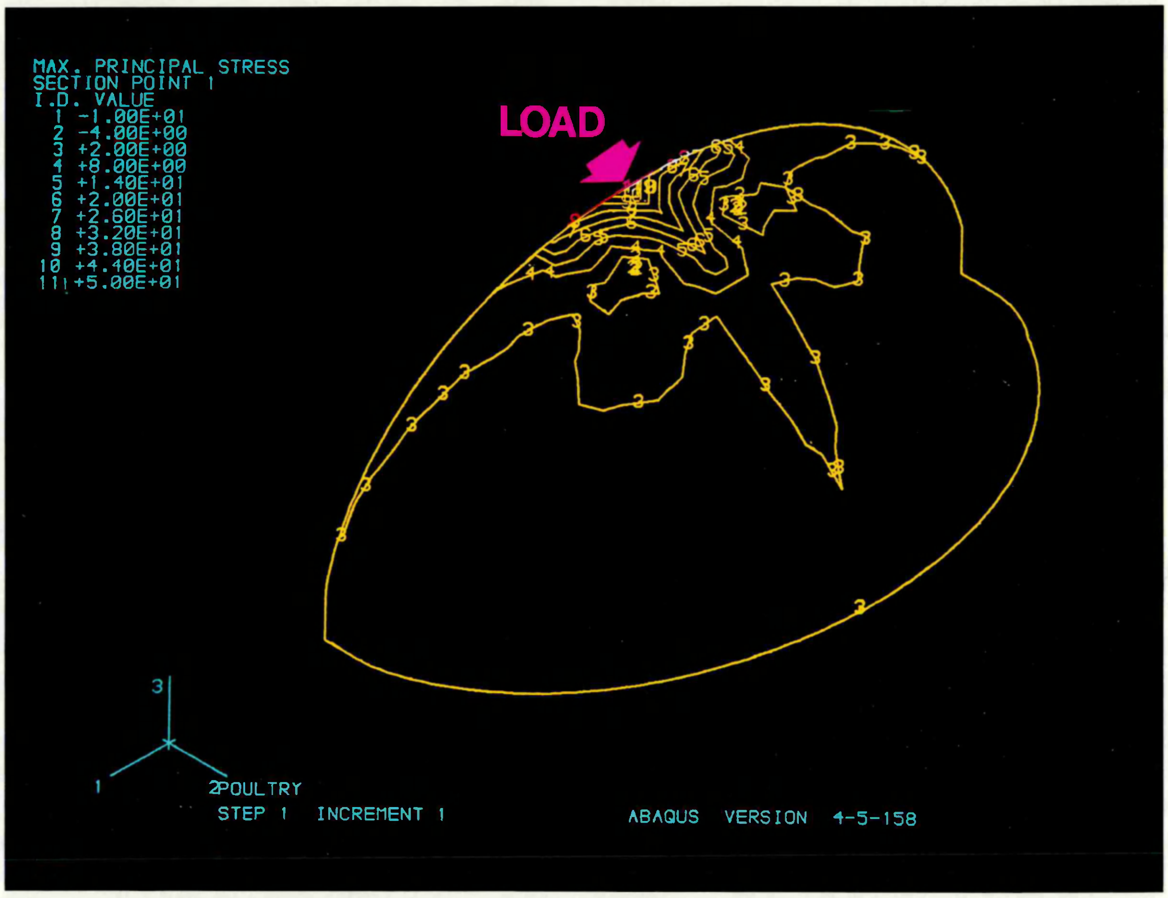


Figure 36 The maximum principal stresses at the inner surface of the "standard" egg shaped model are tensile in nature and give rise to a typical star shaped configuration. (only quarter of the total geometry is illustrated).

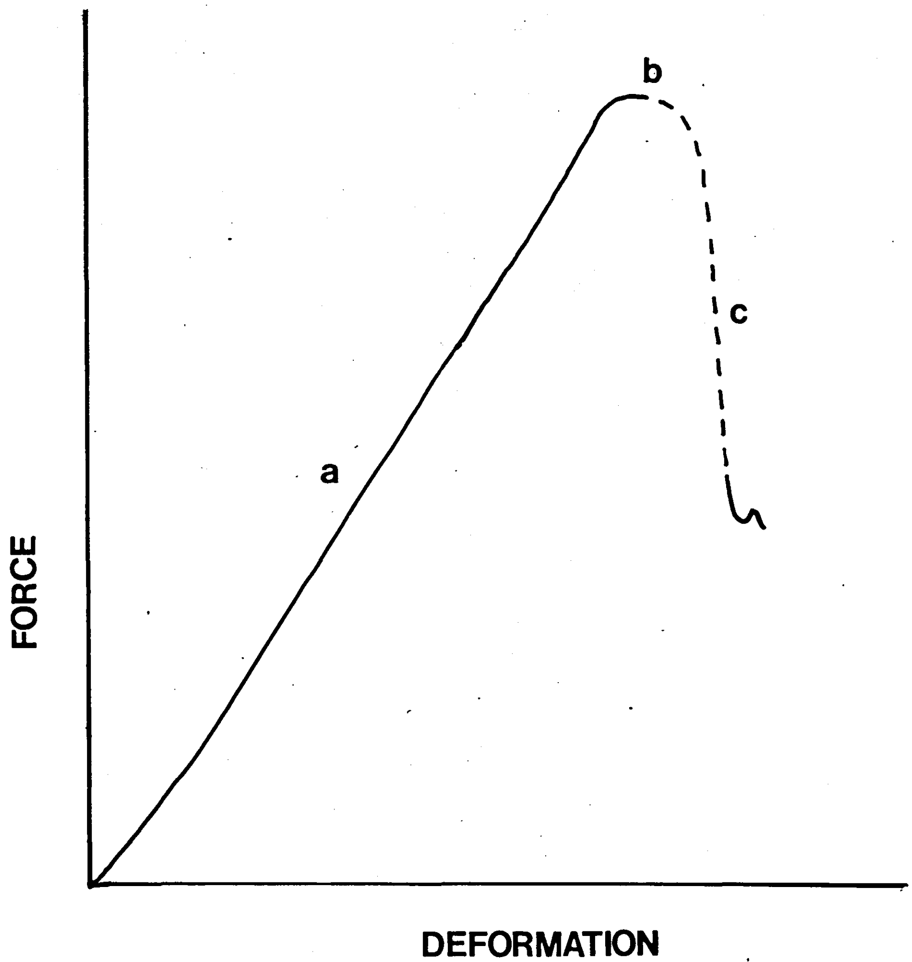
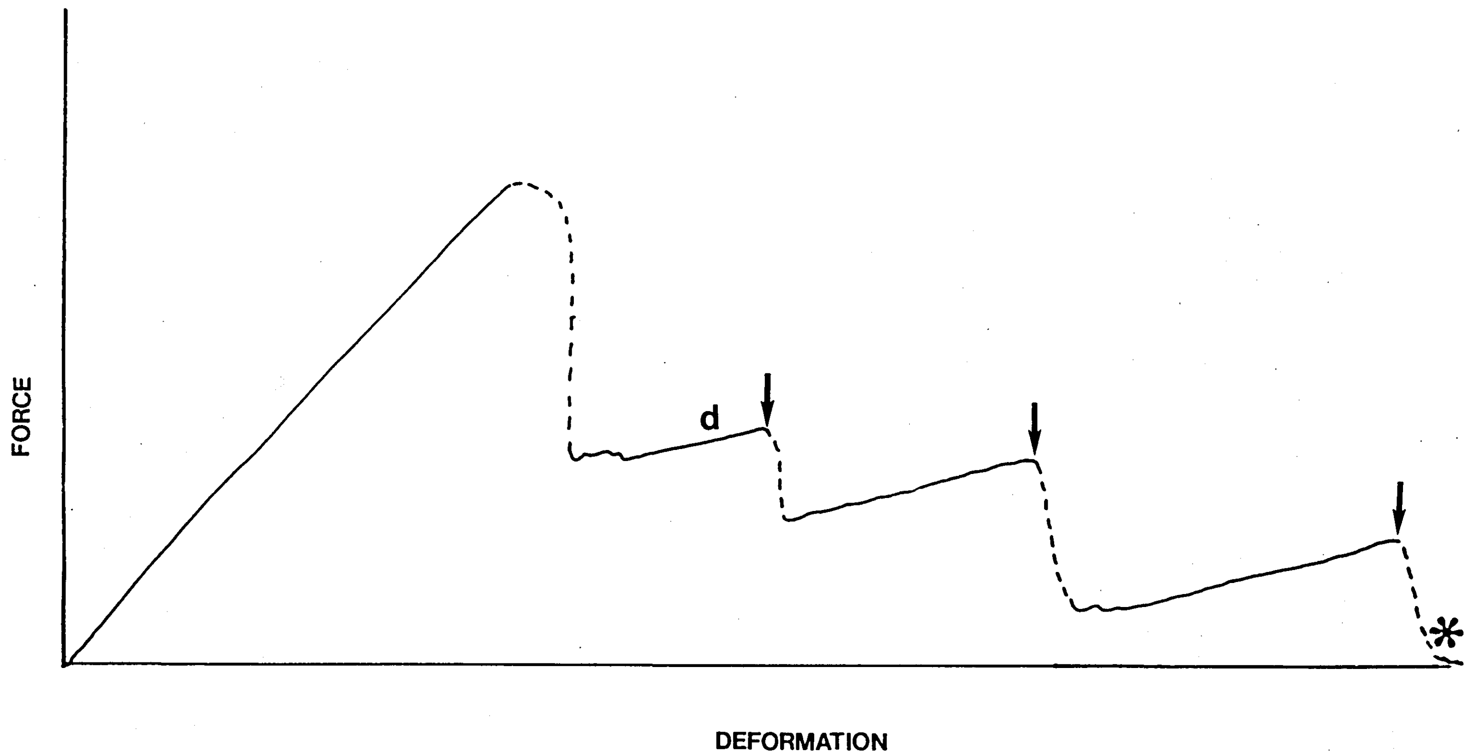


Figure 37 The eggshell fails in a typical brittle elastic manner, in which the force and deformation increase linearly (a) up to the point at which failure occurs(b). The latter is characterised by a large relaxation in the force vs deformation relationship (c).

Figure 38 Post fracture behaviour of eggshells is characterised by the re-establishment of the shells' resistance to crack growth (d). This is then followed by a series of secondary failures (arrowed) and relaxations until the shell eventually implodes (*).



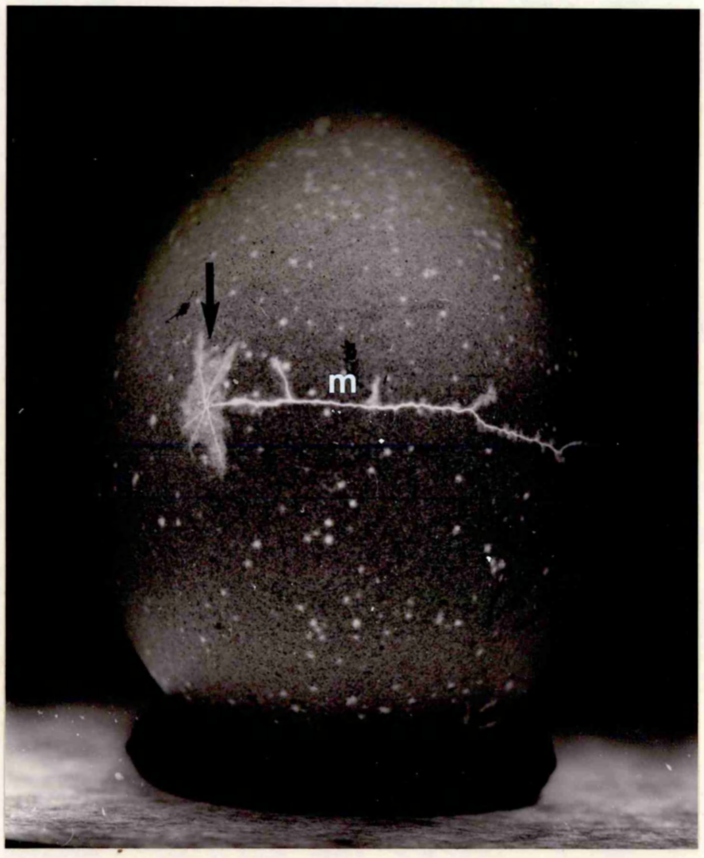


Figure 39 Typical type of damage induced to the shell in quasi-static compression tests. A series of radiating cracks (arrowed) are found within the 'crack initiation zone' and these are accompanied usually by one or more major cracks (m).

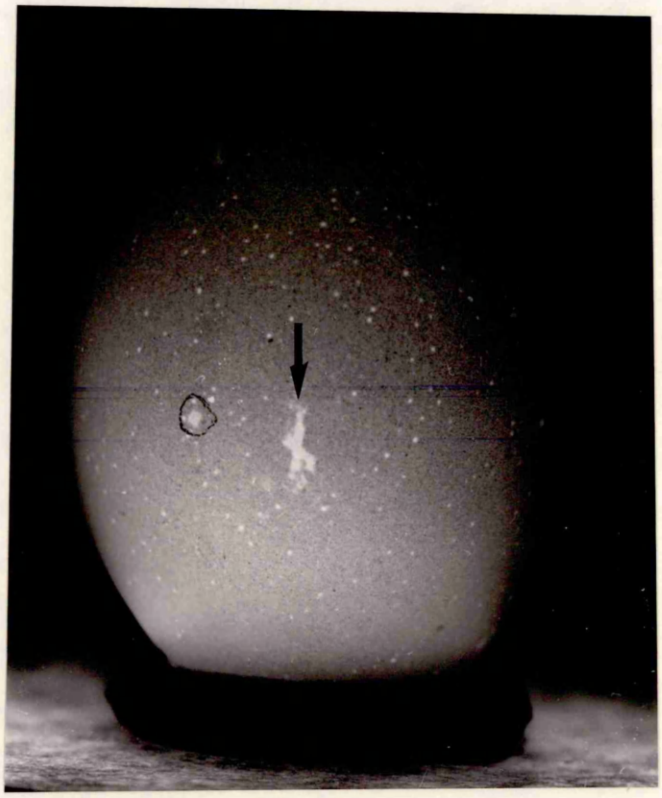


Figure 40 A translucent patch (arrowed) was found on each egg opposite the 'crack initiation zone'. Similar areas were also found at each load site in those eggs tested non-destructively.

Figure 41 SEM revealed the presence of radiating cracks (arrowed) directly beneath the load point in those eggs tested non-destructively x 40.

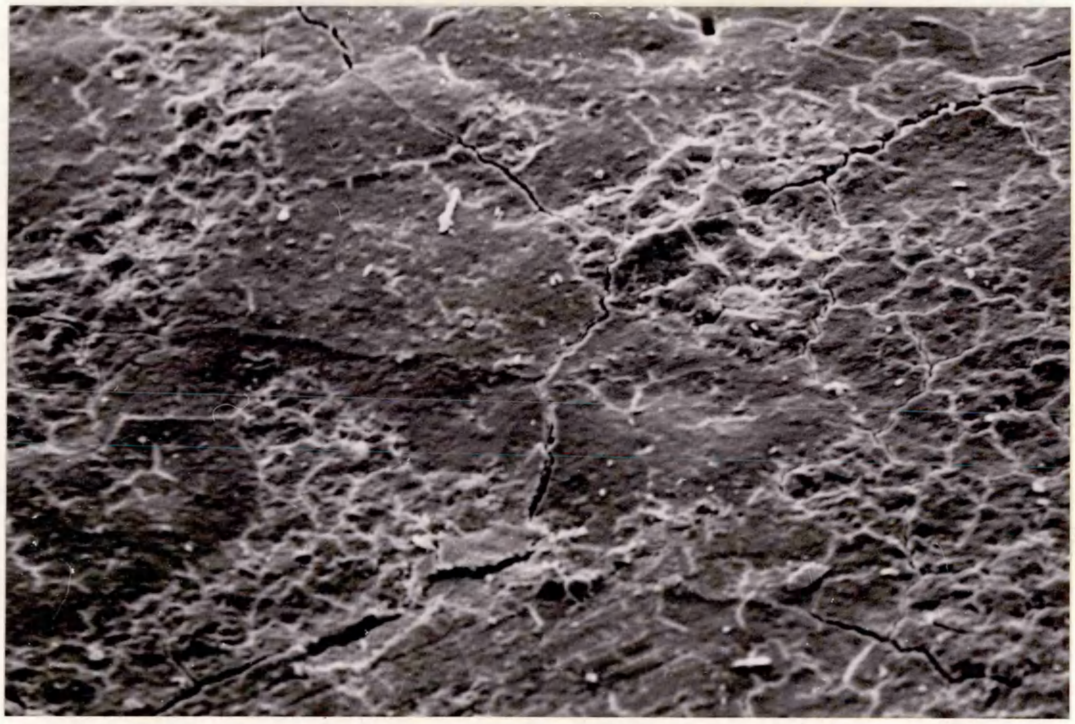


Figure 42 Damage induced to the outer surface of the eggshell as a result of non-destructive quasi-static compression tests x 40.

Figure 43 Cracks (arrowed) tend to select the path of least resistance within the crack initiation zone x 80.

Figure 44 Cracks tend to move around individual mammillae rather than pass through them x 320.

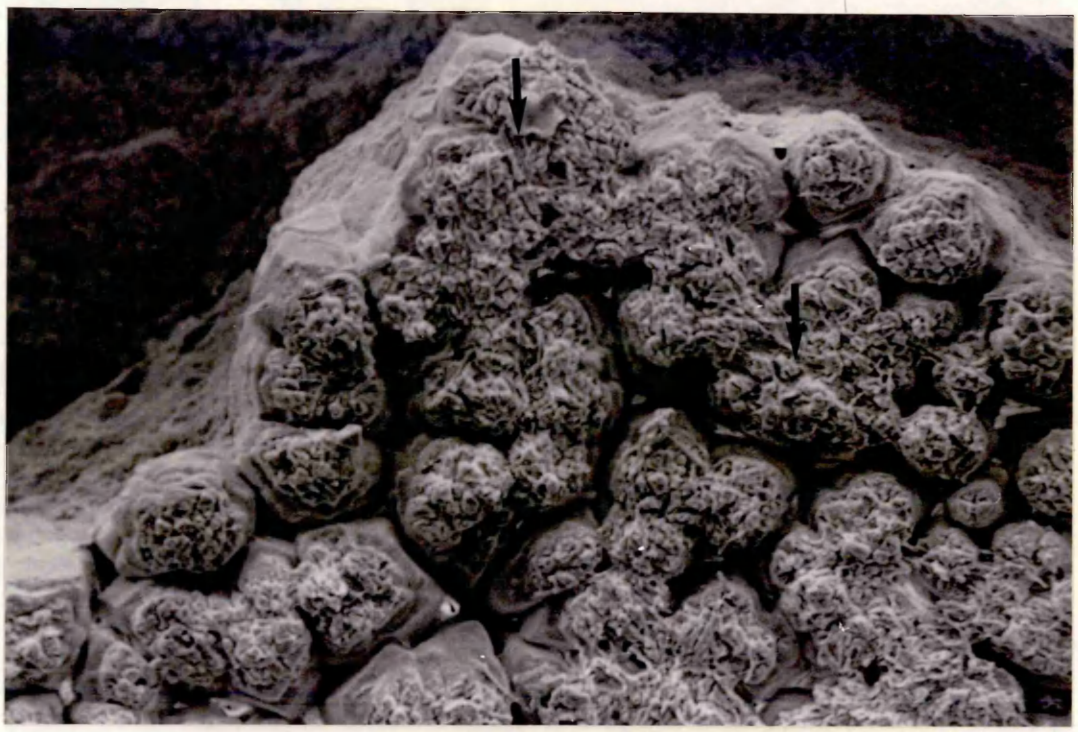


Figure 45 Confluent areas (arrowed) are avoided by the advancing crack x 160.

Figure 46 Poorly structured areas are favoured by the advancing crack x 160.

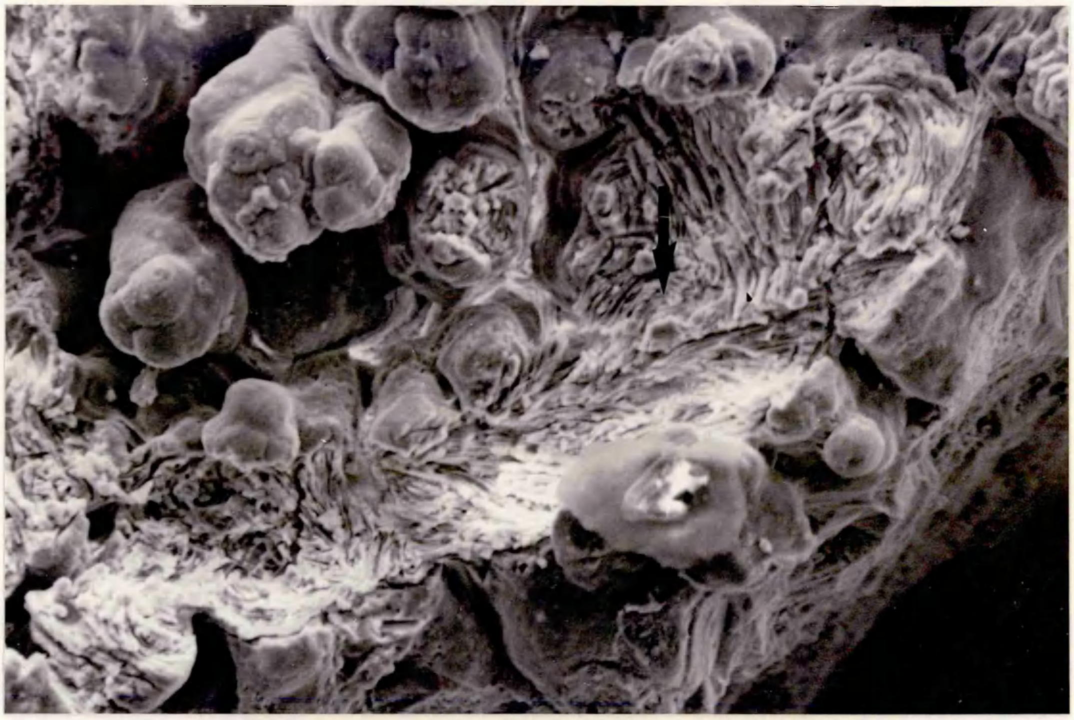


Figure 47 The crack line propagates along areas of structurally inferior mammillae (arrowed) x 320.

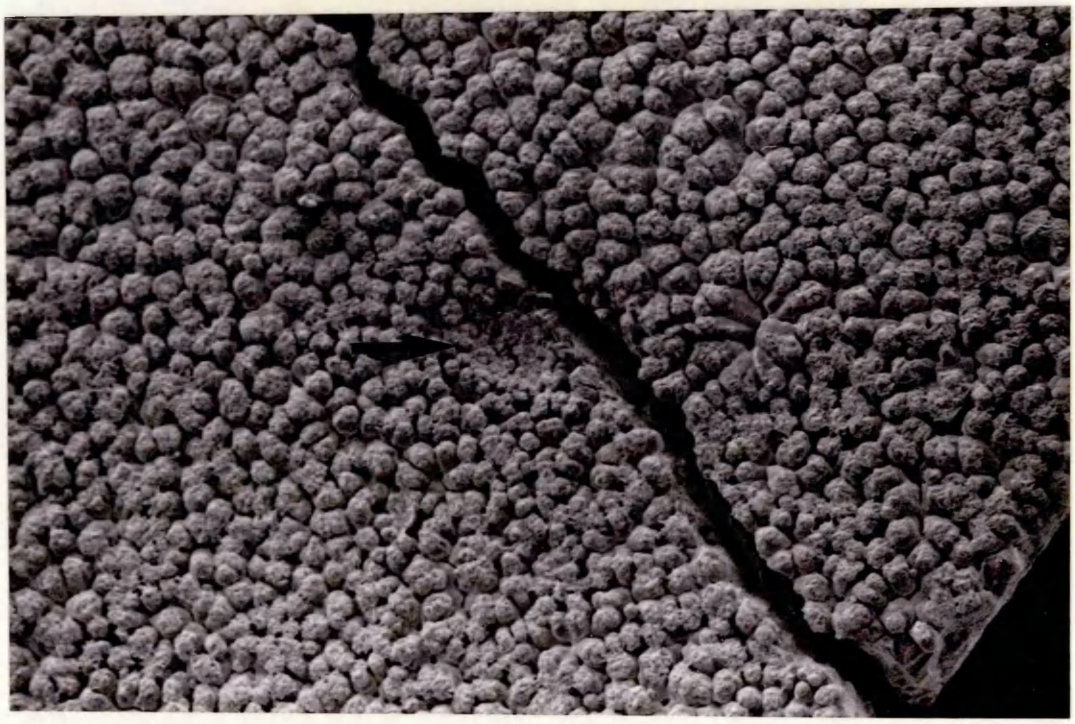


Figure 48 Pitted areas (arrowed) are vulnerable to cracking x 40.

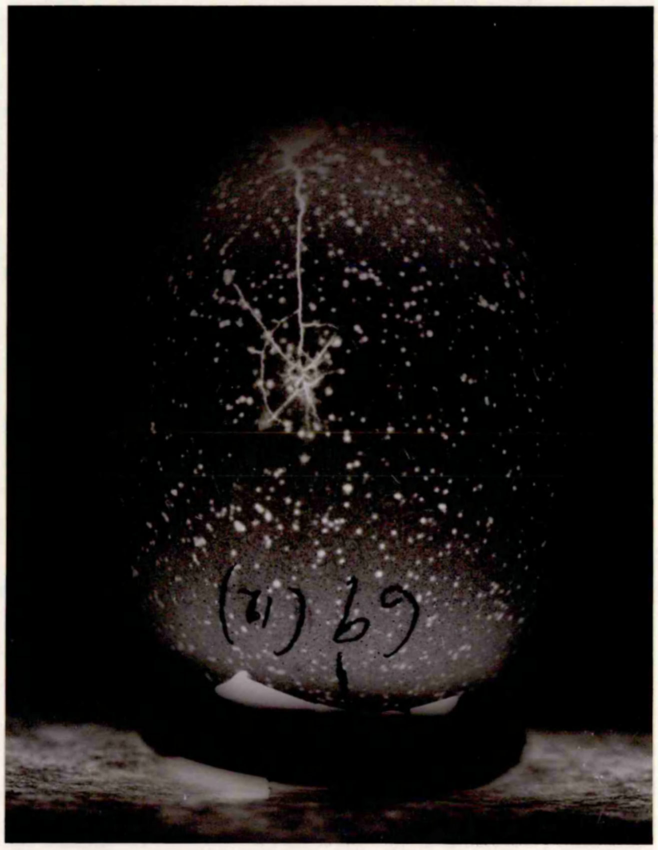


Figure 49 Occasionally circular cracking (arrowed) is also found within the 'crack initiation zone'.

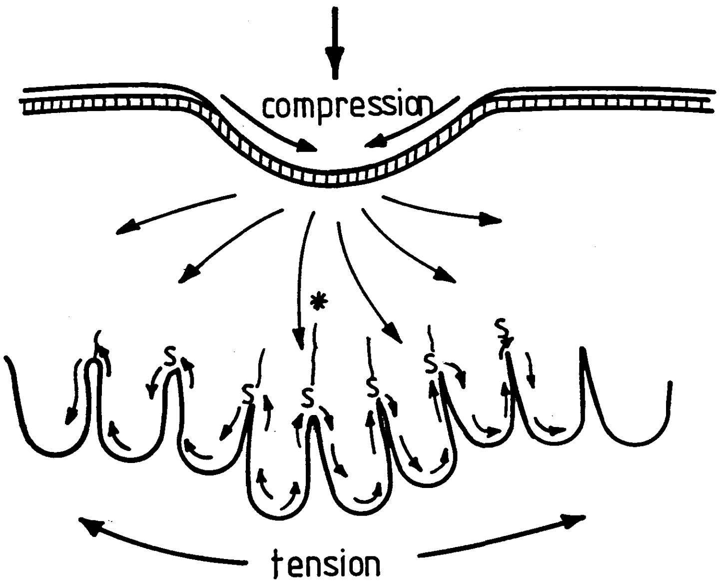


Figure 50 The failure mechanism in eggshells begins with the accumulation of tensional stress (s) where adjacent mammillae fuse. A crack then quickly propagates through the shell wall towards the outer surface (✱).

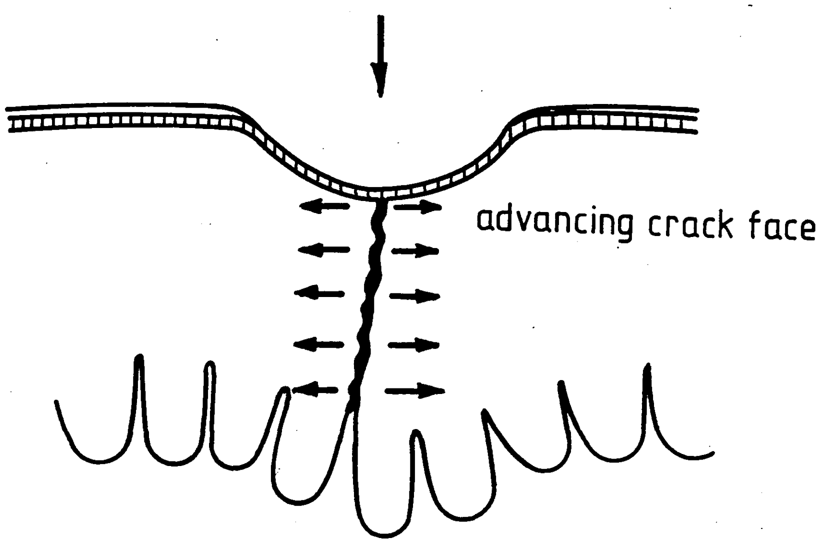
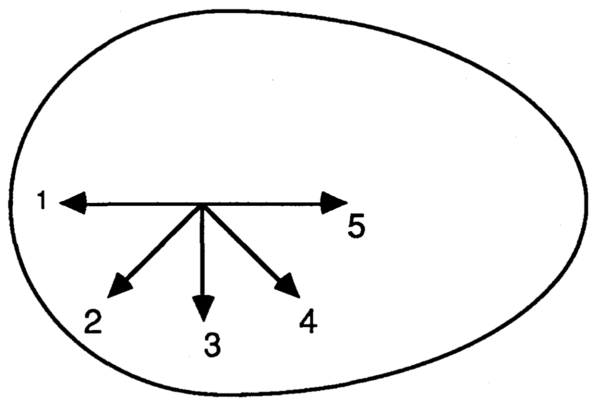


Figure 51 On reaching the surface this crack then proceeds to grow outwards in a radial fashion, at first in a stable manner, but as the interactions between the shell and its loading environment continue, unstable growth of one or more of these radial cracks soon follows.



System of enumerating fracture direction.
(After J.R Hunt and P.W. Voisey 1966)

Crack Direction	Description	Frequency of Occurrence	
1	Equator-Blunt pole	88	(25%)
2	45^{o} Equator/Blunt pole	20	(6%)
3	Equatorial Direction	96	(27%)
4	45^{o} Equator/Pointed pole	37	(11%)
5	Equator-Pointed pole	74	(21%)
x	Multiple Cracking	35	(10%)

Total Eggs		350	

Table 19 The direction and frequency of fractures induced by quasi-static compressive loads.

CHAPTER 4

SECTION 3: CALCULATION OF THE FRACTURE TOUGHNESS OF EGGSHELLS

4.3.1 INTRODUCTION.

Irwin and Kies (1954) showed that the strain energy release rate G could be defined in terms of the applied load F and the change in compliance C associated with a crack length a,

$$G = \frac{F^2}{2}\frac{\partial C}{\partial a} \qquad \text{Eq (17)}$$

Later Irwin (1957) made use of the relationship between the stress intensity factor K and the energy release rate G derived from the work done during virtual crack extension, such that for plane stress,

$$G = \frac{K^2}{E'} \qquad \text{Eq (18)}$$

where E is the elastic modulus. Thus Eq (17) can be redefined as,

$$K = F\left[\frac{E'}{2}\frac{\partial C}{\partial a}\right]^{1/2} \qquad \text{Eq (19)}$$

The determination of the stress intensity factor K from Eq (19) involves measuring the compliance (C) for a range of crack lengths (a). This can be achieved by carrying out standardised mechanical tests on suitably shaped, loaded specimens in which K is established from the derivative of rate of change of the compliance with crack length. This derivative however depends on small changes in the compliance and very accurate experimental measuring techniques (Cartwright and Rooke 1978). Alternatively, theoretical methods such as finite element analysis can be used to determine this relationship by simply incorporating cracks of varying lengths into a suitably defined FE model (Dixon and Pook 1969).

In this section FE analysis is used to derive a suitable technique for calculating the critical stress intensity factor (K_c) of eggshells.

4.3.2 MATERIALS AND METHODS.

All the FE solutions were non-dimensionalised and so in order to calculate the critical stress intensity factor from these data, it was first necessary to modify Eq (19) as follows.

$$K_{nd} = \left[\frac{\partial C_{nd}}{\partial \text{Ø}}\right]^{1/2} \frac{1}{\sqrt{2(1-\nu^2)}} \qquad \text{Eq (20)}$$

Here C_{nd} is the non-dimensionalised compliance, ν is Poisson's ratio, Ø is the crack length (a) divided by the radius of curvature R (which in this case corresponds to half the breadth dimension of the egg), and K_{nd} is the non-dimensionalised form of the stress intensity factor given by

$$K_{nd} = \frac{K}{F\,t^{-3/2}} \qquad \text{Eq (21)}$$

Assuming that Poisson's ratio is equal to 0.3, then Eq (19) becomes,

$$K_{nd} = 0.777 \left[\frac{\partial C_{nd}}{\partial \text{Ø}}\right]^{1/2} \qquad \text{Eq (22)}$$

To establish the relationship between crack length and the non-dimensionalised compliance for eggshells, equatorial cracks of varying lengths were incorporated into the "standard" eggshaped FE model illustrated in Figure 17. This was achieved by simply creating a discontinuous boundary between varying numbers of triangular elements ascribed to surfaces 1 and 2 (see Figure 10) near the equator of this model (Figure 52). Each "cracked" model was then analysed under point load conditions.

Following the analysis, the non-dimensionalised compliance (C_{nd}) of each cracked model was calculated from the displacement (d) directly beneath the load point and the appropriate values of F, E, t and R (see Eq (2) in 2.2.1). In each case E and t were defined in terms of E_{shell} and $t_{effective}$.

A computer programme was then used to curve-fit a polynomial to the resulting compliance vs crack length values. The derivative $(\partial C_{nd}/\partial ø)$ in Eq (22) was subsequently obtained from this function.

4.3.3 RESULTS AND DISCUSSION.

The relationship between crack length (ø) and the non-dimensionalised compliance (C_{nd}) of the cracked "standard" eggshaped FE model is illustrated in Figure 53, and can be summarised by,

$$C_{nd} = 0.754 + 2.388ø + 14.967ø^2 \quad \text{Eq (23)}$$

Differentiating Eq (23) becomes,

$$\frac{\partial C_{nd}}{\partial ø} = 2.388 + 29.934\,ø \quad \text{Eq (24)}$$

and substitution Eq (22), gives

$$K_{nd} = 0.777\,[2.388 + 29.934\,ø]^{1/2} \quad \text{Eq (25)}$$

The stress intensity factor (K_{nd}) can now be established for any crack ø, defined by the ratio of crack length (a) to the eggs' radius of curvature (R).

The critical stress intensity factor (K_c), or fracture toughness, relates to conditions at which the growth of a pre-existing crack becomes unstable. Photographic evidence has already been presented to support the hypothesis that the first macroscopic crack associated with the failure of eggshells results from the unstable growth of one or more previously formed radial cracks within the 'crack initiation zone' (see 4.2). Measurements of the average length of these cracks suggests that unstable crack growth occurs at 6+/-1mm from the load point. Thus for the standard egg (where R = 21.86mm) the non-dimensionalised form of this critical stress intensity factor is of the order,

$$K_{nd} = 2.53 \pm 1.5 \qquad \text{Eq (26)}$$

Assuming that the force recorded at fracture corresponds to the point at which one of these cracks reaches this critical condition, then from Eq (21),

$$F = \frac{1}{2.53} K_c \, t^{3/2} \quad (N) \qquad \text{Eq (27)}$$

where, K_c is the critical stress intensity factor or fracture toughness of the eggshell for a cracklength of 6mm, F is the force required to produce unstable fracture, and t is the effective thickness of shell.

The importance of calculating the fracture toughness of individual eggs is that it enables quantitative relationships to be obtained between the force necessary to produce failure and the size of any defect or pre-cracks that maybe present in two otherwise identical eggshells. This concept is discussed in more detail in the following section.

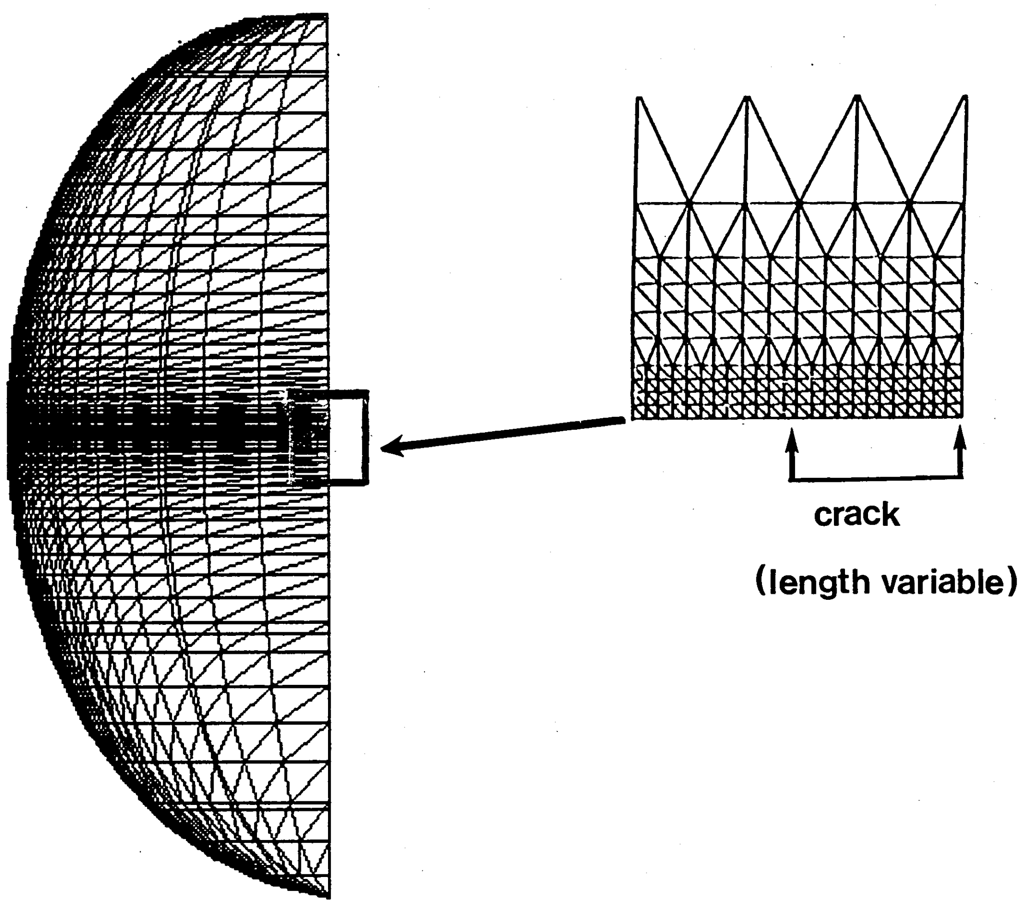


Figure 52 Finite element idealization of an equatorially cracked egg. A crack is incorporated into the model by simply defining a discontinuous boundary between some of the triangular elements near the load point.

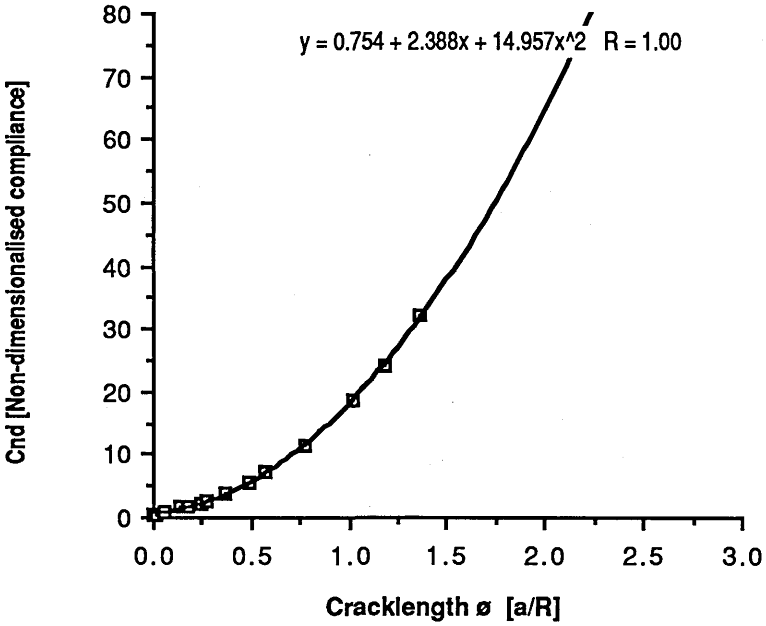


Figure 53 The effect of crack length (ø) on the non-dimensionalised compliance (Cnd) of the "standard" eggshaped FE model.

CHAPTER 4

SECTION 4: VARIATIONS IN STRUCTURAL AND MATERIAL PROPERTIES OF EGGSHELLS SELECTED FROM TWO COMMERCIAL LAYING STOCKS OVER ONE LAYING YEAR.

4.4.1 INTRODUCTION.

According to Voisey and Hamilton (1976) shell thickness, egg size, eggshape or ND deformation separately or in combination account for less than 57% of the variation in shell strength as measured directly by quasi-static compression. In an earlier communication Richards and Staley (1967) had suggested that the proportion of variation in crushing strength accounted for by deformation per unit load, egg width, egg length, shape index, and egg weight was inconsistent from bird to bird and ranged from [R^2.100] 19% to 93%. Thompson *et al* (1981) subsequently obtained a much higher correlation between shell thickness and compression fracture among species than within species (<0.92;<0.44) and suggested that the reason for this lay in the fact that within a single species the range in variables was too small to test properly their relationship with shell strength.

In this section, the relationship between the force required to crush the shell, and variations in shell stiffness characteristics, shell thickness, egg weight, and eggshape is re-examined using the pooled-egg data from each of two commercial laying stocks sampled at the beginning, middle and end of lay. The present study also examines age related changes associated with these properties both within and between the two strains for significance.

The ability of eggshells to withstand externally applied forces is a function of the material and structural strength of the shell (Carter 1970a; Hamilton and Voisey 1980). The fracture toughness and elastic modulus are therefore also considered.

4.4.2 MATERIALS AND METHODS.

[i] Source of eggs:

Eggs were selected at random from two commercial brown egg laying stocks, hereafter referred to as Strain 1 and Strain 2. Both types of bird were housed within the same laying system in plots of 12 cages, with 4 birds per cage. Conditions were otherwise similar to those found commercially.

Pullets come into lay between 20 and 22 weeks of age (Fujii 1981-cited Watt 1989) with peak production occurring at about 28 weeks of age. The birds remain in lay commercially for approximately 52 weeks, and the end of lay, as determined by the reduction in egg numbers and quality, occurs between 60 and 70 weeks of age. Thus to obtain the maximum range of shell quality characteristics, samples were selected from each strain at 24 weeks (beginning of lay), 47 weeks (midlay) and 69 weeks of age (end of lay).

[ii] General procedure:

Each egg was candled to eliminate those which were translucent, cracked, holed or checked. Badly misshapen eggs or eggs with gross abnormalities in texture were also discarded. After candling, the eggs were weighed, then the length and breadth of each egg was measured using hand calipers. The shape index of each egg was derived using the ratio of length/breadth. ND deformation tests were subsequently carried out taking four readings per egg according to the method described in 3.2.2. These values were then converted into units of stiffness (ND stiffness) after correcting for a systematic error of 45% (see 3.2.3).

After allowing the eggs to stand at room temperature overnight, quasi-static (QS) compression tests were carried out to determine the breaking strength of each egg. The optimum test speed at which changes in shell properties with compression speed are minimised, is in the order of 20cm/min (Voisey and Hunt 1969; Voisey and Hamilton 1977) but this speed requires high frequency response recording apparatus. The usual solution to this is to use a precisely controlled slow compression speed for which the response of the available recording equipment is adequate. All compression tests in the current study were therefore carried out using a compression speed of 5cm/min. The force exerted on the shell and the resulting deformation were recorded by means of a 100N load cell and a displacement measuring transducer respectively, coupled to an X-Y chart recorder (see 4.2.2).

The slope of the force vs deformation plots provides a direct measure of shell stiffness (see Figure18). The latter were compared to the ND deformation test estimates of stiffness.

After removal of the egg contents, the loosely adhering inner shell membranes were peeled away. The total thickness (t) and thickness of the mammillary layer (t_m) of each egg were then determined by SEM (see 3.3.2). In each case three equatorial samples per egg were measured, and from these data the average effective thickness ($t_{effective}$) values were obtained. The latter, together with length, breadth and ND stiffness values were then used to calculate the elastic modulus (E_{shell}) for each egg according to the method described in chapter 3.5.3.

The fracture toughness (K_c) of each eggshell was calculated using the method given in chapter4.3. In each case the force (F) was taken to equal the force at fracture, the thickness (t) as $t_{effective}$, the critical crack length (a) was assumed to equal 6mm (see 4.2.3), while the value of R was assumed to equal half the breadth dimensions of each egg.

[iii] Statistical analysis:

Correlation and regression analyses were carried out on a strain specific pooled-egg basis in order to determine which properties of the shell best account for the variability observed in shell breaking strength. A time dependent variable was also introduced into these analyses to test the differences in the various measured parameters for significance with bird age. The eggs were then sorted into six groups according to the strain and age of bird at the time of sampling. Student t-tests were performed on these data to examine in greater detail intra and inter strain differences in shell quality associated with bird age.

4.4.3 RESULTS AND DISCUSSION.

[i] Strain Specific Pooled-egg Statistical Analyses:

Simple correlation coefficients for the pooled-egg analyses are given in Tables 20 and 21. The results of the corresponding multiple regression analyses are summarised in Table 22.

The ND stiffness characteristics and the stiffness characteristics of the shell as measured directly from the slope of the force vs deformation plots during compression tests were found to be correlated with each other in both Strain 1 and Strain 2 pooled-egg analyses (r=0.67; r=0.73). Both methods of assessing stiffness however display inherent faults and as a result the values are not identical. Thus with the compression test it was necessary to ensure firm contact with the upper loading plate before testing commenced. In addition, as the size of eggs was variable it was necessary to alter the position of the displacement transducer at the beginning of each test. In an ideal situation the recording system should have been re-calibrated after each measurement, but in practice this is rarely done. Similarly, the ND deformation test has been criticised on many occasion and correction has already been made in this thesis for the depression in the lower plate. Of prime relevance however is the tendency for many researchers to measure only one area of shell. In the current study, four measures were taken at different points around the equator of each egg.

From Tables 20 and 21, the ND stiffness values appear to be more highly correlated with the effective thickness of the shell, and the egg shape index (see 3.3 and 3.4). The latter values were therefore considered to be providing the more accurate measure of stiffness in the discussion which follows.

The results of the multiple regression analyses given in Table 22 (see Appendix 3) suggest that egg size (including egg weight, length and breadth), eggshape index, and ND stiffness separately or in combination were able to account for less than 63% of the variation in shell strength of all the eggs tested from each strain. The inclusion of thickness in the analyses contributed little to the explanation of shell strength after stiffness had been considered. The proportion of the variability explained by ND stiffness, shape and size however was not consistent between the two strains. In Strain 2 shell stiffness characteristics accounted for 47% of the variability in shell strength, while ND stiffness accounted for only 35% of the variability in Strain 1. The contributions made by the eggshape index, and egg weight to the regression model were also different. For Strain 1, egg weight or eggshape accounted for 17% or 18% of the variation that remained once ND stiffness had been considered, while in Strain 2 egg weight and eggshape accounted for only 8% and 11% of the residual variation. These results therefore highlight the importance of including some measure of shape and size in the routine assessment of shell quality, particularly if the assessment is to be carried out using non-destructive methods.

The force required to break the egg, egg size, and eggshape in both the pooled-egg analyses were found to be highly correlated with the age of the bird at oviposition. It is interesting to note however that the ND stiffness, and thickness measures did not display a similar trend (Tables 20 and 21). Rather, the variation in these measures appears to have remained relatively unaffected by the age of the bird.

<u>[ii] Inter and Intra Strain Statistical analysis:</u>

In Table 23, the mean values for each of the measured variables (see Appendix 4) are given with respect to the strain of bird, and the time in the laying year at which the sample was taken. The standard deviations are also given. The results of the inter and intra strain specific statistical tests are summarised in Table 24.

From Table 24 it is clear that the eggs from Strain 1 were consistently stronger, stiffer and thicker, but less rounded than the eggs from Strain 2, while there was little difference in egg weight, fracture toughness or elastic modulus between the two.

In general as both strains aged, the average egg weight increased, the eggs became less rounded, and the force required to break the shell decreased. It is significant to note however, that this decrease in breaking strength was not paralleled by a concomitant change in ND stiffness.

The stiffness characteristics of an eggshell are determined by its' effective thickness, its' elastic modulus and its' length to breadth ratio (see Chapter 3). Between 24wks and 47wks neither the average effective thickness nor the elastic modulus of the eggs from Strain 1 nor Strain 2 were found to significantly change (see Table 23). A significant increase in the ratio of length to breadth between these two sampling periods however, suggests that the mid lay eggs from both strains were more elongate; but in real terms this difference is slight. Contrastingly, egg size, characterised by an increase in length, breadth and weight, was significantly greater in both the mid lay samples. It could therefore be hypothesised that the decrease in strength in both strains at 47wks was due to a size effect. The computed fracture toughness values of the midlay eggs in each case however were also significantly lower, which suggests that at 47wks the eggs also contained more defects.

Roland (1981) found that shell quality declined with hen age due to a continued increase in egg size, which he suggested forced the constant amount of shell secreted with each egg to be spread thinner. In these current studies, while egg size continued to increase in Strain 1 between 47wks and 69wks of age, the average thickness of the eggs at the end of lay remained statistically consistent with that observed during the midlay period. Brown (pers comm) reported a similar trend in shell thickness measurements in this particular flock.

The ND stiffness characteristics of Strain 1 eggs at 69wks were also statistically consistent with that observed at midlay, despite another small but nevertheless significant increase in the egg shape index. The fracture toughness however continued on its' downwards trend, and as a result provides the more plausible explanation as to why eggshell strength was impoverished in this Strain at 69wks.

The eggs selected from Strain 2 birds in contrast displayed a significant decrease in shell thickness between 47wks and 69wks. Other than a slight increase in length, the end of lay sample did not significantly differ in any other respect from the midlay sample.

It is interesting to note that the fracture toughness of Strain 2 eggshells was statistically superior to that found in the eggshells from Strain 1 at 69wks. Thus, while Strain 1 continued to lay thicker shells than Strain 2, the properties of the eggshell which determine its resistance to crack growth were in fact superior in those eggs from Strain 2 at the end of the laying year.

In conclusion this study has served to highlight the disparity that exists between those properties which affect the stiffness characteristics of eggshells and those which determine an eggs' ultimate strength. It is now clear that while the strength of an eggshell can be correlated to its' stiffness characteristics, those properties which affect its' fracture toughness now provide a more meaningful interpretation of eggshell strength. Implicit in the definition of fracture toughness is the role of ultrastructure. The next section of this thesis attempts to correlate the above decline in fracture toughness with ultrastructural changes at the level of the mammillary layer both within and between these two strains of bird with time.

Data File: STRAIN 1 Physical Data

Force	1.000	-0.452	-0.639	-0.327	-0.579	0.355	0.538	0.588	0.407	0.226	-0.657
weight	-0.452	1.000	0.851	0.926	0.365	0.089	0.079	-0.043	0.011	-0.257	0.769
length	-0.639	0.851	1.000	0.666	0.779	-0.075	-0.076	-0.224	-0.145	-0.253	0.780
breadth	-0.327	0.926	0.666	1.000	0.053	0.104	0.074	-0.019	0.038	-0.213	0.684
shape index	-0.579	0.365	0.779	0.053	1.000	-0.182	-0.165	-0.280	-0.223	-0.168	0.473
t effective	0.355	0.089	-0.075	0.104	-0.182	1.000	0.433	0.700	0.957	-0.019	-0.025
stiffness	0.538	0.079	-0.076	0.074	-0.165	0.433	1.000	0.674	0.447	0.101	-0.063
ND Stiff	0.588	-0.043	-0.224	-0.019	-0.280	0.700	0.674	1.000	0.729	0.189	-0.149
tot thick	0.407	0.011	-0.145	0.038	-0.223	0.957	0.447	0.729	1.000	0.272	-0.097
mam thick	0.226	-0.257	-0.253	-0.213	-0.168	-0.019	0.101	0.189	0.272	1.000	-0.251
age	-0.657	0.769	0.780	0.684	0.473	-0.025	-0.063	-0.149	-0.097	-0.251	1.000

________ ns

-------- p<0.05

p<0.01

Table 20 Simple correlation coeficients. Strain 1 physical data.

Data File: STRAIN 2 Physical Data

force	1.000	-0.390	-0.521	-0.286	-0.503	0.460	0.617	0.689	0.460	0.017	-0.616
weight	-0.390	1.000	0.919	0.944	0.361	-0.094	-0.005	-0.186	-0.055	0.081	0.696
length	-0.521	0.919	1.000	0.797	0.671	-0.115	-0.083	-0.271	-0.076	0.081	0.763
breadth	-0.286	0.944	0.797	1.000	0.093	-0.071	0.026	-0.138	-0.048	0.047	0.624
shape index	-0.503	0.361	0.671	0.093	1.000	-0.100	-0.157	-0.284	-0.066	0.072	0.499
t effective	0.460	-0.094	-0.115	-0.071	-0.100	1.000	0.454	0.563	0.888	-0.200	-0.198
stiffness	0.617	-0.005	-0.083	0.026	-0.157	0.454	1.000	0.730	0.492	0.097	-0.071
ND stiff	0.689	-0.186	-0.271	-0.138	-0.284	0.563	0.730	1.000	0.608	0.118	-0.267
tot thick	0.460	-0.055	-0.076	-0.048	-0.066	0.888	0.492	0.608	1.000	0.272	-0.180
mam thick	0.017	0.081	0.081	0.047	0.072	-0.200	0.097	0.118	0.272	1.000	0.034
age	-0.616	0.696	0.763	0.624	0.499	-0.198	-0.071	-0.267	-0.180	0.034	1.000

______ ns
------- $p<0.05$
$p<0.01$

Table 21 Simple correlation coeficients. Strain 2 physical data.

STRAIN 1 Pooled-eggs Analysis					R^2x100%	r
ND stiffness	Eggsize[a]	Eggshape	Thickness[b]	Age	72%	0.847
ND stiffness	Eggsize[a]	Eggshape	Thickness[b]		62%	0.788
ND stiffness	Eggsize[a]	Eggshape	-		62%	0.785
ND stiffness	Eggsize[a]	-	-		62%	0.785
ND stiffness	-	Eggshape	-		53%	0.729
ND stiffness	Weight	-	-		52%	0.724
ND stiffness	Weight	Eggshape	-		61%	0.782
ND stiffness	-	-	-		35%	0.588

STRAIN 2 Pooled-eggs Analysis						
ND stiffness	Eggsize[a]	Eggshape	Thickness[b]	Age	71%	0.844
ND stiffness	Eggsize[a]	Eggshape	Thickness[b]		63%	0.795
ND stiffness	Eggsize[a]	Eggshape	-		62%	0.787
ND stiffness	Eggsize[a]	-	-		61%	0.784
ND stiffness	-	Eggshape	-		58%	0.760
ND stiffness	Weight	-	-		55%	0.739
ND stiffness	Weight	Eggshape	-		61%	0.779
ND stiffness	-	-	-		47%	0.689

ND Corrected Non-destructive estimates.
a Eggweight, Length and Breadth.
b Total thickness, Mammillary thickness, t effective

Table 22 Coefficients of multiple determination [R^2.100%] for regressions of force at failure on physical properties of eggshells. Strain specific pooled-egg analyses. **(see Appendix 3)**

		24wks		47wks		69wks		[t Test Sig Beg/End]
CATEGORY	STRAIN	MEAN	S.D	MEAN	S.D	MEAN	S.D	
WEIGHT	STRAIN 2	54.65	4.59	66.25	4.88 **	68.44	6.59 ns	[**]
[g]	STRAIN 1	54.30	3.18	63.73	4.38 **	67.02	4.45**	[**]
LENGTH	STRAIN 2	54.2	1.8	59.0	1.7 **	60.2	2.2 **	[**]
[mm]	STRAIN 1	54.6	1.4	58.7	1.9 **	60.8	2.4 **	[**]
BREADTH	STRAIN 2	42.4	1.1	44.9	1.3 **	45.3	1.6 ns	[**]
[mm]	STRAIN 1	42.0	0.9	44.0	1.2 **	44.6	1.1**	[**]
SHAPE INDEX	STRAIN 2	1.28	0.02	1.31	0.04 **	1.33	0.04 ns	[**]
	STRAIN 1	1.30	0.04	1.33	0.05 **	1.36	0.06**	[**]
THICKNESS	STRAIN 2	0.301	0.020	0.303	0.028 ns	0.290	0.026**	[**]
[mm]	STRAIN 1	0.344	0.022	0.339	0.036 ns	0.336	0.037 ns	[ns]
Teffective	STRAIN 2	0.240	0.020	0.244	0.026 ns	0.228	0.026**	[**]
[mm]	STRAIN 1	0.272	0.020	0.271	0.032 ns	0.270	0.040 ns	[ns]
MAM.THICK	STRAIN 2	0.061	0.005	0.059	0.016 ns	0.062	0.012 ns	[ns]
[mm]	STRAIN 1	0.072	0.008	0.068	0.010**	0.066	0.010 ns	[**]
FRACTURE	STRAIN 2	3.31	0.52	2.70	0.49 **	2.32	0.55 **	[**]
FORCE [Kg]	STRAIN 1	3.83	0.52	3.33	0.58 **	2.60	0.64 **	[**]
QS STIFFNESS	STRAIN 2	145.2	22.7	144.6	29.1 ns	140.1	35.6 ns	[ns]
[Nmm-1]	STRAIN 1	184.7	24.8	195.7	37.3 ns	179.9	43.7 ns	[ns]
ND STIFFNESS	STRAIN 2	139.9	19.1	133.3	20.9 ns	124.8	26.5 ns	[**]
[Nmm^{-1}]	STRAIN 1	180.1	23.3	177.9	31.4 ns	168.4	37.6 ns	[ns]
FRACTURE	STRAIN 2	721	96	566	110 **	533	104 ns	[**]
TOUGHNESS	STRAIN 1	700	96	607	139 **	469	107**	[**]
[$Nmm^{-3/2}$]								
ND Eshell	STRAIN 2	29000	3900	28700	5300 ns	30500	6000 ns	[ns]
[Nmm^{-2}]	STRAIN 1	29400	4900	31000	4600 ns	30400	6300 ns	[ns]

Within strain statistical tests for significance: ** $P < 0.01$ ns Not Significant
[Student t-tests] * $P < 0.05$ [ ] beg/end of lay comparisons

Table 23 A comparative study of Eggshell Quality within two brown egg laying strains of hen over one laying year. Means and standard deviation are given according to each sample date. Those samples within each strain which differ significantly are indicated.

BEGINNING OF LAY:

	STRAIN 1	STRAIN 2	Probability
BREADTH		X	0.05
SHAPE INDEX	X		0.01
THICKNESS	X		0.01
MAM THICK	X		0.01
FRACTURE FORCE	X		0.01
STIFFNESS	X		0.01

MIDDLE OF LAY:

	STRAIN 1	STRAIN 2	Probability
WEIGHT		X	0.01
BREADTH		X	0.01
SHAPE INDEX	X		0.05
THICKNESS	X		0.01
MAM THICK	X		0.01
FRACTURE FORCE	X		0.01
STIFFNESS	X		0.01
ELASTIC MODULUS	X		0.05

END OF LAY:

	STRAIN 1	STRAIN 2	Probability
BREADTH		X	0.05
SHAPE INDEX	X		0.01
THICKNESS	X		0.01
FRACTURE FORCE	X		0.02
STIFFNESS	X		0.01
FRACTURE TOUGHNESS		X	0.01

Table 24 Strain differences in eggshell quality at the beginning, middle and end of lay. X denotes which strain displays the greatest value in each case.

CHAPTER 4

SECTION 5: A RELATIONSHIP BETWEEN THE FRACTURE TOUGHNESS OF EGGSHELLS AND THE STRUCTURAL ORGANISATION OF THE MAMMILLARY LAYER.

4.5.1 INTRODUCTION.

Fracture toughness describes the relationship between the stress necessary to cause a structure to fail and the size of any defect or pre-crack that may be present (Knott 1978). Similarly, the mammillary layer of the eggshell is characterised by the presence of many naturally occurring fissures and it has already been suggested that the latter play an influential role both in the initiation of the first crack through the shell wall (Figure 50) and thereafter in the propagation of radial cracks which subsequently arise from this (Figure 51).

In a survey of eggs collected from retail outlets, Watt (1985) found that there was a high proportion of structural abnormalities in the cone layer of those eggs which were cracked or broken. Robinson and King (1970) and Bunk and Balloun (1977;1978) had previously described similar structural variations in intrinsically weak and low quality shells. This section therefore sets out to determine if a relationship can be established between the fracture toughness of those eggs described in the previous section and a decline in cone layer quality characteristics.

4.5.2 MATERIALS AND METHODS.

[i] Surface Coatings:

Eggs with superficial coatings have been reported in the field. Solomon (pers comm) has indicated that coated eggs usually also display an abnormal cone layer. The external appearance of each egg was therefore assessed using a modified version of the method described by Hughes *et al* (1986), before specimens were removed and prepared for SEM assessment following the procedure given in 4.2.2.

[ii] Procedure for Quantifying Shell ultra-structure by Scanning Electron Microscopy (SEM):

After plasma treatment (4.2.2), fifteen structural features of the cone layer were assessed per shell according to the scoring system illustrated in Figure 54. This semi-quantitative scoring system was not arbitrarily chosen but was derived as a result of the collaborative input from both a statistician and the experience of fellow workers who have worked in the field of eggshell quality for several years. At the time of writing it was nevertheless accepted that modifications to this system would be required as more information on the influence of these structural traits on shell performance became available.

In general each structural trait was assigned a series of possible scores weighted in terms of whether a high or low incidence of that particular feature was considered to be good or bad with respect to a shells' overall performance. The range of scores assigned to each individual characteristic were subsequently weighted against one another, such that those traits considered to be more detrimental were given the greatest possible range of values.

[iii] Scanning Electron Microscopy:

First the organisation of the mammillary layer was assessed in terms of its overall visual appearance. Aligned mammillae, i.e those which were arranged in an orderly fashion, were considered to be a poor shell quality characteristic as cracks tend to move along rather than between the cones (Figure 43). The presence or absence of pitting was also noted at this stage and scored according to its severity. Mammillary counts (after Reid 1984) were then taken at three randomly chosen areas per sample at a magnification of x160 and a working distance of 13.

Basal cap morphology was assessed using the criteria described by Reid (1984) and later modified by Watt (1985;1989) and Solomon (1990). Each egg was given a basal cap score in terms of the predominant degree of etching found (after Watt 1985), and the distance between neighboring caps in several fields of view. Shells with flat, poorly etched caps, or a low cap to cone ratio were given the highest and therefore the worst score. Next, the predominant type of fusion found in each sample was assessed. Parsons (1982) suggested that early fusion of adjacent mammillae might be desirable in terms of improving shell strength and so this was given a lower score than where the adjacent mammillary columns predominantly fused later. Finally, the incidence of aberrant crystal forms, viz: Type B's, cubics, aragonite and cuffing, were evaluated in terms of their frequency of occurrence in several fields of view at a magnification of x320.

To eliminate bias the ultra-structural score of each egg was assessed blind, that is, it was not known to which strain or sampling date a particular specimen belonged until the analysis of all samples had been completed. A total of 300 samples were analysed in this way, such that 50 eggs from each strain were assessed at times corresponding to the beginning, middle and end of lay.

[iv] Statistical Analysis:

The mean values for each structural characteristic were calculated according to sample date and strain. Student t-tests were then carried out on these data to determine if inter or intra strain differences in the incidence of some or all these structural traits could be identified.

4.5.3 RESULTS AND DISCUSSION.

[i] A Possible Link Between Surface Spoilage and Structural Imperfections in the Cone Layer:

The incidence of eggs displaying surface abnormalities from Strains 1 and 2 at 24wks, 47wks and 69wks of age are summarised in Table 25.

In general, the incidence of surface spoilage in Strain 1 remained consistent throughout the laying year, but in terms of specific abnormalities, differences did exist. For example, while "pinks" (18%) and "fine dusting" (39%) accounted for most of the variation at the beginning of lay, eggs with surface accretions (32%) and calcium splashes (16%) were more commonly found in this flock towards the end of lay. The eggs from Strain 2, in contrast did not show a consistent pattern in the incidence of surface abnormalities. Rather, eggs with a normal surface appearance were more common in this flock at 24wks (63%) and 47wks of lay (66%), while over 70% of the eggs were classified as abnormal at 69wks. This dramatic change was again almost entirely due to an increase in the incidence of surface accretions (34%).

It is assumed that these surface accretions (Figure 55) correspond to the pimpling phenomenon described by Ball *et al* (1973) and Roland *et al* (1975b). Ball *et al* (1973) suggested that pimpling may arise from masses of albumen debris produced in the magnum which become attached to the outer shell membranes prior to the onset of shell mineralisation. Roland *et al* (1975b) subsequently found that pimpling could be induced by the introduction of calcium carbonate into the uterus. Although it is still not certain which factors predispose a bird to lay pimpled eggs, it is nevertheless clear that the mechanism is quite different from that which results in a "finely dusted" egg. Hughes *et al* (1986) suggest that the latter arise from a delay in oviposition perhaps due to stress or some external disturbance upon the bird. This delay then allows additional calcium carbonate or phosphate to be deposited on top of the cuticle. In this respect it is of interest to note that structural modifications in the cone layer were essentially absent in the current study in those eggs described as "finely dusted", while the underlying palisade layer was found to be disrupted in those eggs which had surface accretions (Figure 55). These observations are consistent with the findings of Watt (1989) and bear witness to the different provenance of these extraneous deposits.

[ii] Cone Layer Structural Variations:

The criteria, according to Watt (1985) which must be satisfied if the cone layer of the shell is to perform its' diverse functions are summarised in Figure 56.

Variations from the normal basal cap morphology have been described in detail (Reid 1984, Watt 1985;1989; Solomon 1985a,1990) and these can be classified as follows; spicular and confluent caps; Type A's; changed membrane; denuded or sheared caps; Type B's; cuffing; aragonite; cubics; and pitting (which includes depressions, erosions, and pin holes). The full range of these structural abnormalities are illustrated in Figures 57 to 81.

Type A's and spicular caps (Figure 57 and 58) appear to be associated only with the basal cap crystals of the mammillae as indicated by little or no evidence of contact with the membrane fibres. While the latter does not interfere with the formation of cone and palisade layers, a high incidence of these abnormalities constitutes a tenuous bond between the organic and inorganic components of the shell.

A more severe form of the spicular cap phenomenon is illustrated in Figures 59 and 60. Here the individual mammillary caps have become confluent with one another.

The changed membrane abnormality (Figures 61-64) reflects both morphological and chemical changes in the nature of the shell membranes and as a result the membrane fibres are not removed by the process of plasma etching. Rather, the latter remain adhering to the mammillary caps and in some instances any resemblance to a fibrous morphology is absent (Figure 62). Such remnants are considered to be rich in sulphur (Watt 1985) and are often found in association with denuded or sheared cones. The latter appear to have a line of weakness below the basal cap which fractures if the bond between the membrane fibres and the cap is disturbed (Figures 63 and 64).

Pitting is potentially one of the most severe types of structural fault found in the shell and was first described in white egg laying stock (Solomon pers comm). Pitting can exist in three forms: as a depression (Figure 65); as an erosion (Figure 66); or as a distinct hole extending through the entire thickness of the shell (Figures 67, 68 and 69).

Type B's, cuffing, aragonite and cubic crystals all reflect changes in the rate of crystal growth and are most commonly found in the inter-mammillary spaces that exist between adjacent cones. Type B's (after Reid 1984) are small, spherical bodies which do not make any direct contribution to the palisade layer (Figures 70 and 71). These rounded forms occasionally show evidence of membrane contact but more commonly are found growing from the sides of established cones. In shells where Type B's occur in isolated clumps, fusion of adjacent columns tends to be impaired (Figures 72 and 73).

Cuffing appears as a secondary crystallisation between the cones and is believed to be formed at some point after the mammillary knobs have begun to fuse (Figure 74).

The $CaCO_3$ crystals in the avian eggshell are predominantly calcitic but individual crystals as such do not exist (Erben 1970). Nevertheless isolated cubic crystals typical of free growing calcite are occasionally found (Figure 75). Aberrant crystal forms more typical of the aragonite morphology have also been isolated in the avian eggshell (Watt 1985) (Figure 76 and 77). The aragonitic form of $CaCO_3$ is however more typically associated with reptilian eggshells (Solomon and Watt 1985).

In these current investigations, the spatial relationships of individual mammillae, the numbers and relative size of mammillae per unit area, and the degree of early and late fusion were also considered to be of importance. Examples of alignment and early and late fusion are presented in Figures 78 to 81.

[iii] Structural Profiles of the Eggshells Obtained From Strains 1 and 2 at the Beginning, Middle and End of Lay:

The mean and standard deviations of the fifteen shell characteristics derived for each group of 50 eggs as determined by the procedure given in Figure 54 are summarised in Table 26. Intra-strain differences which were statistically significant are also indicated. The results of the statistical analysis carried out between strains are summarised in Table 27. The relative frequencies of scores associated with each individual characteristic feature are given in Table 28.

Strain Differences: A comparison of the structural profiles of eggs from Strain 1 and Strain 2 at any point within the laying year suggests that in some cases genetic differences exist. In general the mammillary density and cuffing scores were found to be consistently higher in those eggs from Strain 2 birds while late fusion tended to be a feature more characteristic found in the eggs from Strain 1 (Table 27).

Age Effects: According to the total structural scores the greatest decline in quality occurred in both Strains between 47 and 69wks of age. However the individual shell characteristics which make up this total structural score were different both within and between each strains as the birds aged.

Between 24 and 47wks, eggs from both Strains 1 and 2 displayed a significant increase in pitting, late fusion, and numbers of mammillae per unit area, while the incidence of confluence, and alignment decreased. Despite these similarities, it is interesting to note from Table 26 that at midlay, the eggs from Strain 2 also had improved caps, fewer Type A's but more changed membrane, while the incidence of early fusion appears to have increased in the eggs from Strain 1.

Between 47 and 69wks, late fusion and pitting continued to increase in the eggs from both flocks. Late fusion however, was statistically shown only to be significant in the eggs from Strain 1, while the increase in pitting was found to be significant only in those eggs obtained from Strain 2. Nevertheless, it is interesting to note that without exception the type of pitting, namely erosions, became potentially more damaging in both flocks during this period (Figure 82 and Table 27).

The eggs from Strain 1 also typically displayed a higher incidence of aragonite and Type B's at 69wks and as a result had a much more open framework (Figure 83). These structural changes have been related to environmental stress (Watt 1989).

In contrast, those eggs obtained from Strain 2 at 69wks typically displayed a higher incidence of changed membrane and poorer basal caps (Figure 84). Solomon (pers comm) suggests that these abnormalities relate to oviducal dysfunction.

[iv] Relating Cone Layer Abnormalities to the Fracture Toughness of Eggshells:

In transverse section, the avian eggshell reveals a characteristically "notched" appearance at the junction between the "true" shell and the shell membranes (Figures 22 and 23). The number of these notches or fissures is related to the numbers of mammillae per unit area, and in chapter 4.2 it was hypothesised that failure begins with the propagation of one or more of these cracks through the shell wall (Figure 50).

Robinson and King (1970), Simons (1971), and Bunk and Balloun (1978) were all of the opinion that good quality shells generally have a higher mammillary density. Simons (1971) in addition to this suggested that the smaller crystal column diameter found in the guinea fowl egg may account for their greater strength compared to the hens' egg. Van Toledo *et al* (1982) proposed that a low mammillary density was generally a feature of stronger shells.

Unlike the others, Van Toledo *et al* (1982) carried out their investigations with eggs of the same overall thickness.

In calculating the fracture toughness of eggshells, thickness and geometry are eliminated from comparisons. Thus, one might expect a relationship to exist between the number of mammillae per unit area and the fracture toughness of eggshells viz; the more mammillae per unit area the greater the chance of stress concentrations forming at the points of fusion between adjacent cones.

The average mammillary density increased in both the eggs from Strain 1 and Strain 2 between 24 and 47wks and according to the results given in Table 23, there was also a decrease in the fracture toughness between these two sample dates. At 69wks however, the average mammillary density of Strain 1 eggs did not differ from that observed at 24wks, yet according to Table 23, the fracture toughness continued downwards in this flock. Further, it is interesting to note that despite having consistently more mammillae per unit area, the average fracture toughness of the eggs from Strain 2 did not statistically differ from that of Strain 1 (with the exception of those eggs selected from the end of lay). It can therefore only be concluded that the mammillary density *per se* does not influence the fracture toughness of eggshells, at least not in isolation.

According to Hancock (pers comm) the size and depth of the worst defect is of greater importance to a materials' fracture toughness; thus a solid with a deep crack will break before an otherwise identical solid in which the fissures are merely superficial. One might therefore anticipate that stress concentrations would occur more rapidly in eggshells where the fusion of adjacent mammillary columns was delayed or where a large defect occurred directly beneath the load point. In this respect it is encouraging to find that in three out of the four experimental periods the decline in fracture toughness can now be explained in terms of an increase in late fusion of adjacent mammillary columns (and the types of abnormality which accompany this), and an increase pitting. Nevertheless the eggshells from Strain 2 at the end of lay still do not conform to this pattern and so an alternative explanation must be sought.

Structurally the eggs from Strain 2 displayed a similar decline in quality to that observed in Strain 1 between 47 and 69wks, but in this case the latter was not accompanied by a significant decrease in fracture toughness (Table 23). The reason for this is unclear in terms of the above hypothesis but maybe due to the fact that at 69wks the type of abnormalities found in this group of eggs, other than pitting, were more typically of the poor basal cap and changed membrane variety.

In a good quality shell, the membrane fibres penetrated into the cones to a depth of some 20μm (Simons 1971), and in this way the shell is firmly anchored. A poor basal cap to cone contact, and a higher incidence of changed membrane therefore represent an imperfection in this relationship. Bunk and Balloun (1977) and Solomon (1985a) argued that without a proper foundation for the calcium salts to crystallize upon, poor shell quality would occur irrespective of an adequate and balanced dietary intake. In this respect it is interesting to note that at 69wks the eggshells from Strain 2 were also of inferior thickness. Perhaps then this cap to membrane relationship directly affects shell thickness (a structural property) rather than the fracture toughness (a material property). In addition this may explain why the eggs from Strain 1 also had a higher fracture toughness than the eggs from Strain 1 at 69wks.

EGG NO. __________

BREED INFORMATION. _

						SCORE:
MAM. DENSITY.	AREA 1.	AREA 2.	AREA 3.		MEAN: / STD. DEV.	
CONFLUENCE.	NONE (3)	ISOL. (4)	MOD. (6)	EXT. (1)		
CAPS.	G (1)	G- (3)	P+ (6)	P (8)	P- (10)	
EARLY FUSION.	EXT. (1)	MOD. (2)	ISOL. (4)			
LATE FUSION.	EXT. (6)	MOD. (3)	ISOL. (1)			
MAM.ORGAN.	NONE. (1)	ISOL. (2)	MOD. (4)	EXT. (7)		
TYPE B'S.	NONE. (1)	ISOL. (2)	MOD. (5)	EXT. (8)		
PITTED.	NONE. (1)	DEP. (5)	EROS. (7)	HOLE.(12)		
ARAGONITE.	NONE. (1)	ISOL. (2)	MOD. (5)			
TYPE A'S.	NONE. (1)	ISOL. (2)				
CUBICS.	NONE. (1)	ISOL. (2)	MOD. (5)			
CUFFING.	NONE. (5)	ISOL. (4)	MOD. (1)			
CHANGED MEMB.	NONE. (1)	ISOL. (4)	MOD. (8)	EXT.(14)		

TOTAL: ______

Figure 54 Procedure for quantifying shell quality by SEM. Numbers in brackets represent the score ascribed to each individual characteristic.

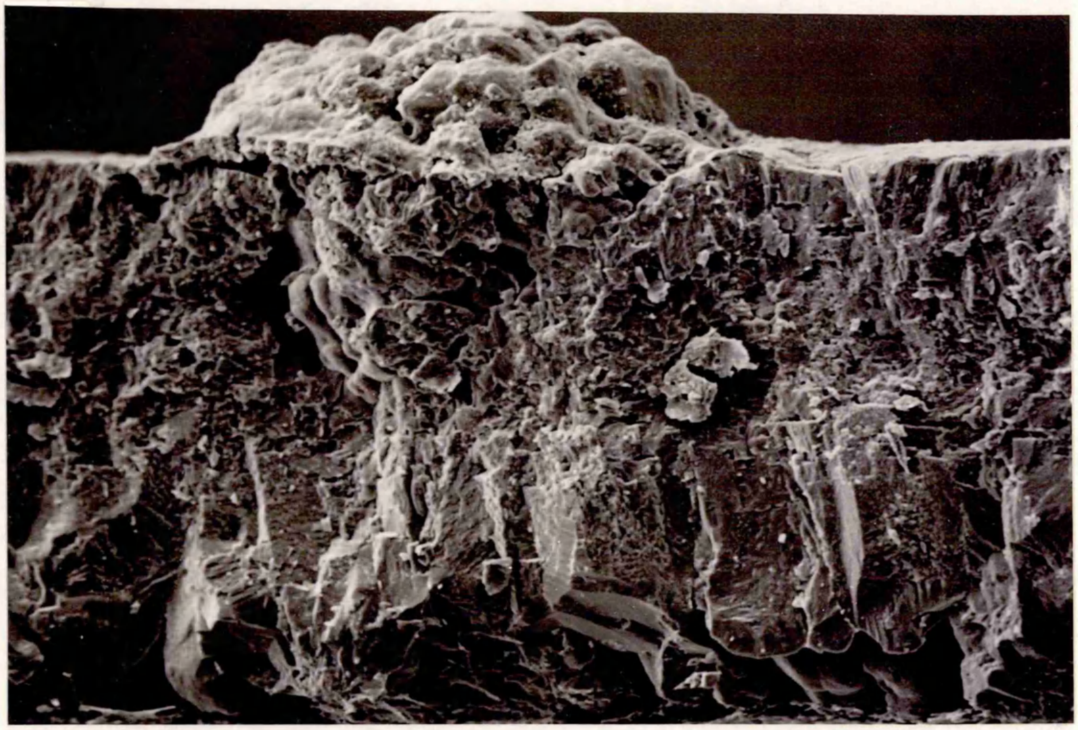


Figure 55 Transverse view showing a surface accretion. The underlying palisade layer is also disrupted x160.

Figure 56 Definition of normality with respect to the cone layer (after Watt 1985):

i) A relatively large cap area providing a large area of contact between true shell and the outer shell membranes.
ii) A cap area (C) in which the fibre tracks (F) have been deeply etched showing a very close association of individual membrane fibres and the initial calcium carbonate crystals, thus giving a strong bond between the organic and inorganic shell components.
iii) A cap area (C) which is rounded, and conforms to the 'eisopherites' described by Tyler (1969).
iv) No remnants of inorganic membranous ash: all organic membrane in normal shells is removed by the plasmaprep technique (Reid1983).
v) Cone area which have the normal honeycomb appearance.
vi) No obvious regularly occuring aberrant crystal forms in between adjacent mammillary knobs.

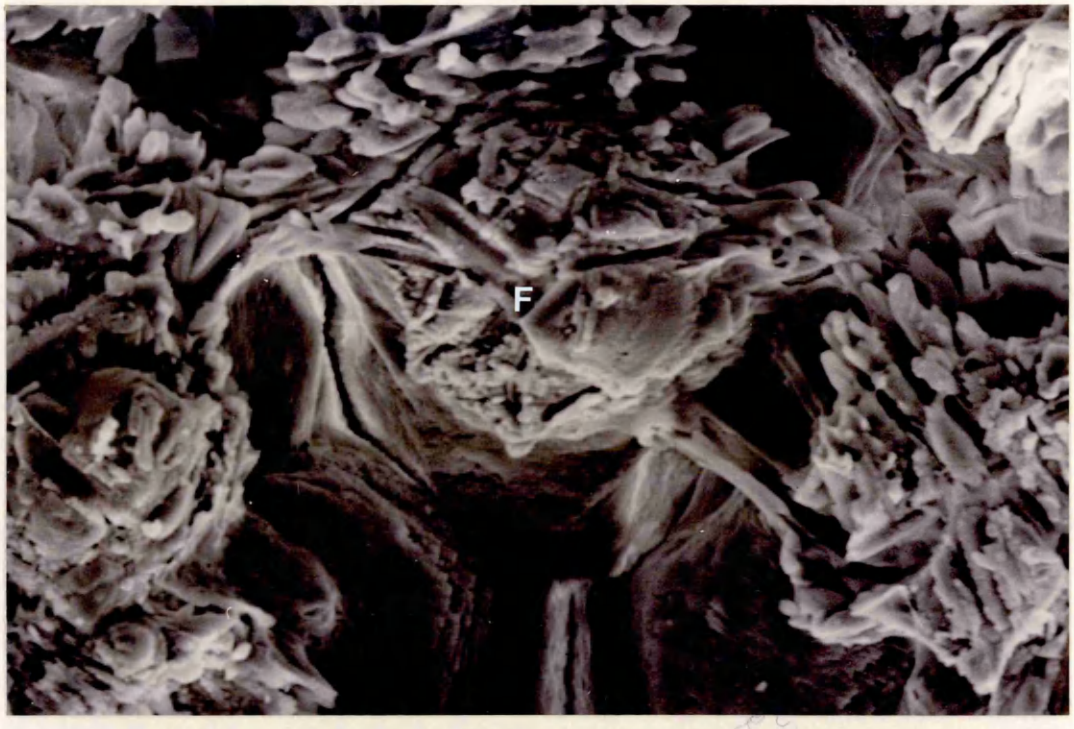


Figure 57 Spicular cap abnormality displays superficial fibre tracks thus constituting a tenuous bond between the inorganic and organic components of the shell. F = fibre track x640.

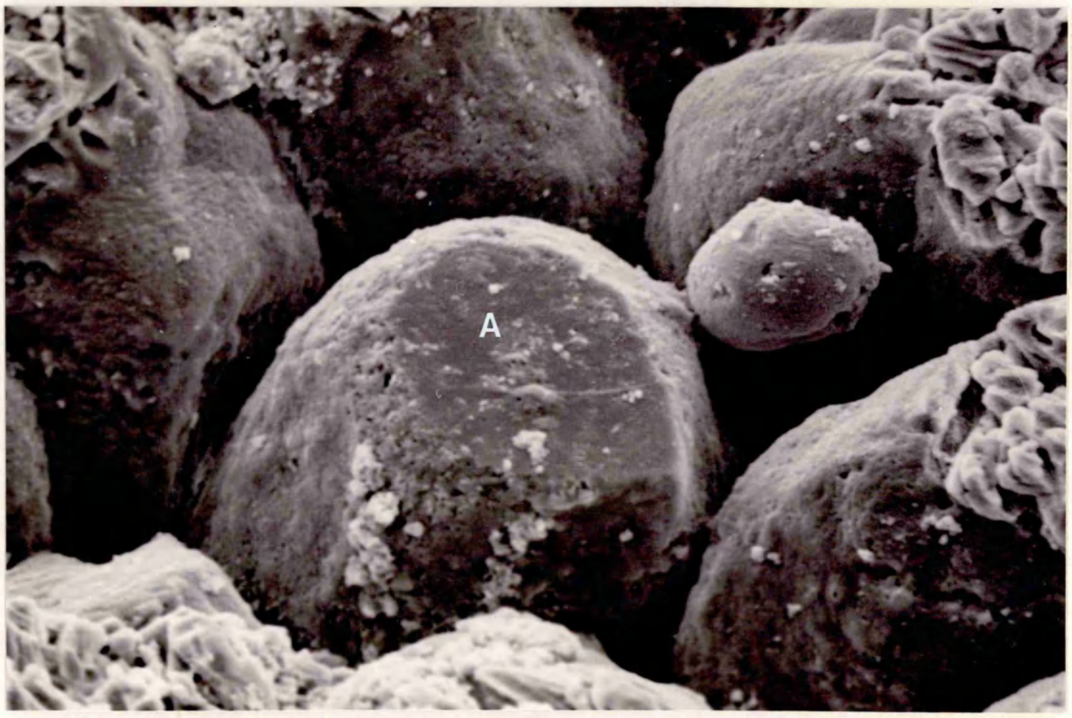


Figure 58 "Type A" mammillary bodies have no basal cap. The formation of cone and palisade columns proceed as normal x640.

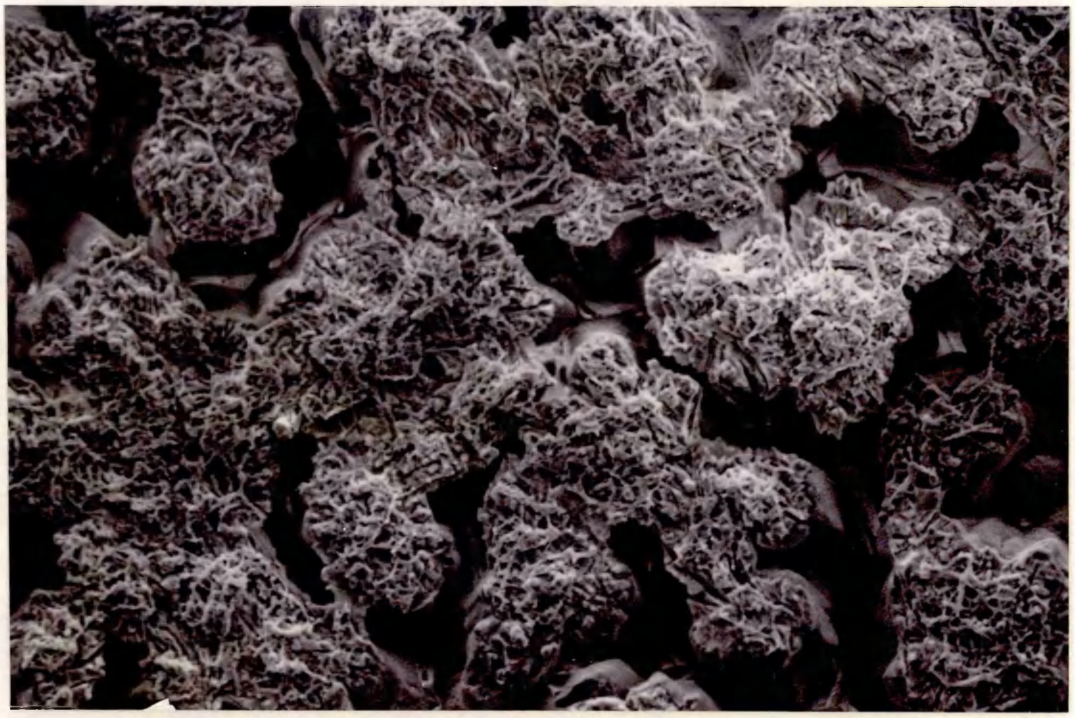


Figure 59 Moderate confluence coverage x160.

Figure 60 Extensive confluence. Individual mammillae can no longer be identified x160.

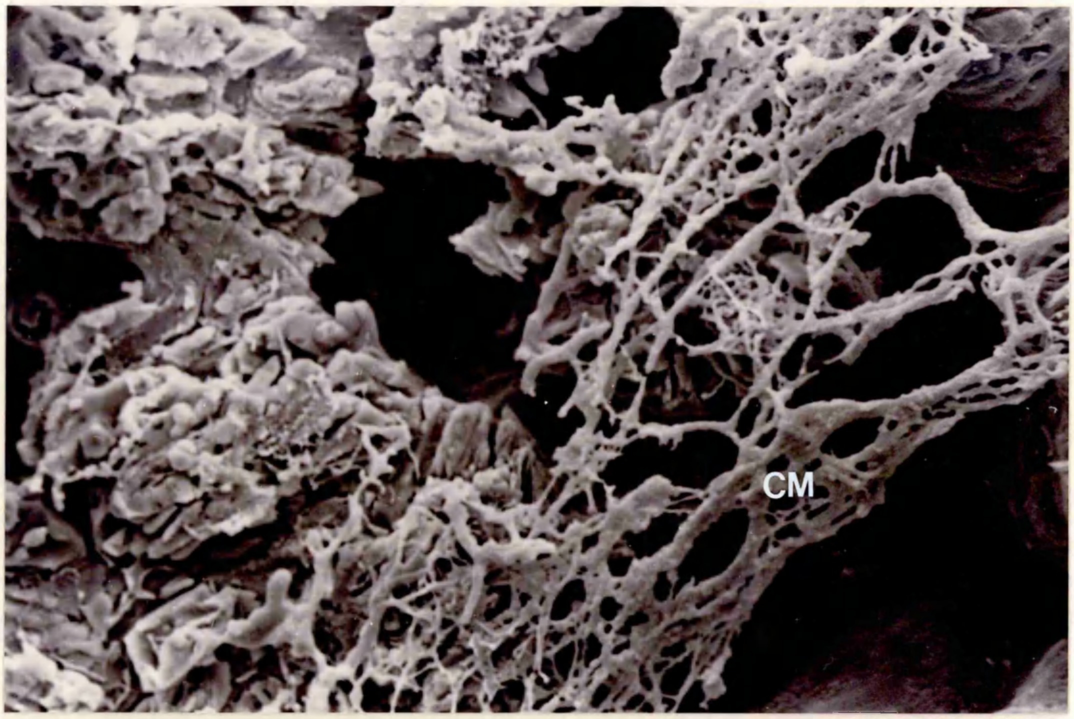


Figure 61 Changed membrane abnormality. Individual membrane fibres can still be identified. (CM = changed membrane) x320.

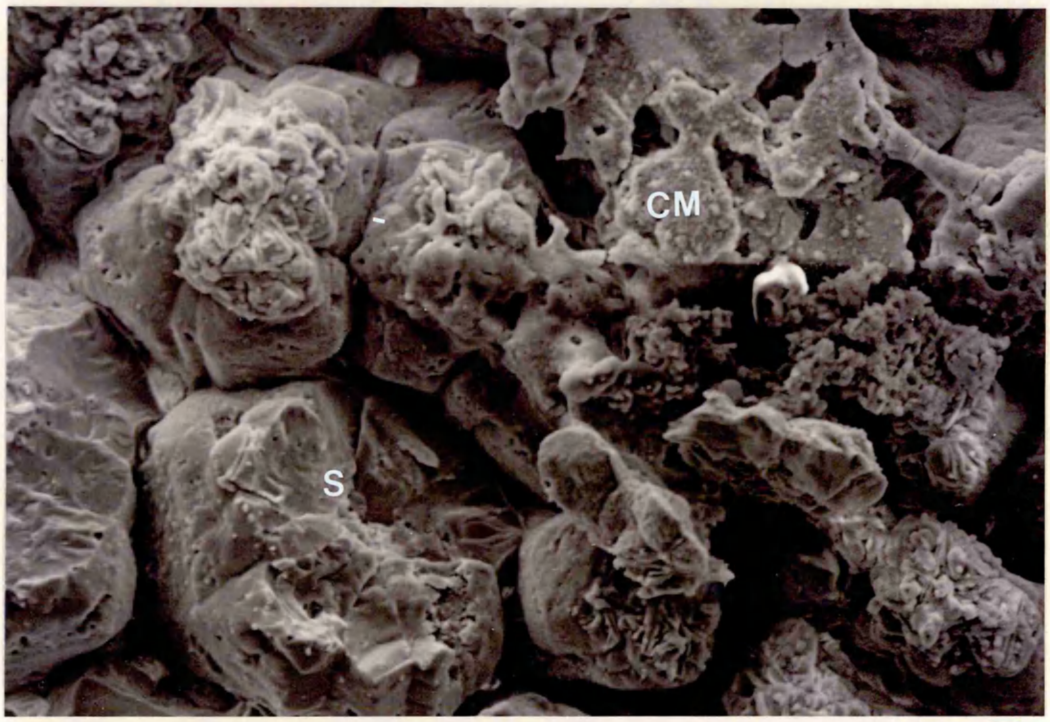


Figure 62 Changed membrane remnants often have a molten appearance, as a result the underlying mammillary caps cannot be identified. There also appears to be a relationship between the incidence of changed membrane and the shearing abnormality. (CM = changed membrane; S = sheared cap) x320.

Figure 63 Mammillary cones in the process of shearing (arrowed). A line of weakness occurs in the underlying cone layer if the bond between the membrane fibres and the mammillary cap is altered. (CM = changed membrane; S = sheared cap; F = fracture) x160.

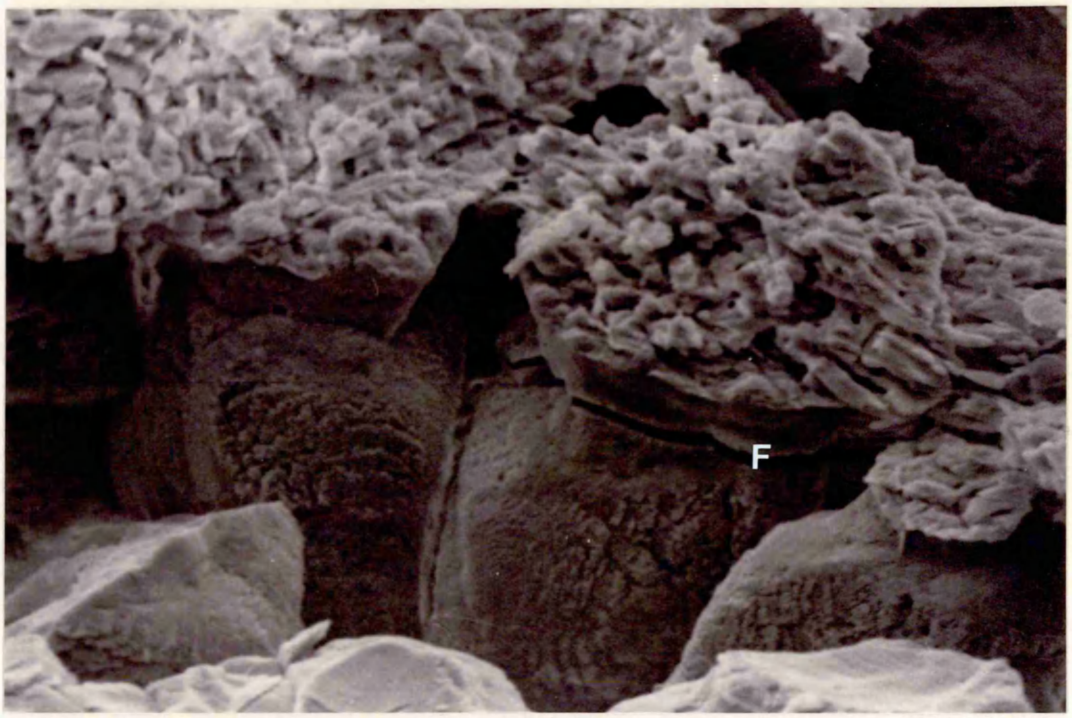


Figure 64 Higher magnification of weakness induced in the cone layer. (F = fracture) x640.

Figure 65 Pitting Type 1: Depressions represent areas which display concave distortion of normal mammillary appearance x160.

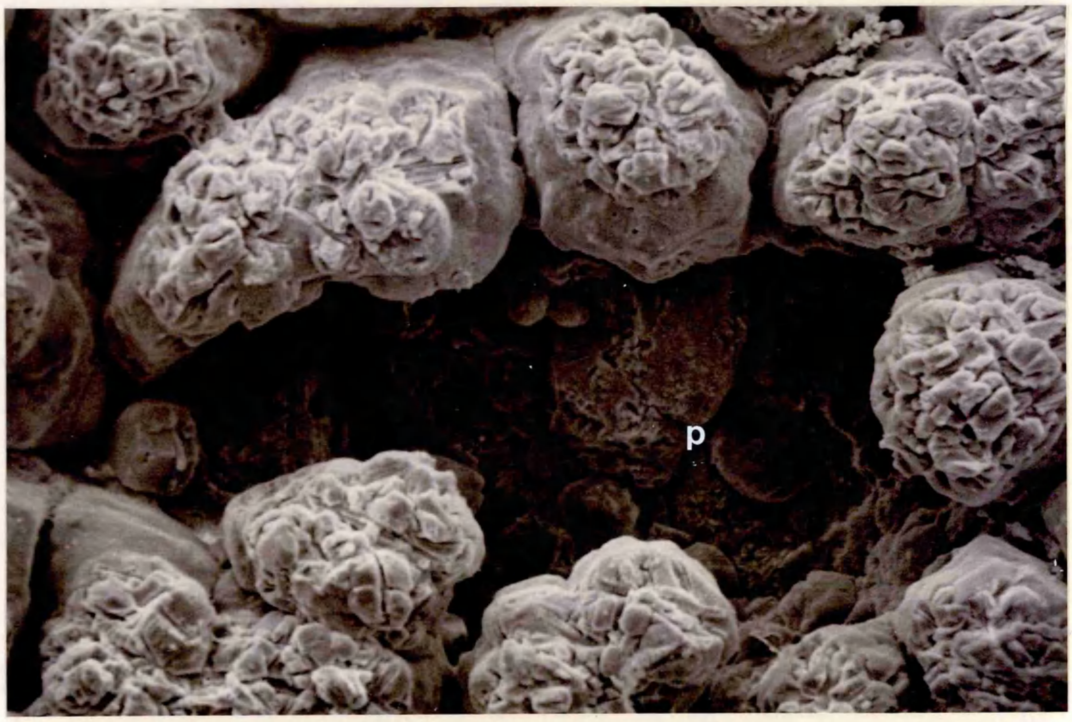


Figure 66 Pitting Type 2: Erosions typically lack normal basal cap and cone structure formations. The underlying palisade material is therefore exposed. (p = palisade) x640.

Figure 67 Pitting Type 3: Cuticular surface appearance of pin hole. Lack of shell debris and a smooth binding surface suggest that this abnormality was not mechanically induced x20.

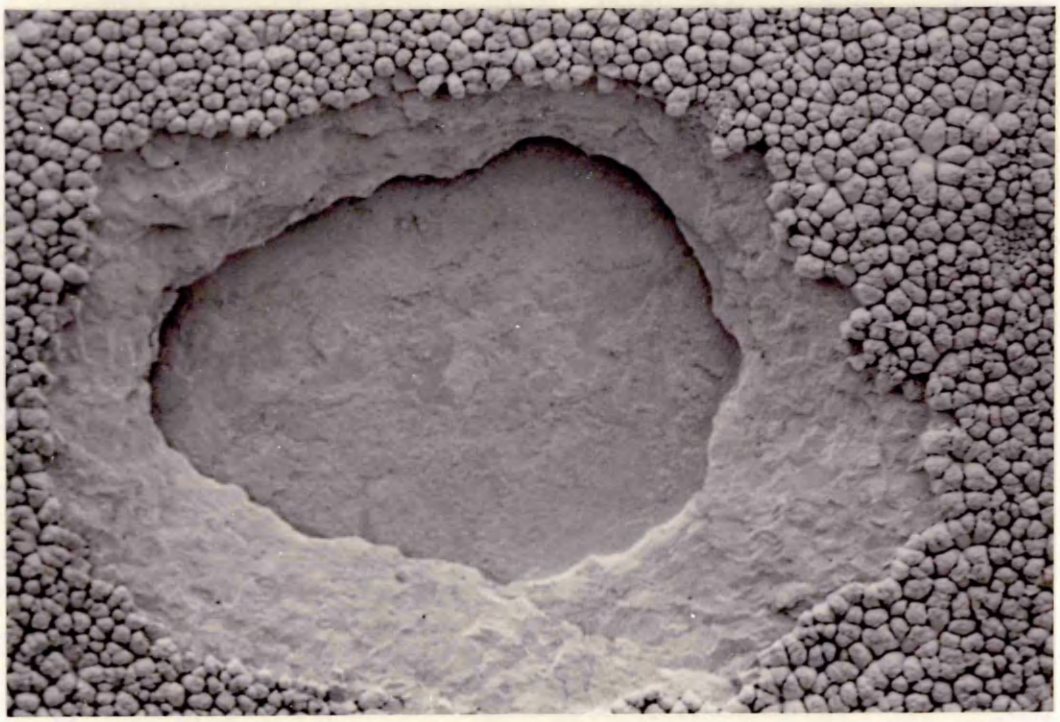


Figure 68 Pitting Type 3: Damage is not localised as evidenced by the gap between the edge of the pin hole and the beginning of the mammillary layer x20.

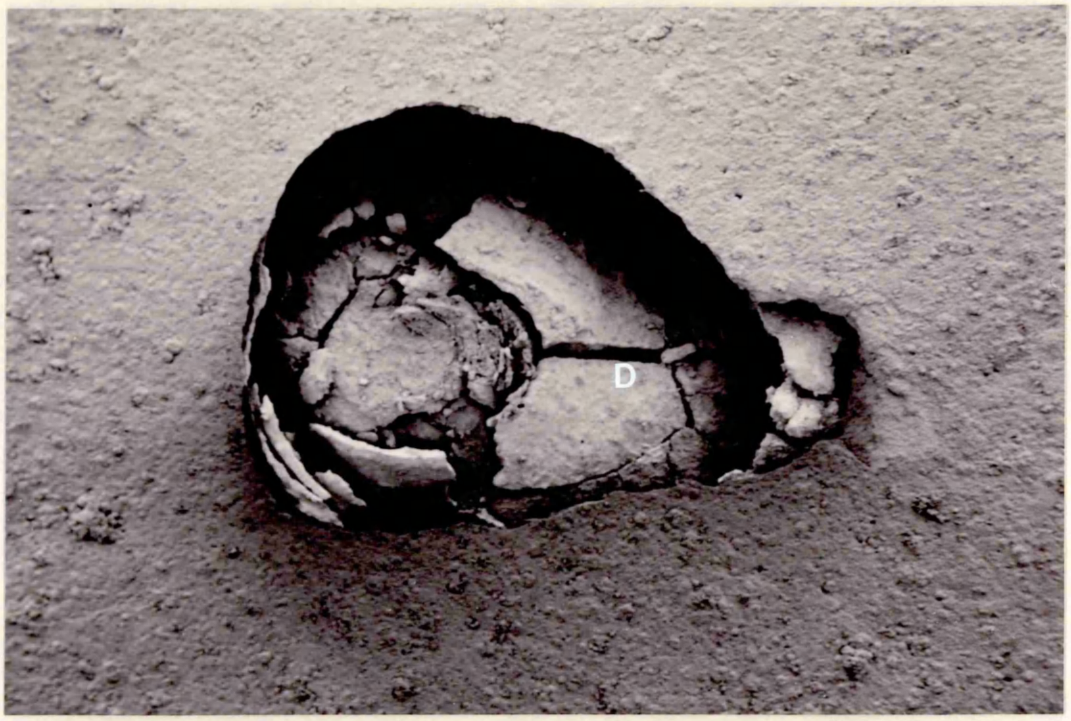
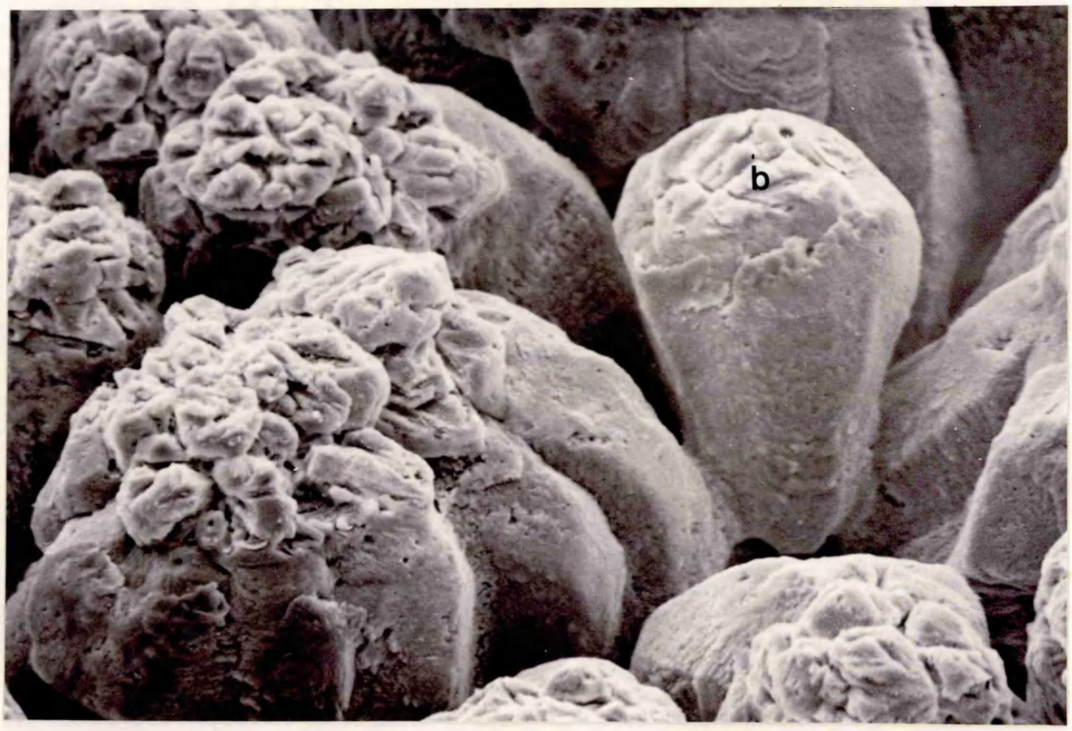


Figure 69 Cuticular surface appearance of cage damaged shell. Shell debris suggests that this type of hole was mechanically induced. (D = debris) x20.



Figures 70 and 71 "Type B" bodies occasionally show evidence of contact with the shell membranes but make no meaningful contribution to the formation of the palisade layer x640.

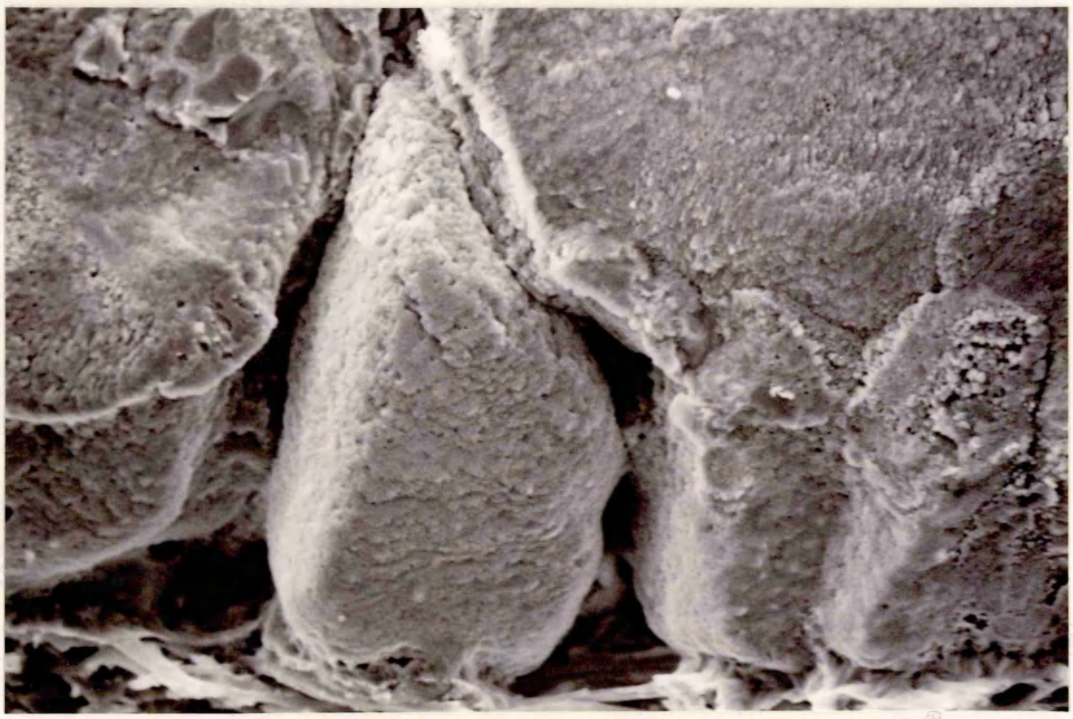


Figure 72 "Type B" bodies are typically located between adjacent mammillae. In older birds most of the cone layer may consist of these round calcified bodies x160.

Figure 73 Isolated clumps of "Type B" bodies hinder the early fusion of adjacent mammillary columns x320.

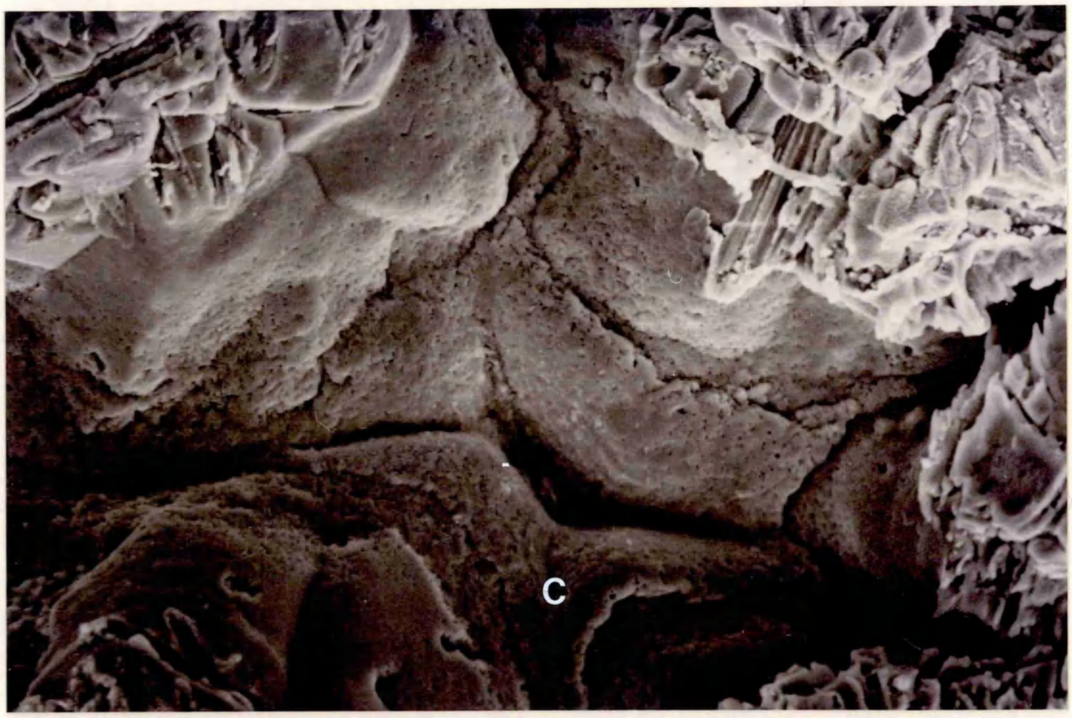


Figure 74 Cuffing. A secondary crystallisation takes place around and between adjacent mammillary knobs. (C = cuffing) x640.

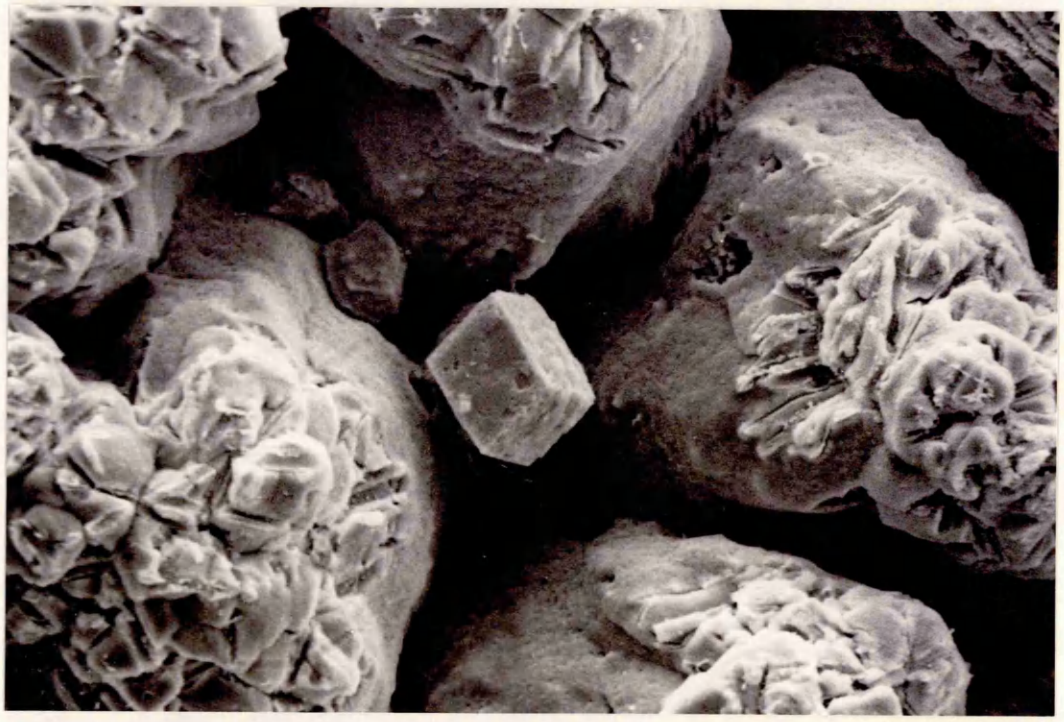
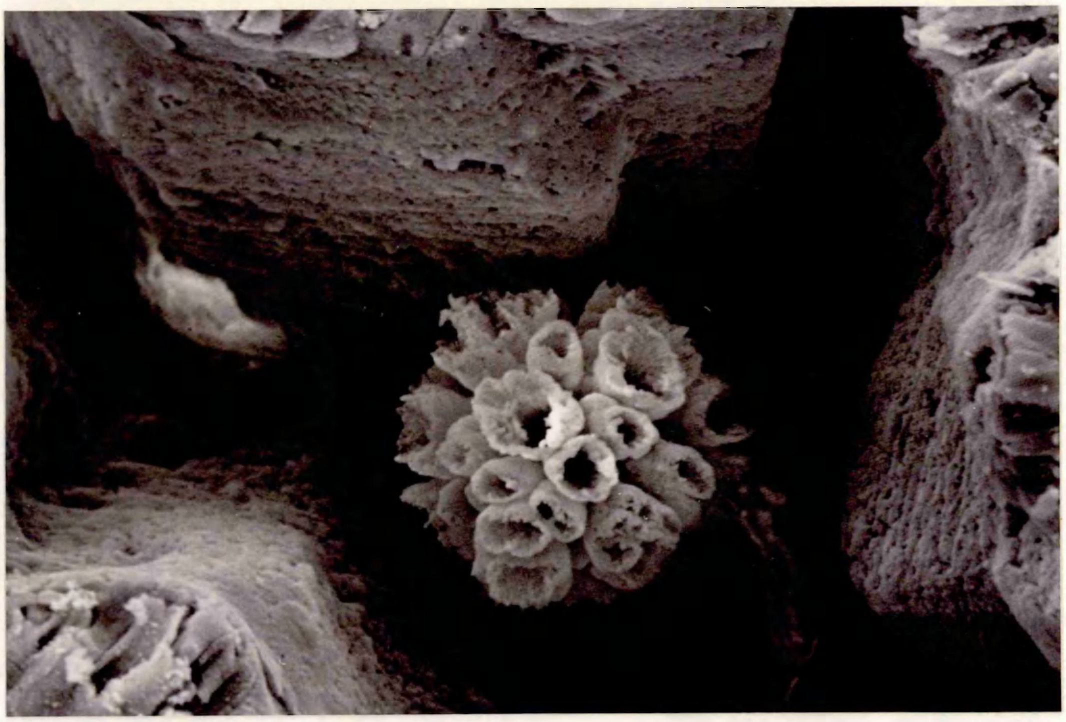


Figure 75 Cubic crystals typical of free growing calcite are occasionally found x640.



Figures 76 and 77 The normal hens eggshell consists of calcium carbonate in its calcite form. Nevertheless, isolated clusters of aragonite do occasionally occur. These figures illustrate the diverse morphology of aragonite x1250.

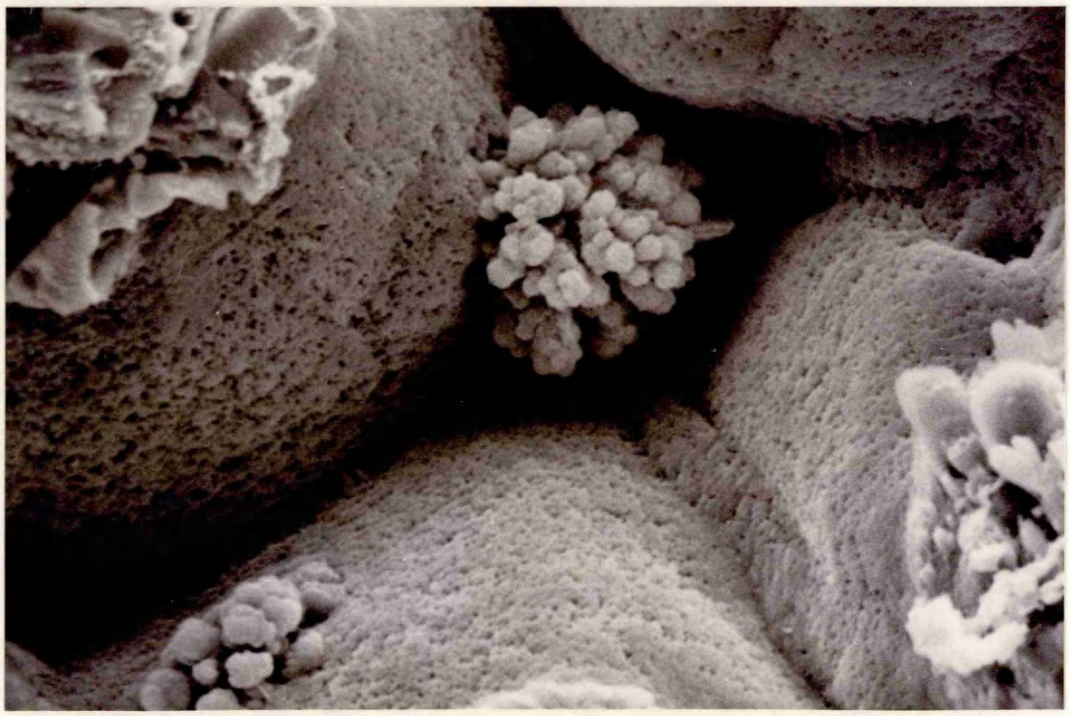


Figure 78 Extensive alignment. Aligned mammillae offer a low resistance to crack growth x20.

Figure 79 Higher magnification of aligned mammillae (arrowed) x40.

Figure 80 Early fusion of mammillary columns x160.

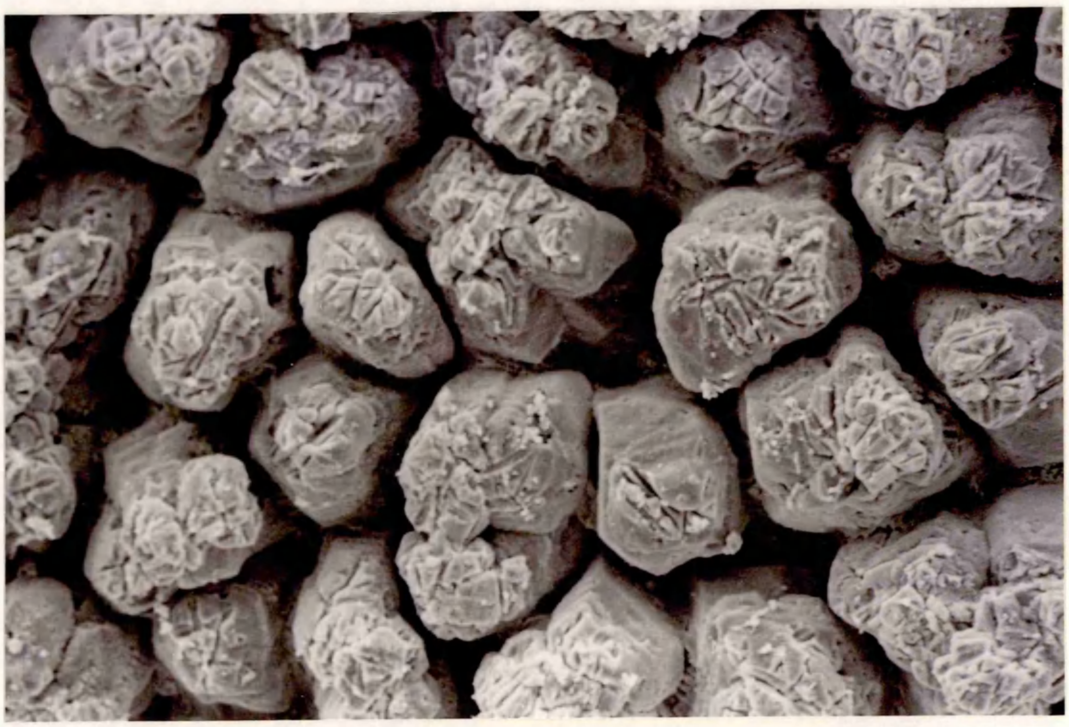


Figure 81 Late fusion of mammillary columns x160.

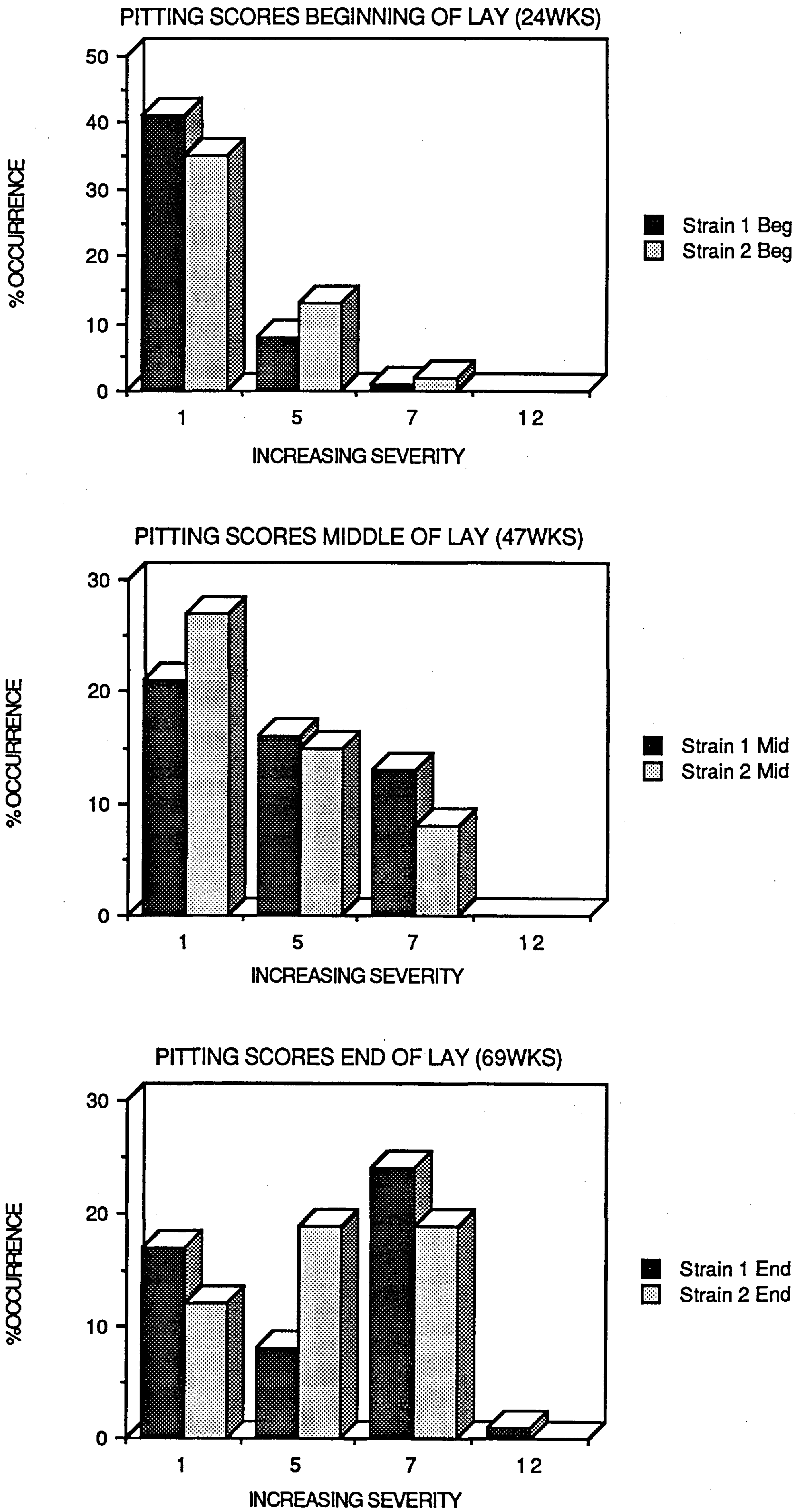


Figure 82 The severity of Pitting increases in both strains with age.

Figure 83 Typical appearance of mammillary layer of eggs laid by Strain 1 at 69wks of age. Late fusion (L) and "Type B" bodies (b) result in an open framework x160.

Figure 84 The mammillary layer of eggs laid by Strain 2 at 69wks typically display poor basal cap morphology (arrowed). Cuffing [C] and early fusion [e] of columns are also indicated x320.

STRAIN 1: BEG LAY

TOTAL EGGS	=	94	
NORMAL	=	34	(36%)
ABNORMAL	=	60	(64%)
FINE DUSTED	=	37	(39%)
HEAVY DUSTED	=	1	(1%)
SPECKLED	=	3	(3%)
PINKS	=	17	(18%)
CALCIUM SPLASH	=	2	(2%)

STRAIN 2: BEG LAY

TOTAL EGGS	=	83	
NORMAL	=	52	(63%)
ABNORMAL	=	31	(37%)
FINE DUSTED	=	15	(18%)
HEAVY DUSTED	=	1	(1%)
SPECKLED	=	10	(12%)
PINKS	=	4	(5%)
CALCIUM SPLASH	=	1	(1%)

STRAIN 1: MID LAY

TOTAL EGGS	=	72	
NORMAL	=	29	(40%)
ABNORMAL	=	43	(60%)
FINE DUSTED	=	7	(10%)
HEAVY DUSTED	=	0	(-)
SPECKLED	=	6	(8%)
PINKS	=	2	(3%)
CALCIUM SPLASH	=	5	(7%)
ACCRETIONS	=	9	(12%)
SLAB SIDED/EQ BULGE	=	2	(2%)

STRAIN 2: MID LAY

TOTAL EGGS	=	59	
NORMAL	=	39	(66%)
ABNORMAL	=	20	(34%)
FINE DUSTED	=	3	(5%)
HEAVY DUSTED	=	0	(-)
SPECKLED	=	10	(17%)
PINKS	=	0	(-)
CALCIUM SPLASH	=	5	(8%)
ACCRETIONS	=	2	(3%)
SLAB SIDED/EQ BULGE	=	0	(-)

STRAIN 1: END LAY

TOTAL EGGS	=	69	
NORMAL	=	22	(32%)
ABNORMAL	=	47	(68%)
FINE DUSTED	=	5	(7%)
HEAVY DUSTED	=	1	(1%)
SPECKLED	=	1	(1%)
PINKS	=	3	(4%)
CALCIUM SPLASH	=	11	(16%)
ACCRETIONS	=	22	(32%)
PIN HOLE	=	1	(1%)
ABNORMAL PIGMENT	=	5	(7%)

STRAIN 2: END LAY

TOTAL EGGS	=	56	
NORMAL	=	17	(30%)
ABNORMAL	=	39	(70%)
FINE DUSTED	=	6	(11%)
HEAVY DUSTED	=	1	(2%)
SPECKLED	=	2	(4%)
PINKS	=	2	(4%)
CALCIUM SPLASH	=	6	(11%)
ACCRETIONS	=	19	(34%)
EQ BULGE/WRINKLED	=	5	(8%)

Table 25 Visual appraisal of surface spoilage.

	STRAIN 1				STRAIN 2			
	BEG	MID	END	[beg/end]	BEG	MID	END	[beg/end]
CONFLUENCE	4.5+/-0.9	3.9+/-1.0 **	3.8+/-1.0 ns	[**]	4.5+/-1.1	4.0+/-0.9**	4.1+/-1.2 ns	[ns]
BASAL CAPS	5.5+/-1.6	5.3+/-1.9 ns	5.5+/-2.2 ns	[ns]	5.9+/-1.4	5.0+/-1.6**	6.3+/-1.6**	[ns]
EARLY FUSION	3.7+/-0.7	3.1+/-1.1**	3.4+/-1.1 ns	[ns]	2.9+/-1.2	2.7+/-1.2 ns	2.8+/-1.2 ns	[ns]
LATE FUSION	1.7+/-1.1	2.4+/-1.6**	3.1+/-1.7 *	[**]	1.5+/-0.9	2.1+/-1.5 *	2.6+/-1.7 ns	[**]
OVERALL FUSION	5.4+/-1.4	5.6+/-2.1 ns	6.5+/-2.3 *	[**]	4.4+/-1.6	4.8+/-2.2 ns	5.4+/-2.6 ns	[*]
ALIGNMENT	3.3+/-1.1	2.8+/-1.2 *	2.7+/-1.1 ns	[**]	3.3+/-1.5	2.5+/-1.2 **	2.7+/-1.5 ns	[ns]
TYPE B'S	2.0+/-0.9	2.0+/-1.0 ns	3.2+/-2.2 **	[**]	1.9+/-0.6	2.0+/-1.3 ns	2.2+/-1.1 ns	[ns]
PITTING	1.8+/-1.7	3.8+/-2.6**	4.7+/-2.9 ns	[**]	2.3+/-2.0	3.2+/-2.5 *	4.8+/-2.3**	[**]
ARAGONITE	1.1+/-0.3	1.1+/-0.3 ns	1.6+/-1.2 **	[**]	1.0+/-0.0	1.0+/-0.2 ns	1.2+/-0.4**	[**]
TYPE A'S	1.7+/-0.5	1.6+/-0.5 ns	1.7+/-0.5 ns	[ns]	1.7+/-0.5	1.4+/-0.5**	1.5+/-0.5 ns	[ns]
CUBICS	1.1+/-0.3	1.1+/-0.6 ns	1.2+/-0.4 ns	[ns]	1.0+/-0.1	1.0+/-0.1 ns	1.0+/-0.1 ns	[ns]
CUFFING	4.6+/-0.5	4.6+/-0.9 ns	4.5+/-0.9 ns	[ns]	3.8+/-1.3	3.8+/-1.4 ns	3.6+/-1.6 ns	[ns]
CHANGED MEMB	2.3+/-1.7	2.9+/-2.0 ns	2.7+/-1.7 ns	[ns]	1 .7+/-1.5	2.8+/-2.7 **	4.3+/-2.6**	[**]
TOTAL SCORE	33.1+/-4.2	34.6+/-5.3 ns	38.1+/-6.9**	[**]	31.4+/-4.0	31.7+/-5.1ns	37.3+/6.1**	[**]
MAM DENSITY	80.5+/-13.5	89.8+/-16.2 **	80.6+/-16.3**	[ns]	95.6+/-12.2	107.9+/-25.4**	111.2+/-21.5 ns	[**]

** P < 0.01 ns = not significant
* P< 0.05 [ ] = beg/end comparisons.

Table 26 Structural scores (mean +/- s.d.) for each individual characteristic of the cone layer in eggs from Strain 1 and 2. Values correspond to beginning, middle and end of lay.

BEGINNING OF LAY:

	STRAIN 1	STRAIN 2	PROBABILITY
MORE EARLY FUSION		X	0.01
OVERALL LATER FUSION	X		0.01
MAMMILLARY DENSITY HIGHER		X	0.01
ARAGONITE	X		0.05
CUFFING		X	0.01

MIDDLE OF LAY:

	STRAIN 1	STRAIN 2	PROBABILITY
MAMMILLARY DENSITY HIGHER		X	0.01
CUFFING		X	0.01
POORER OVERALL SCORE	X		0.01

END OF LAY:

	STRAIN 1	STRAIN 2	PROBABILITY
MORE EARLY FUSION		X	0.01
FUSION OVERALL LATER	X		0.05
MAMMILLARY DENSITY HIGHER		X	0.01
ARAGONITE	X		0.05
CUBICS	X		0.01
TYPE B'S	X		0.01
TYPE A'S	X		0.05
CAPS SIG POORER		X	0.05
CUFFING		X	0.01
CHANGED MEMBRANE		X	0.01

Table 27 Comparison of ultrastructural scores between strains.
X denotes the strain displaying the characterisitic.

	STRAIN 1			STRAIN 2		
CONFLUENCE	BEG	MID	END	BEG	MID	END
NONE	2	22	25	7	11	16
ISOLATED	35	20	18	27	31	20
MODERATE	13	8	7	16	8	13
EXTENSIVE	0	0	0	0	0	1
CAP APPEARANCE	BEG	MID	END	BEG	MID	END
GOOD	0	2	3	0	0	1
GOOD-	13	14	12	7	18	4
POOR+	31	26	26	36	29	29
POOR	6	8	6	7	3	16
POOR-	0	0	3	0	0	0
EARLY FUSION	BEG	MID	END	BEG	MID	END
ISOLATED	43	31	38	26	22	24
MODERATE	7	14	7	19	20	17
EXTENSIVE	0	5	5	5	8	9
LATE FUSION	BEG	MID	END	BEG	MID	END
ISOLATED	35	23	12	38	29	21
MODERATE	14	21	28	12	17	21
EXTENSIVE	1	6	10	0	4	8
ALIGNMENT	BEG	MID	END	BEG	MID	END
NONE	0	4	4	0	3	5
ISOLATED	20	26	27	24	37	29
MODERATE	29	19	19	22	8	13
EXTENSIVE	1	1	0	4	2	3
TYPE B'S	BEG	MID	END	BEG	MID	END
NONE	9	12	6	9	16	9
ISOLATED	38	34	28	40	30	35
MODERATE	3	4	10	1	3	6
EXTENSIVE	0	0	6	0	1	0
PITTING	BEG	MID	END	BEG	MID	END
NONE	41	21	17	35	27	12
DEPRESSION	8	16	8	13	15	19
EROSION	1	13	24	2	8	19
HOLE	0	0	1	0	0	0
ARAGONITE	BEG	MID	END	BEG	MID	END
NONE	45	46	35	50	48	38
ISOLATED	5	4	10	0	2	12
MODERATE	0	0	5	0	0	0
TYPE A'S	BEG	MID	END	BEG	MID	END
NONE	17	20	14	15	29	24
ISOLATED	33	30	36	35	21	26

Table 28 Frequencies of structural scores within each group of eggs assessed by SEM

	STRAIN 1			STRAIN 2		
CUBICS	BEG	MID	END	BEG	MID	END
NONE	45	47	41	49	49	49
ISOLATED	5	2	9	1	1	1
MODERATE	0	1	0	0	0	0
CUFFING	BEG	MID	END	BEG	MID	END
NONE	29	34	30	15	17	18
ISOLATED	21	14	18	27	24	19
MODERATE	0	2	2	8	9	13
CHANGED MEMBRANE	BEG	MID	END	BEG	MID	END
NONE	30	23	23	40	28	13
ISOLATED	19	24	26	9	17	23
MODERATE	1	3	1	1	4	14
EXTENSIVE	0	0	0	0	1	0
TOTAL SCORE	BEG	MID	END	BEG	MID	END
E < 20	0	0	0	0	0	0
G 20-29	12	8	3	12	19	17
G- 30-34	17	18	12	29	21	9
P+ 35-39	18	18	18	8	6	17
P 40-49	3	5	14	1	4	16
P- ≥ 50	0	1	3	0	0	1
TOTAL EGGS	50	50	50	50	50	50

Table 28 (continued)

CHAPTER 5.

GENERAL DISCUSSION AND CONCLUSIONS.

5.1.1 THE DUAL ROLE OF THE EGGSHELL.

The conflict between the role of the shell as an embryonic chamber and as a consumer package will always exist with reference to hatchibility rates and monetary turnover from eggs and egg related products. The eggshell must perform a dual function but whether it can achieve this to maximum efficiency remains a matter of debate.

Primarily the eggshell is nature's way of protecting the avian embryo during its development outwith the hen and in this respect the shell is mechanically efficient. The shell must be strong enough to withstand the weight of the broody hen, yet be sufficiently fragile to allow the chick to break out at the end of incubation. It must be stiff to resist distortion but must also be elastic (returning to its original shape after a load has distorted it) so that it can transmit and distribute this energy.

As a consumer package the eggshell is less suited to the type of trauma experienced en route to the supermarket shelf and to satisfy this market the eggshell might perform better if it were made of a more spongy or elastic material. More realistically this thesis suggests that an egg would be less vulnerable if it were round rather than elongate, thicker rather than thinner, but more importantly if it possessed those inherent ultrastructural modifications which increased its' resistance to crack growth (fracture toughness), viz: early fusion, cuffing, and possibly a lower mammillary density. Strain differences appear to exist in all of these. However, in terms of the set egg excessive thickness is to be avoided since it will impair the process of hatching. The shell in this capacity is first and foremost a source of calcium and other trace elements but must also act as a barrier to pathogen transfer. Superimposed upon these functions is the process of gas exchange; thus while early fusion of columns offers a higher resistance to crack propagation, this together with cuffing or confluence are to be avoided in the set egg as they will impair hatching and the process of gaseous exchange.

It has been estimated that fifty percent or more of the variability in egg shell quality is not genetically determined (Poggenpoel 1986). Rather management, environment, nutrition and disease all appear to play a more significant role (Hughes *et al* 1985; Mohamed 1986; Washburn 1982; Spackman 1985). Nevertheless, despite intensive research in these fields, there is still no consensus on how eggshell strength may be improved or how the production of eggs with shells of low breaking strength may be avoided. Ultrastructural studies provide another level at which to examine the effects of genetical, nutritional and environmental manipulation upon egg formation and in this respect some progress has now been made to interpret these observations in terms of their effect on the fracture toughness of eggs.

5.1.2 BONE AND EGGSHELLS ARE MECHANICALLY SIMILAR.

In many respects the eggshell is similar to other calcified tissues such as bone. Both are composed of crystals, but both also possess one other common property, and that is that they tend to be bound together by an organic matrix. The essential difference between ossification and calcification of the eggshell is that bone as a tissue has cells (osteoblasts) which not only organise the osteoid matrix but are thought to start crystal formation (Pritchard 1979). In contrast the matrix proteins associated with the eggshell are manufactured in the liver and are thereafter transported to the surface epithelial cells lining the SGP (Board pers comm). The subsequent role of the matrix fibres in shell formation is still open to debate but according to Wyburn *et al* (1973), these fibres are to the shell of the hens egg what collagen is to bone.

Calcified tissues have been compared to composites such as fibreglass where the two components glass and resin have different properties from the composite. Certainly bone has a modulus of elasticity intermediate between that of its mineral and organic constituents and a tensile strength greater than both (Pritchard 1979).

In this thesis the elastic modulus of eggshells was found to be similar to that of bone and was subsequently found to be remarkably consistent from one strain of bird to another. Considerable variation however was found when the modulus of individual eggs were compared.

Tung *et al* (1969) reported a considerable variation in the elastic modulus of eggs from individual birds. Environmental stress has been shown by Watt (1989) to alter the acidity of the secretions released from the epithelial cells in the oviduct which in turn alter the process of mineralisation. It might therefore be hypothesised that in stressed birds the efficiency of other protein producing organs might also be affected. Adrenalin, for example, has been shown to inhibit ovulation and suppress yolk formation (Solomon *et al* 1987; Watt 1989). These observation indicate that, albeit under heightened stress levels, liver dysfunction does occur. Perhaps further consideration should be given to the amino acid composition of the matrix of eggs from such provenance as this may explain the above variations in the modulus values.

Trace elements such as potassium, magnesium and phosphate are also thought to be of importance in proper shell formation. The mechanism of the latter is probably in the process of matrix formation since trace minerals are also known to influence the formation of osteoid matrix in bone (Longstaff and Hill 1972). Tung *et al* (1968,1969) suggested that the variation in hardness through the thickness of the shell was due to a variation in magnesium content. Future studies might therefore also consider the role and distribution of these trace elements in relation to shell stiffness and strength.

Baird *et al* (1975) suggests that both brown and white laying species contain protoporphyrin as an integral part of the calcite matrix and that the latter may have a separate function from the additional pigment which was found in the cuticular layer of brown eggs. According to Solomon (1985b) these flat porphyrin molecules are chemically similar to the phthalocyanins used by engineers as solid state lubricants, but it remains open to speculation as to whether the insertion of these porphyrin molecules into the growing calcite actually confers any resilience on the shell. From current work, it is clear that the cuticle and the pigment contained therein have little effect on the stiffness characteristics of brown eggs.

5.1.3 COMPUTER MODELLING AND FINITE ELEMENT ANALYSIS.

Throughout this study finite element analysis has proved invaluable in the development of our understanding of eggshell strength. It has facilitated the consideration of geometry in isolation from other features and so highlighted the importance of the inclusion of eggshape in quality assessment; it has provided a means of calculating the elastic modulus which not only takes into consideration the effective thickness of the shell but also the modifying effects on this caused by geometry; it has proved useful in the interpretation of eggshell behaviour under quasi-static loads with particular reference to the concept of the shells resistance to unstable crack growth (fracture toughness); it has provided a means of calculating the fracture toughness of eggshells from quasi-static compression test data; and it has provided a means of converting the values obtained from the non-destructive deformation tests into units of stiffness.

Implicit in the use of this method were the assumptions that the egg is symmetrical about its longitudinal axis and that the shell consists of two homogeneous and isotropic layers, viz layer 1 which included the cuticle, vertical crystal layer (VCL) and palisade, and layer 2 the mammillary knob layer. Theoretically the FE model of the eggshell should have consisted of at least four layers, however current methods of thinning the shell did not permit a more detailed study of the elastic properties of the cuticle, VCL and palisade layers. The mammillary layer in contrast was unequivocally found in chemical etching experiments not to contribute in a meaningful way to stiffness characteristics of the remaining layers.

Carter (1976) questioned the application of engineering theories to the eggshell in so far as they did not take into account what he described as an inner layer of shell material which was weak in tension (Carter 1970a;1971b), and in many cases the egg was also assumed to be spherical. In both respects some success can now be claimed, but perhaps of more relevance these current investigations have also called into question the assumption which was made by Voisey and Hamilton and their associates (1964-82); namely that the eggshell be regarded as a classical case of a "thin shell". For R/t<300, both the shear and normal components were predicted by FE analysis to be important in the generation an eggshells' resistance to load. For the eggshell 50<R/t<100.

5.1.4 A CRITICAL APPRAISAL OF THE PROCEDURE USED TO QUANTIFY THE ASSESSMENT OF SHELL ULTRASTRUCTURE BY SCANNING ELECTRON MICROSCOPY.

Any method of assessment based purely on observation is subjective. Nevertheless, the method used in the current investigations to quantify the ultrastructural assessment of shell quality, attempted not only to provide a set of guidelines on which to base these observations, but also to summarise the structural features of each specimen in terms of an overall or total score. In this respect some success can be claimed, with both strains displaying higher total scores towards the end of lay when the ultrastructure of an egg is notoriously poor. Nevertheless, during the analysis it became increasingly apparent that rarely does one shell contain all of the more harmful traits, and so it was more realistic in terms of fracture toughness to consider the average score for each individual abnormality. Perhaps the greatest difficulty encountered with the current method of quantifying shell ultrastructure however was that no provision had been made for the fact that the numbers and the relative depth and size of pits varied from one sample to the next. One might anticipate that a large number of erosions would have had a more detrimental effect on an eggs performance than for example a single isolated depression.

This possibility was apparently overlooked in the derivation of the scoring system perhaps because pitting had previously only been found in the eggs from white egg laying stock.

It has also come to light that in order to apply this method of quantifying shell ultrastructure to other aspects of shell performance, for example in relation to bacterial penetration, then further modifications to the current scoring system might also be necessary (Nascimento pers comm).

The statistical test applied to these data may also be open to criticism. One needs no reminding that the Students t-test was designed primarily for use on data which have a normal distribution about a mean value. Nevertheless, the above system was designed with the help of a statistician who by assigning a weighted scoring system to each abnormality has attempted to fit these data to a normal distribution curve (see central limit theorem Moran 1984).

5.1.5 A RE-INTERPRETATION OF EGGSHELL STRENGTH.

Eggshell strength can now be defined in terms of those properties which influence its' stiffness characteristics and those properties which influence its' resistance to crack growth.

The stiffness characteristics of the eggshell are determined by it's elastic modulus and the modifying effects on this caused by geometry which includes the effective thickness of the shell. In this thesis it has been shown that while the modulus of eggshells on an individual egg basis can be variable, strain/age specific differences in stiffness characteristics are best explained in terms of an increase or decrease in the effective thickness of the shell. Eggshape to a limited extent also has a significant role to play.

It has been argued that a relationship exists between the stiffness characteristics of an eggshell and its' ultimate strength but in addition to this it is now clear that those factors which influence the shells' resistance to crack growth must also be considered if shell strength is to be improved. For example, it is a well recognised fact that as the bird ages, the strength of the shell produced diminishes. Several theories have been suggested to explain this phenomenon. Peterson (1965) suggested that as a hen ages its calcium metabolism becomes less efficient in that it cannot absorb and retain as much calcium from the diet. This however was refuted later by Roland *et al* (1975a) and Roland (1981). The latter suggested that as the bird ages the amount of shell produced remains constant. However, since the average size of the egg increases with bird age then the same amount of material must spread over a larger area and as a result those eggs at the end of lay are thinner. In the current work shell thickness and hence stiffness remained comparatively unchanged throughout the laying year in all but one of the intra strain specific comparisons which were carried out, while the greatest change in egg size took place between the first and second sampling periods. Shell strength in contrast declined throughout the study period.

It should be re-emphasised at this point that eggshell characteristics are subject to both genetic and environmental conditions, with variation in the latter having perhaps the most profound effect. The above results must therefore be interpreted in the first instance with reference to the conditions prevailing at the West of Scotland Agricultural College where the birds used in this study were housed. The same birds housed elsewhere might perform differently. For example, before this work was carried out, it was the consensus of opinion that the Strain 1 bird would produce eggs with better quality characteristics as measured by physical parameters such as egg weight and shell thickness, and in this capacity the authors expectations were fulfilled. Previously the Strain 1 birds also emerged as best with regard to shell ultrastructure (Solomon pers comm). However in the current investigations those characteristics which influence the shells' resistance to crack growth were in fact superior in Strain 2 at 69wks.

The time of sampling and the number of eggs analysed on each occasion are also open to debate. With reference to the latter the time scale in this programme of research restricted sample size to some extent. The mid point sampling period in these investigations was chosen as that time when shell ultrastructure is known to be at its best (Solomon pers comm). Nevertheless, it might be of interest in future work to sample at peak production and to consider the role of individual bird variation and the sequence of eggs within the clutch (Belyavin *et al* 1985).

It is well established that failure is initiated in a structure where strain energy tends to be concentrated. The characteristic notched appearance of the mammillary layer therefore provides the ideal crack initiation site. Crack initiation and shell breakage however are not synonymous which partly explains why previous attempts to predict shell failure from the ultimate tensile strength of eggshells (Voisey and Hunt 1967b) under-estimated the actual force required to break eggs in subsequent experimental tests. Crack initiation must now be defined as localised trauma. While these sites may weaken the shell, this type of damage is not likely to be detected even by the most experienced candler as it takes several hours for the shell contents to penetrate these traumatised sites. In contrast the broken shell by virtue of its gross imperfection fails to function in any capacity and in most cases will be identified at the processing plant or by the consumer before buying. In terms of the risk of food spoilage the traumatised eggshell is therefore potentially the more hazardous. Thus future consideration should be directed towards reducing the risks of trauma but more importantly improving the toughness of the end product.

The fracture toughness of an eggshell is a measure of its resistance to unstable crack growth and in this respect there appears to be a link between latter and the structural organisation of the mammillary layer. Thus where fusion is late, crack propagation through the shell wall, and thereafter outwards from the load point will occur more rapidly.

The mammillary layer also provides the foundation for subsequent shell formation and as illustrated in this thesis the eggs from Strain 2 birds at 69wks were as a result of a tenuous link between the true shell and the membrane fibres correspondingly thinner. The role of the mammillary layer is therefore two fold with respect to shell strength (Table 29).

Inter and intra strain differences were found in the rate of fusion, the numbers of mammillae per unit area, the incidence of pitting, confluence, cuffing and Type B's, and as a result one might have anticipated inter strain differences in fracture toughness after correcting for thickness and geometry. At this point in our interpretation of quality however it would be naive to cross strain boundaries and so introduce into genetically different lines those features which appear to have had an efficient effect on quality in one particular strain. Cuffing and a high mammillary density for example, appear to have worked for a genetically determined thin shell but will these features have the same influence in a genetically determined thick shell? More work is required before any decisive conclusions in this respect can be made.

5.1.6 QUALITY ASSESSMENT.

The non-destructive deformation test remains one of the most widely used methods of assessing shell quality. However in this work the correlation between stiffness and shell strength was found to be variable from one group of eggs to another. In the light of the foregoing discussion this is perhaps not surprising since factors other than the stiffness characteristics of the eggshell are now known to affect its performance. This measure should therefore be interpreted with caution.

While a study of this type has been both time consuming and comparatively costly, it is nevertheless essential to an industry that has witnessed dramatic changes in shell ultrastructure consistent with modern intensive husbandry practices. If effective methods are to be adopted in solving the ongoing problem of down grading and indeed the more recent problem of bacterial penetration, then a re-interpretation of strength and quality in terms of the eggshells' true ultrastructural complexity is well overdue.

Structural variations which increase the fracture toughness of eggshells:

Early fusion
Cuffing
Confluent mammillae
A low mammillary density

Structural variations which decrease the fracture toughness of eggshells:

Late fusion
Type B's = open framework
Aragonite = open framwork
Pitting: depressions, erosions, pin holes.
Alignment of mammillae
A high mammillary density

Other:

Changed membrane
Cap to cone contact
Type A's

Table 29 Categorisation of mammillary layer abnormalities into those which increase, and those which decrease an eggshells' fracture toughness. Those remaining are of the basal cap to membrane variety and these have a direct influence on shell thickness.

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APPENDICES

APPENDIX 1

Dimensional Data for Various Eggshaped Models

MODEL REF	DESCRIPTION	LENGTH/2 [mm]	BREADTH/2 [mm]	L:B	X [mm]
Standard	l ≠ b; X≠Midaxis	29.41	21.90	1.34	1.78
Sphere	l = b; X=Midaxis	21.86	21.86	1.00	0.00
L:B 1	l ≠ b; X=Midaxis	26.23	21.86	1.19	0.00
L:B 2	l ≠ b; X=Midaxis	30.60	21.86	1.39	0.00
L:B 3	l ≠ b; X=Midaxis	34.97	21.86	1.60	0.00
L:B 5	l ≠ b; X=Midaxis	39.35	21.86	1.80	0.00
L:B 6	l ≠ b; X=Midaxis	48.09	21.86	2.20	0.00
L:B 7	l ≠ b; X=Midaxis	52.60	21.86	2.41	0.00
Rounded	l = b; X≠Midaxis	21.86	21.86	1.00	1.78
Pro.Pole	l ≠ b; X≠Midaxis	34.73	21.05	1.65	5.90
Elongated	l ≠ b; X=Midaxis	34.73	21.05	1.65	0.00

Input Data and Results of the Analyses Carried out on Different Eggshaped FE Models.

1.The Effect of Increasing the Length to Breadth Ratio on the Non-dimensionalised Compliance.

Model Ref	L:B	R/t	t [mm]	E [MN/mm^2]	v	F [N]	d [mm]	Compliance* [X 1/2]	CPU Hr:min:sec
Sphere	1.0	87	0.251	0.036	0.3	5	0.0423	0.444	02:08:01.50
L:B 1	1.2	87	0.251	0.036	0.3	5	0.0511	0.536	02:02:55.40
L:B 2	1.4	87	0.251	0.036	0.3	5	0.0565	0.592	02:05:35.57
L:B 3	1.6	87	0.251	0.036	0.3	5	0.0589	0.618	02:04:36.35
L:B 4	1.8	87	0.251	0.036	0.3	5	0.0621	0.651	02:04:10.11
L:B 5	2.2	87	0.251	0.036	0.3	5	0.0653	0.685	02:04:18.18
L:B 6	2.4	87	0.251	0.036	0.3	5	0.0674	0.707	02:02:45.49

* Compliance has been halved in each case to take account of the fact that only quarter of the model was generated in each case.

2. The Effect of Moving the Maximum Breadth Away From the Midaxis.

Model Reference	R [1/2 B]	t [mm]	E [MN/mm^2]	v	F [N]	d [mm]	Compliance* [x 1/2]	CPU Hr:Min:Sec.
Standard	21.92	0.342	0.158	0.3	25	0.0339	0.573	02:05:48.44
Sphere	21.86	0.342	0.158	0.3	25	0.0263	0.445	02:08:01.50
Rounded	21.86	0.342	0.158	0.3	25	0.0263	0.444	02:07:52.11
Pro.Pole	22.81	0.342	0.158	0.3	25	0.0432	0.701	02:09:31.23
Elongated	22.81	0.342	0.158	0.3	25	0.0413	0.669	02:05:34.38

* Compliance has been halved in each case to take account of the fact that only quarter of the model was generated in each case.

Appendix 2

Derivation of the non-dimensionalised compliance of different eggshapes according to thick shell theory.

From Chapter 2. 2 and chapter 3.4, C is the FE estimation of the non-dimensionalised compliance, the value of which is dependant upon both the ratio of R/t (Figure 15) and the shape of the egg (Figure 33). The data in Figure 15 can be described by,

$$C_{sphere} = 0.408 + 3.026t/R \qquad [0>t/R\leq 300;\ L:B=1] \qquad (1)$$

where C_{sphere} is the thick shell equivalent to Koitres (1963) "thin shell" solution. To correct for eggshape this equation becomes,

$$C = C_{sphere} \times A \qquad [0<t/R\leq 300;\ 1.0\leq L:B\leq 2.2] \qquad (2)$$

where A is a correction factor defined by,

$$A = \frac{C_{eggshape}}{0.444} \qquad [R/t=87] \qquad (3)$$

From Figure 33 ,

$$C_{eggshape} = -0.666 + 1.866\ (L:B) - 0.907\ (L:B)^2 + 0.153\ (L:B)^3 \qquad [R/t=87;\ 1.0\leq L:B\leq 2.2] \qquad (4)$$

Some values of A are given below.

L:B Ratio	Value of A
1.0	1.00
1.1	1.10
1.2	1.19
1.3	1.21
1.4	1.32

Assuming that a similar correction factor will exist irrespective of the value of R/t, the non-dimensionalised compliance C for any R/t and L:B ratio can now be calculated from (2) without having to generate and analyse additional FE models.

APPENDIX 3

Multiple Regression Analysis.

Data File: STRAIN 1 Physical Data   Dependent Variable: Force

Variable Name	Coefficient	Std. Err. Estimate	t Statistic	Prob > t
Constant	-71.459	153.675	-0.465	0.643
weight	-0.055	0.272	-0.204	0.839
length	-2.001	2.508	-0.798	0.426
breadth	3.145	3.523	0.893	0.373
shape index	60.164	110.530	0.544	0.587
t effective	-676.575	1361.121	-0.497	0.620
ND Stiff	0.119	0.017	7.009	0.000
tot thick	665.466	1361.372	0.489	0.626
mam thick	-683.201	1365.915	-0.500	0.618
Age	-5.079	0.741	-6.859	0.000

Data File: STRAIN 1 Physical Data

Source	Sum of Squares	Deg. of Freedom	Mean Squares	F-Ratio	Prob>F
Model	6270.442	9	696.716	38.595	0.000
Error	2455.041	136	18.052		
Total	8725.482	145			

Coefficient of Determination (R^2)	0.719
Adjusted Coefficient (R^2)	0.700
Coefficient of Correlation (R)	0.848
Standard Error of Estimate	4.249
Durbin-Watson Statistic	2.091

Data File: STRAIN 1 Physical Data   Dependent Variable: Force

Variable Name	Coefficient	Std. Err. Estimate	t Statistic	Prob > t
Constant	65.752	176.120	0.373	0.709
weight	-0.026	0.314	-0.081	0.935
length	-1.580	2.898	-0.545	0.586
breadth	0.706	4.051	0.174	0.862
shape index	8.242	127.461	0.065	0.949
t effective	-634.941	1573.295	-0.404	0.687
ND Stiff	0.118	0.020	6.022	0.000
tot thick	626.334	1573.587	0.398	0.691
mam thick	-626.446	1578.822	-0.397	0.692

Data File: STRAIN 1 Physical Data

Source	Sum of Squares	Deg. of Freedom	Mean Squares	F-Ratio	Prob>F
Model	5421.211	8	677.651	28.096	0.000
Error	3304.271	137	24.119		
Total	8725.482	145			

Coefficient of Determination (R^2)	0.621
Adjusted Coefficient (R^2)	0.599
Coefficient of Correlation (R)	0.788
Standard Error of Estimate	4.911
Durbin-Watson Statistic	1.785

Data File: STRAIN 1 Physical Data   Dependent Variable: Force

Variable Name	Coefficient	Std. Err. Estimate	t Statistic	Prob > t
Constant	65.001	172.713	0.376	0.707
weight	-0.016	0.308	-0.051	0.959
length	-1.584	2.837	-0.558	0.578
breadth	0.678	3.979	0.170	0.865
shape index	8.428	124.906	0.067	0.946
ND Stiff	0.112	0.014	8.030	0.000

Data File: STRAIN 1 Physical Data

Source	Sum of Squares	Deg. of Freedom	Mean Squares	F-Ratio	Prob>F
Model	5381.547	5	1076.309	45.620	0.000
Error	3350.198	142	23.593		
Total	8731.744	147			

Coefficient of Determination (R^2)	0.616
Adjusted Coefficient (R^2)	0.603
Coefficient of Correlation (R)	0.785
Standard Error of Estimate	4.857
Durbin-Watson Statistic	1.791

Data File: STRAIN 1 Physical Data Dependent Variable: Force

Variable Name	Coefficient	Std. Err. Estimate	t Statistic	Prob > t
Constant	76.385	36.830	2.074	0.040
weight	-0.013	0.303	-0.042	0.967
length	-1.394	0.333	-4.181	0.000
breadth	0.417	0.908	0.459	0.647
ND Stiff	0.112	0.014	8.058	0.000

Data File: STRAIN 1 Physical Data

Source	Sum of Squares	Deg. of Freedom	Mean Squares	F-Ratio	Prob>F
Model	5381.439	4	1345.360	57.424	0.000
Error	3350.305	143	23.429		
Total	8731.744	147			

Coefficient of Determination (R^2)	0.616
Adjusted Coefficient (R^2)	0.606
Coefficient of Correlation (R)	0.785
Standard Error of Estimate	4.840
Durbin-Watson Statistic	1.793

Data File: STRAIN 1 Physical Data   Dependent Variable: Force

Variable Name	Coefficient	Std. Err. Estimate	t Statistic	Prob > t
Constant	97.541	12.042	8.100	0.000
shape index	-63.618	8.324	-7.643	0.000
ND Stiff	0.113	0.014	7.787	0.000

Data File: STRAIN 1 Physical Data

Source	Sum of Squares	Deg. of Freedom	Mean Squares	F-Ratio	Prob>F
Model	4640.043	2	2320.021	82.216	0.000
Error	4091.702	145	28.219		
Total	8731.744	147			

Coefficient of Determination (R^2)	0.531
Adjusted Coefficient (R^2)	0.525
Coefficient of Correlation (R)	0.729
Standard Error of Estimate	5.312
Durbin-Watson Statistic	1.336

Data File: STRAIN 1 Physical Data Dependent Variable: Force

Variable Name	Coefficient	Std. Err. Estimate	t Statistic	Prob > t
Constant	38.242	4.822	7.932	0.000
weight	-0.486	0.065	-7.449	0.000
ND Stiff	0.139	0.014	9.936	0.000

Data File: STRAIN 1 Physical Data

Source	Sum of Squares	Deg. of Freedom	Mean Squares	F-Ratio	Prob>F
Model	4572.577	2	2286.288	80.251	0.000
Error	4159.449	146	28.489		
Total	8732.026	148			

Coefficient of Determination (R^2)	0.524
Adjusted Coefficient (R^2)	0.517
Coefficient of Correlation (R)	0.724
Standard Error of Estimate	5.338
Durbin-Watson Statistic	1.925

Data File: STRAIN 1 Physical Data   Dependent Variable: Force

Variable Name	Coefficient	Std. Err. Estimate	t Statistic	Prob > t
Constant	95.947	11.000	8.722	0.000
weight	-0.350	0.064	-5.467	0.000
shape index	-46.839	8.197	-5.714	0.000
ND Stiff	0.118	0.013	8.885	0.000

Data File: STRAIN 1 Physical Data

Source	Sum of Squares	Deg. of Freedom	Mean Squares	F-Ratio	Prob>F
Model	5343.377	3	1781.126	75.695	0.000
Error	3388.367	144	23.530		
Total	8731.744	147			

Coefficient of Determination (R^2)	0.612
Adjusted Coefficient (R^2)	0.604
Coefficient of Correlation (R)	0.782
Standard Error of Estimate	4.851
Durbin-Watson Statistic	1.790

Data File: STRAIN 2 Physical Data   Dependent Variable: force

Variable Name	Coefficient	Std. Err. Estimate	t Statistic	Prob > t
Constant	-136.502	78.203	-1.745	0.083
weight	-0.067	0.208	-0.323	0.747
length	-2.650	1.261	-2.101	0.037
breadth	4.000	1.761	2.271	0.025
shape index	98.161	55.045	1.783	0.077
t effective	-13.392	869.752	-0.015	0.988
ND stiff	0.144	0.017	8.251	0.000
tot thick	33.615	867.527	0.039	0.969
mam thick	-34.477	868.409	-0.040	0.968
Age	-3.647	0.583	-6.251	0.000

Data File: STRAIN 2 Physical Data

Source	Sum of Squares	Deg. of Freedom	Mean Squares	F-Ratio	Prob>F
Model	4626.905	9	514.101	38.462	0.000
Error	1871.317	140	13.367		
Total	6498.222	149			

Coefficient of Determination (R^2)	0.712
Adjusted Coefficient (R^2)	0.694
Coefficient of Correlation (R)	0.844
Standard Error of Estimate	3.656
Durbin-Watson Statistic	1.917

Data File: STRAIN 2 Physical Data Dependent Variable: force

Variable Name	Coefficient	Std. Err. Estimate	t Statistic	Prob > t
Constant	-47.645	86.662	-0.550	0.583
weight	0.089	0.233	0.382	0.703
length	-2.790	1.421	-1.964	0.051
breadth	2.613	1.969	1.327	0.187
shape index	67.042	61.779	1.085	0.280
t effective	-249.208	979.241	-0.254	0.799
ND stiff	0.140	0.020	7.127	0.000
tot thick	285.675	976.599	0.293	0.770
mam thick	-274.358	977.693	-0.281	0.779

Data File: STRAIN 2 Physical Data

Source	Sum of Squares	Deg. of Freedom	Mean Squares	F-Ratio	Prob>F
Model	4104.662	8	513.083	30.225	0.000
Error	2393.560	141	16.976		
Total	6498.222	149			

Coefficient of Determination (R^2)	0.632
Adjusted Coefficient (R^2)	0.611
Coefficient of Correlation (R)	0.795
Standard Error of Estimate	4.120
Durbin-Watson Statistic	1.703

Data File: STRAIN 2 Physical Data   Dependent Variable: force

Variable Name	Coefficient	Std. Err. Estimate	t Statistic	Prob > t
Constant	-57.948	86.946	-0.666	0.506
weight	0.063	0.232	0.273	0.785
length	-2.963	1.423	-2.082	0.039
breadth	2.944	1.973	1.492	0.138
shape index	77.269	61.915	1.248	0.214
ND stiff	0.165	0.016	10.585	0.000

Data File: STRAIN 2 Physical Data

Source	Sum of Squares	Deg. of Freedom	Mean Squares	F-Ratio	Prob>F
Model	4024.823	5	804.965	46.865	0.000
Error	2473.399	144	17.176		
Total	6498.222	149			

Coefficient of Determination (R^2)	0.619
Adjusted Coefficient (R^2)	0.606
Coefficient of Correlation (R)	0.787
Standard Error of Estimate	4.144
Durbin-Watson Statistic	1.734

Data File: STRAIN 2 Physical Data Dependent Variable: force

Variable Name	Coefficient	Std. Err. Estimate	t Statistic	Prob > t
Constant	44.531	28.632	1.555	0.122
weight	0.085	0.232	0.365	0.715
length	-1.235	0.327	-3.777	0.000
breadth	0.625	0.664	0.941	0.348
ND stiff	0.163	0.016	10.497	0.000

Data File: STRAIN 2 Physical Data

Source	Sum of Squares	Deg. of Freedom	Mean Squares	F-Ratio	Prob>F
Model	3998.072	4	999.518	57.969	0.000
Error	2500.150	145	17.242		
Total	6498.222	149			

Coefficient of Determination (R^2)	0.615
Adjusted Coefficient (R^2)	0.605
Coefficient of Correlation (R)	0.784
Standard Error of Estimate	4.152
Durbin-Watson Statistic	1.728

Data File: STRAIN 2 Physical Data   Dependent Variable: force

Variable Name	Coefficient	Std. Err. Estimate	t Statistic	Prob > t
Constant	69.828	11.593	6.024	0.000
shape index	-49.443	8.261	-5.985	0.000
ND stiff	0.170	0.016	10.620	0.000

Data File: STRAIN 2 Physical Data

Source	Sum of Squares	Deg. of Freedom	Mean Squares	F-Ratio	Prob>F
Model	3753.184	2	1876.592	100.494	0.000
Error	2745.038	147	18.674		
Total	6498.222	149			

Coefficient of Determination (R^2)	0.578
Adjusted Coefficient (R^2)	0.572
Coefficient of Correlation (R)	0.760
Standard Error of Estimate	4.321
Durbin-Watson Statistic	1.568

Data File: STRAIN 2 Physical Data   Dependent Variable: force

Variable Name	Coefficient	Std. Err. Estimate	t Statistic	Prob > t
Constant	17.383	3.939	4.413	0.000
weight	-0.220	0.046	-4.788	0.000
ND stiff	0.183	0.016	11.285	0.000

Data File: STRAIN 2 Physical Data

Source	Sum of Squares	Deg. of Freedom	Mean Squares	F-Ratio	Prob>F
Model	3544.824	2	1772.412	88.219	0.000
Error	2953.398	147	20.091		
Total	6498.222	149			

Coefficient of Determination (R^2)	0.546
Adjusted Coefficient (R^2)	0.539
Coefficient of Correlation (R)	0.739
Standard Error of Estimate	4.482
Durbin-Watson Statistic	1.778

Data File: STRAIN 2 Physical Data Dependent Variable: force

Variable Name	Coefficient	Std. Err. Estimate	t Statistic	Prob > t
Constant	67.979	11.239	6.049	0.000
weight	-0.149	0.045	-3.286	0.001
shape index	-40.333	8.466	-4.764	0.000
ND stiff	0.165	0.016	10.615	0.000

Data File: STRAIN 2 Physical Data

Source	Sum of Squares	Deg. of Freedom	Mean Squares	F-Ratio	Prob>F
Model	3942.185	3	1314.062	75.059	0.000
Error	2556.037	146	17.507		
Total	6498.222	149			

Coefficient of Determination (R^2)	0.607
Adjusted Coefficient (R^2)	0.599
Coefficient of Correlation (R)	0.779
Standard Error of Estimate	4.184
Durbin-Watson Statistic	1.746

APPENDIX 4

Physical Data, Eshell and Fracture Toughness Values for Strains 1 and 2: Beginning, Middle and End of Lay.

Strain 1 (24weeks)

	egg no	weight	length	breadth	shape index
1	1	53.12	56.0	40.8	1.37
2	5	55.88	55.7	42.6	1.31
3	6	55.07	52.4	43.6	1.20
4	8	56.18	56.6	42.3	1.34
5	9	53.70	54.5	42.4	1.28
6	10	55.20	56.4	41.9	1.35
7	11	49.09	53.6	40.2	1.33
8	12	54.85	55.2	41.8	1.32
9	13	53.54	54.2	41.7	1.30
10	14	64.46	57.7	44.7	1.29
11	15	56.36	56.6	42.5	1.33
12	16	49.63	54.3	40.5	1.34
13	18	56.15	54.4	42.8	1.27
14	19	56.36	55.1	42.1	1.31
15	20	52.93	54.0	41.7	1.29
16	23	58.94	56.7	42.5	1.33
17	24	53.62	54.7	41.7	1.31
18	25	56.31	53.7	43.0	1.25
19	26	57.19	54.8	42.6	1.29
20	29	51.05	53.6	41.0	1.31
21	30	56.37	56.0	42.0	1.33
22	31	56.14	54.8	42.6	1.29
23	34	51.59	55.0	41.0	1.34
24	36	53.47	54.8	41.8	1.31
25	38	58.25	56.9	42.3	1.34
26	39	46.74	53.0	39.7	1.33
27	41	55.68	54.4	42.6	1.28
28	43	47.30	51.0	40.4	1.26
29	45	50.95	53.4	41.4	1.29
30	47	51.15	55.0	41.0	1.34
31	48	58.98	57.5	42.8	1.34
32	51	56.36	54.0	42.9	1.26
33	54	54.67	53.9	42.3	1.27
34	55	55.61	54.4	42.8	1.27
35	56	53.50	53.6	42.0	1.28
36	59	55.92	54.0	43.0	1.26
37	62	52.58	54.0	41.7	1.29
38	63	57.75	58.2	42.0	1.39
39	66	51.40	52.9	41.7	1.27
40	67	50.47	52.4	41.2	1.27
41	69	51.12	53.7	41.6	1.29
42	72	53.52	54.0	42.0	1.29
43	74	52.29	53.1	42.1	1.26
44	76	51.29	54.3	41.0	1.32
45	78	56.35	54.4	42.9	1.27
46	80	56.03	56.3	42.2	1.33
47	81	53.51	54.5	41.9	1.30
48	86	53.39	53.4	42.4	1.26
49	88	57.44	55.7	42.9	1.30
50	90	55.46	54.0	43.0	1.26

Strain 1 (24weeks)

	Force	stiffness	ND Stiff	tot thick	mam thick
1			220.0		
2	40.8	165.9	170.0	0.335	0.056
3	42.3	190.5	199.0	0.352	0.073
4	29.4	172.9	179.0	0.332	0.071
5	33.5	158.8	140.0	0.325	0.080
6	33.0	162.6	174.0	0.310	0.081
7	33.0	177.4	179.0	0.332	0.066
8	47.0	217.6	200.0	0.316	0.062
9	34.7	164.5	179.0	0.355	0.072
10	45.1	197.8	189.0	0.351	0.070
11	34.0	167.5	155.0	0.320	0.073
12	37.4	174.0	175.0	0.332	0.067
13	45.3	207.8	181.0	0.347	0.067
14	53.7	246.3	227.0	0.393	0.077
15	43.1	195.9	195.0	0.398	0.074
16	50.2	231.3	272.0	0.325	0.064
17	41.7	214.9	203.0	0.353	0.077
18	37.5	190.4	147.0	0.422	0.085
19	32.7	179.7	171.0	0.343	0.066
20	42.4	161.2	199.0	0.339	0.082
21	30.0	144.9	159.0	0.369	0.071
22	31.1	152.5	162.0	0.335	0.070
23	34.7	168.4	164.0	0.335	0.072
24	38.9	164.8	180.0	0.316	0.062
25	35.1	151.9	188.0	0.371	0.092
26	37.2	166.8	145.0	0.318	0.072
27	38.3	155.7	167.0	0.349	0.072
28	37.5	228.7	186.0	0.350	0.067
29	33.3	163.2	181.0	0.336	
30	40.7	190.2	172.0	0.356	0.081
31	41.4	187.3	181.0	0.360	0.083
32	38.7	207.0	181.0	0.353	0.073
33	36.4	182.0	157.0	0.343	0.081
34	36.7	181.7	181.0	0.342	0.079
35	35.5	189.8	181.0	0.336	0.071
36	36.3	139.6	153.0	0.328	0.068
37	32.3	173.7	153.0	0.319	0.065
38	35.7	198.3	168.0	0.317	0.057
39	39.8	170.8	164.0	0.327	0.066
40	44.1	176.4	216.0	0.358	0.080
41	42.9	178.0	172.0	0.339	0.068
42	41.6	191.7	177.0	0.346	0.075
43	42.6	242.0	203.0	0.368	0.071
44	38.9	192.6	192.0	0.360	0.066
45	43.1	189.0	192.0	0.364	0.086
46	34.4	237.2	203.0	0.327	0.064
47	40.4	192.4	181.0	0.343	0.064
48	37.4	185.1	172.0	0.327	0.068
49	34.9	178.1	150.0		
50	30.4	192.4	172.0	0.320	0.074

Strain 1 (24weeks)

	Kc [6mm]	ND Eshell	t effective
1			
2	707.1	2.66e+4	0.279
3	727.0	2.97e+4	0.279
4	565.0	3.19e+4	0.261
5	707.3	2.75e+4	0.245
6	774.2	3.98e+4	0.229
7	629.0	2.93e+4	0.266
8	944.7	3.97e+4	0.254
9	594.0	2.66e+4	0.283
10	759.0	3.02e+4	0.281
11	708.6	3.10e+4	0.247
12	714.6	2.93e+4	0.265
13	779.6	2.79e+4	0.280
14	775.9	2.78e+4	0.316
15	602.3	2.23e+4	0.324
16	963.1	4.84e+4	0.261
17	741.2	3.18e+4	0.276
18	487.9	1.56e+4	0.337
19	572.9	2.69e+4	0.277
20	844.2	3.51e+4	0.257
21	473.7	2.18e+4	0.298
22	582.3	2.73e+4	0.265
23	667.4	2.80e+4	0.263
24	781.9	3.30e+4	0.254
25	610.3	2.98e+4	0.279
26	801.0	2.74e+4	0.246
27	671.0	2.59e+4	0.277
28	649.8	2.62e+4	0.283
29			
30	732.1	2.71e+4	0.275
31	724.1	2.94e+4	0.277
32	665.4	2.76e+4	0.280
33	695.5	2.67e+4	0.262
34	693.8	3.13e+4	0.263
35	668.5	3.04e+4	0.265
36	697.0	2.69e+4	0.260
37	650.3	2.79e+4	0.254
38	691.7	3.07e+4	0.260
39	769.3	2.80e+4	0.261
40	778.5	3.22e+4	0.278
41	784.3	2.78e+4	0.271
42	757.5	2.84e+4	0.271
43	675.5	2.72e+4	0.297
44	633.0	2.63e+4	0.294
45	749.1	2.97e+4	0.278
46	654.1	3.56e+4	0.263
47	704.8	2.78e+4	0.279
48	726.5	3.01e+4	0.259
49			
50	634.2	3.36e+4	0.246

Strain 1 (47weeks)

		egg^{no}	weight	length	breadth	shape index
1	47wks	101	59.91	57.5	43.6	1.32
2		102	59.97	58.3	42.7	1.36
3		103	54.35	58.0	42.3	1.37
4		104	58.73	56.9	43.4	1.31
5		105	63.05	59.8	43.4	1.38
6		106	63.92	61.0	42.9	1.42
7		107	70.50	64.0	44.3	1.45
8		108	63.52	57.0	44.0	1.29
9		110	63.96	60.6	43.4	1.40
10		111	62.13	58.3	43.4	1.34
11		112	68.70	59.3	45.5	1.30
12		114	64.08	58.7	44.4	1.32
13		116	64.77	58.4	44.2	1.32
14		117	62.38	58.4	44.4	1.31
15		119	56.77	57.8	41.2	1.40
16		120	66.18	59.0	44.5	1.33
17		121	64.29	59.8	44.5	1.34
18		123	67.77	59.0	45.0	1.31
19		124	56.01	54.6	43.0	1.27
20		125	60.22	59.0	42.7	1.38
21		126	62.91	57.0	44.0	1.29
22		127	66.08	61.8	44.0	1.41
23		129	70.46	58.6	46.6	1.26
24		130	59.29	57.0	43.0	1.33
25		131	62.97	57.6	44.3	1.30
26		132	65.22	60.0	44.0	1.36
27		133	64.42	58.0	44.9	1.29
28		134	59.10	57.7	42.0	1.37
29		137	68.01	61.5	44.7	1.38
30		138	63.97	58.4	43.8	1.33
31		139	63.86	62.4	42.7	1.46
32		140	70.79	58.8	46.5	1.26
33		141	69.37	61.5	45.0	1.37
34		142	69.11	59.0	45.7	1.29
35		143	68.60	56.9	46.0	1.24
36		144	65.69	58.3	45.4	1.28
37		145	61.60	56.3	43.9	1.28
38		146	70.58	61.0	45.6	1.34
39		147	64.83	59.3	43.8	1.35
40		148	60.39	55.4	44.2	1.25
41		149	67.89	58.6	45.5	1.29
42		152	64.14	59.8	43.6	1.37
43		153	63.82	58.6	43.7	1.34
44		154	54.40	57.3	42.6	1.34
45		156	65.57	57.2	44.7	1.28
46		157	69.21	61.9	44.4	1.39
47		158	53.33	54.9	41.6	1.32
48		160	60.63	56.8	43.8	1.30
49		161	62.94	59.3	43.6	1.36
50		162	66.20			

Strain 1 (47weeks)

	Force	stiffness	ND Stiff	tot thick	mam thick
1	38.5	219.8	184.0	0.358	0.071
2	40.0	232.6	216.0	0.367	0.070
3	34.6	233.9	192.0	0.358	0.070
4	39.7	205.9	188.0	0.350	0.066
5	31.3	211.4	133.0	0.277	0.061
6	28.7	217.6	144.0	0.280	0.061
7	34.1	232.0	206.0	0.374	0.078
8	37.7	279.2	220.0	0.340	0.082
9	32.6	210.1	179.0	0.363	0.074
10	27.2	141.9	148.0	0.305	0.042
11	40.5	204.5	212.0	0.372	0.053
12	35.2	198.0	192.0	0.373	0.067
13	38.8	189.0	175.0	0.315	0.063
14	23.2	110.7	114.0	0.283	0.049
15	29.8	185.9	188.0	0.367	0.070
16	27.8	127.9	148.0	0.286	0.054
17	38.0	207.6	188.0	0.357	0.071
18	26.8	169.3	162.0	0.315	0.060
19	29.2	156.4	144.0	0.287	0.067
20	30.0	159.6	241.0	0.396	0.089
21	43.5	241.7	136.0	0.274	0.060
22	37.5	200.5	170.0	0.331	0.052
23	40.8	191.3	192.0	0.367	0.060
24	38.5	190.6	181.0	0.328	0.062
25	29.4	159.2	212.0	0.414	0.087
26	27.5	180.7	162.0	0.326	0.060
27	36.6	231.8	188.0	0.359	0.082
28	29.9	209.4	199.0	0.325	0.067
29	32.0	200.0	162.0	0.337	0.082
30	31.2	201.2	126.0	0.280	0.062
31	19.8	118.6	110.0	0.347	0.064
32	39.1	186.2	188.0	0.326	0.081
33	29.2	184.9	152.0	0.298	0.069
34	30.7	204.6	188.0	0.354	0.062
35	36.9	177.3	179.0	0.345	0.064
36	31.2	146.4	162.0	0.343	0.069
37	37.1	212.2	212.0	0.365	0.079
38	30.7	155.0	157.0	0.292	0.060
39	22.8	186.6	212.0	0.385	0.066
40	40.1	222.7	188.0	0.326	0.082
41	29.4	157.5	131.0	0.297	0.056
42	26.5	170.8	188.0	0.377	0.081
43	39.6	182.5	159.0	0.332	0.065
44	31.4	190.5	172.0	0.322	0.065
45	49.0	314.2	272.0	0.429	0.070
46	31.4	210.9	199.0	0.370	0.065
47	37.6	203.4	188.0	0.354	0.069
48	35.4	249.2	167.0	0.326	0.071
49	27.0	214.1	192.0	0.358	0.070
50	32.0	200.0	175.0	0.329	0.079

Strain 1 (47weeks)

	Kc [6mm]	ND Eshell	t effective
1	630.9	2.80e+4	0.288
2	627.5	3.05e+4	0.298
3	573.7	2.88e+4	0.288
4	669.0	2.92e+4	0.283
5	791.0	3.55e+4	0.216
6	713.4	3.77e+4	0.219
7	532.7	3.16e+4	0.296
8	726.2	4.15e+4	0.258
9	532.3	3.37e+4	0.289
10	511.6	2.67e+4	0.263
11	560.1	2.71e+4	0.319
12	522.7	2.63e+4	0.306
13	772.2	3.46e+4	0.252
14	511.9	2.60e+4	0.235
15	476.4	2.64e+4	0.297
16	620.7	3.42e+4	0.233
17	623.9	2.97e+4	0.286
18	520.4	3.23e+4	0.255
19	720.3	3.50e+4	0.220
20	450.1	3.25e+4	0.307
21	1109.2	3.63e+4	0.214
22	642.3	2.85e+4	0.279
23	591.9	2.63e+4	0.307
24	714.4	3.16e+4	0.266
25	395.5	2.52e+4	0.327
26	506.0	2.92e+4	0.266
27	624.9	3.10e+4	0.278
28	586.1	3.67e+4	0.258
29	626.7	3.26e+4	0.254
30	775.0	3.29e+4	0.218
31	335.6	1.78e+4	0.283
32	796.4	4.00e+4	0.245
33	666.3	3.74e+4	0.229
34	484.0	2.86e+4	0.292
35	614.6	2.85e+4	0.281
36	539.6	2.72e+4	0.275
37	612.8	3.20e+4	0.286
38	684.0	3.79e+4	0.232
39	320.0	2.68e+4	0.319
40	842.8	3.85e+4	0.243
41	619.2	3.42e+4	0.241
42	416.8	2.76e+4	0.296
43	726.4	2.80e+4	0.267
44	615.6	3.20e+4	0.257
45	571.1	2.70e+4	0.359
46	470.8	2.83e+4	0.304
47	637.4	2.78e+4	0.285
48	695.1	3.17e+4	0.255
49	442.4	2.94e+4	0.288
50			0.250

Strain 1 (69weeks)

	egg no	weight	length	breadth	shape index
1	201	67.68	59.2	45.1	1.31
2	202	70.69	64.6	45.0	1.44
3	203	59.92	58.9	43.4	1.36
4	204	60.65	58.3	43.3	1.35
5	205	63.14	61.1	43.5	1.41
6	206	62.92	59.0	43.9	1.34
7	207	62.04	61.0	42.4	1.44
8	208	69.49	65.5	43.6	1.50
9	209	70.40	59.8	45.9	1.30
10	210	70.11	59.4	46.0	1.29
11	211	63.42	60.5	43.4	1.39
12	212	69.50	60.5	45.3	1.34
13	213	68.52	60.9	44.8	1.36
14	214	67.10	60.2	44.7	1.35
15	215	62.48	59.0	44.2	1.33
16	216	67.70	60.0	45.0	1.33
17	217	72.38	63.1	45.0	1.40
18	223	66.16	60.4	44.5	1.36
19	224	65.59	60.8	43.9	1.39
20	225	63.17	60.2	43.4	1.39
21	226	68.00	60.8	44.7	1.36
22	227	73.13	62.0	45.6	1.36
23	228	63.75	68.4	44.9	1.52
24	230	64.92	58.1	44.8	1.30
25	233	65.79	62.4	43.0	1.45
26	234	67.57	59.8	45.0	1.33
27	235	64.44	60.6	44.9	1.35
28	236	73.17	64.9	45.2	1.44
29	237	76.26	64.6	46.9	1.38
30	238	57.89	56.7	42.6	1.33
31	239	71.85	61.0	46.3	1.32
32	240	68.46	57.6	46.2	1.25
33	241	60.92	62.0	42.1	1.47
34	242	69.43	61.4	45.0	1.36
35	243	69.76	59.1	46.0	1.28
36	244	63.51	57.4	44.3	1.30
37	245	73.92	61.0	46.6	1.31
38	246	74.06	64.5	45.5	1.42
39	247	69.92	62.3	44.8	1.39
40	248	66.91	58.7	45.6	1.29
41	250	64.03	58.5	44.3	1.32
42	252	61.90	58.4	44.4	1.31
43	253	72.41	62.0	46.2	1.34
44	254	73.69	65.3	45.7	1.43
45	255	57.35	57.7	42.1	1.37
46	256	67.86	60.5	44.6	1.36
47	257	63.22	60.0	43.9	1.37
48	258	68.97	60.3	45.0	1.34
49	260	69.35	61.6	44.4	1.39
50	261	65.55	58.8	44.8	1.31

Strain 1 (69weeks)

	Force	stiffness	ND Stiff	tot thick	mam thick
1	35.0	213.4	199.0	0.377	0.067
2	26.8	202.6	172.0	0.355	0.075
3	20.5	136.7	159.0	0.311	0.065
4	32.2	177.2	164.0	0.326	0.062
5	19.0	126.7	108.0	0.263	0.053
6	29.0	179.0	167.0	0.257	0.073
7	25.8	214.6	206.0	0.344	0.061
8	20.8	178.9	164.0	0.336	0.071
9	26.0	160.5	134.0	0.326	0.050
10	22.0	159.4	181.0	0.356	0.075
11	26.8	173.7	175.0	0.326	0.079
12	31.5	185.3	152.0	0.299	0.076
13	25.5	153.6	145.0	0.320	0.084
14	28.0	162.8	184.0	0.375	0.063
15	19.2	106.9	157.0	0.327	0.057
16	35.5	216.5	212.0	0.368	0.076
17	16.8	299.1	188.0	0.306	0.068
18	33.5	188.2	188.0	0.343	0.071
19	31.0	218.3	203.0	0.381	0.079
20	21.0	140.0	136.0	0.328	0.064
21	32.5	222.6	184.0	0.358	0.079
22	37.2	229.9	220.0	0.390	0.071
23	14.0	111.1	84.0	0.250	0.070
24	29.8	181.4	195.0	0.374	0.079
25	17.0	96.6	138.0	0.272	0.065
26	30.0	202.7	203.0	0.369	0.063
27	16.8	102.1	109.0	0.317	0.069
28	26.2	201.9	189.0	0.353	0.062
29	19.2	152.8	150.0	0.325	0.057
30	23.8	133.4	159.0	0.319	0.073
31	28.5	163.8	220.0	0.375	0.053
32	36.2	235.4	241.0	0.373	0.051
33	15.5	107.6	96.0	0.286	0.054
34	26.2	198.9	175.0	0.360	0.056
35	21.8	149.0	126.0	0.286	0.068
36	38.0	243.6	225.0	0.369	0.059
37	34.5	233.1	216.0	0.403	0.051
38	27.5	202.2	134.0	0.337	0.064
39	22.8	177.7	159.0	0.351	0.059
40	29.5	210.7	179.0	0.351	0.073
41	24.5	200.8	170.0	0.336	0.079
42	19.5	121.9	104.0	0.286	0.095
43	26.5	200.7	138.0	0.311	0.062
44	23.0	188.5	152.0	0.338	0.055
45	20.8	172.9	152.0	0.332	0.049
46	32.5	239.0	212.0	0.377	0.076
47	22.5	144.2	164.0	0.320	0.060
48	24.5	200.8	184.0	0.344	0.059
49	17.5	132.6	108.0	0.295	0.063
50	38.0	243.6	241.0	0.415	0.059

Strain 1 (69weeks)

	Kc [6mm]	ND Eshell	t effective
1	506.6	2.68e+4	0.310
2	452.3	2.96e+4	0.280
3	426.3	3.28e+4	0.246
4	602.8	2.94e+4	0.264
5	500.5	3.09e+4	0.210
6	928.2	6.06e+4	0.184
7	438.8	3.30e+4	0.283
8	386.2	3.12e+4	0.265
9	445.2	2.28e+4	0.276
10	366.4	2.98e+4	0.281
11	553.9	3.65e+4	0.247
12	746.6	3.91e+4	0.223
13	557.1	3.36e+4	0.236
14	402.8	2.47e+4	0.312
15	344.6	2.73e+4	0.270
16	562.6	3.22e+4	0.292
17	361.8	4.37e+4	0.238
18	593.0	3.29e+4	0.272
19	471.9	2.90e+4	0.302
20	392.8	2.49e+4	0.264
21	552.9	3.08e+4	0.279
22	514.0	2.90e+4	0.319
23	458.8	3.48e+4	0.180
24	465.8	2.84e+4	0.295
25	459.5	4.09e+4	0.207
26	443.2	2.82e+4	0.306
27	340.4	2.28e+4	0.248
28	416.6	3.05e+4	0.291
29	340.6	2.85e+4	0.268
30	498.2	3.19e+4	0.246
31	385.9	2.83e+4	0.322
32	490.6	3.42e+4	0.322
33	356.0	2.25e+4	0.232
34	390.9	2.51e+4	0.304
35	531.3	3.36e+4	0.218
36	553.8	2.96e+4	0.310
37	407.7	2.34e+4	0.352
38	480.4	2.43e+4	0.273
39	361.9	2.47e+4	0.292
40	501.1	2.96e+4	0.278
41	473.0	3.24e+4	0.257
42	587.1	3.53e+4	0.191
43	528.2	2.93e+4	0.249
44	380.0	2.59e+4	0.283
45	354.6	2.35e+4	0.283
46	493.8	3.05e+4	0.301
47	428.8	3.10e+4	0.260
48	402.6	2.95e+4	0.285
49	393.6	2.58e+4	0.232
50	448.1	2.48e+4	0.356

Strain 2 (24weeks)

	egg no	weight	length	breadth	shape index
1	1	54.54	53.8	42.4	1.27
2	3	59.09	56.4	43.3	1.30
3	4	53.18	54.7	41.6	1.31
4	8	53.17	53.6	42.0	1.28
5	10	54.64	53.6	42.7	1.25
6	12	56.63	54.4	43.0	1.26
7	13	56.18	54.9	42.6	1.29
8	15	56.18	54.4	42.4	1.28
9	16	56.88	55.7	42.5	1.31
10	17	55.51	54.6	42.9	1.27
11	18	54.45	51.7	40.8	1.27
12	19	59.87	55.2	43.8	1.26
13	20	48.36	54.7	42.0	1.30
14	22	45.88	52.0	39.9	1.30
15	27	59.88	55.8	43.6	1.28
16	28	55.20	55.0	42.0	1.31
17	29	50.65	53.8	41.0	1.31
18	30	52.30	54.1	41.7	1.30
19	31	57.06	55.4	43.0	1.29
20	33	57.05	56.3	43.0	1.31
21	35	57.98	55.0	43.5	1.26
22	37	53.27	53.9	42.0	1.28
23	38	54.63	54.2	42.5	1.27
24	39	53.84	54.8	41.8	1.31
25	40	54.73	54.9	42.8	1.28
26	41	55.30	53.6	42.6	1.26
27	42	54.76	56.0	43.0	1.30
28	43	58.21	53.6	42.8	1.25
29	45	55.41	54.0	42.8	1.26
30	46	59.04	56.0	43.7	1.28
31	47	51.43	53.6	42.0	1.28
32	50	51.18	53.2	41.8	1.27
33	51	48.30	51.4	41.0	1.25
34	53	50.79	53.0	41.4	1.28
35	57	48.04	51.3	40.9	1.25
36	59	48.88	52.8	41.2	1.28
37	60	50.64	53.0	41.3	1.28
38	61	50.51	51.2	42.3	1.21
39	62	51.37	52.0	42.3	1.23
40	63	58.00	54.7	43.8	1.25
41	66	62.31	57.6	44.1	1.31
42	71	61.15	56.5	44.0	1.28
43	72	59.19	56.8	43.4	1.31
44	74	51.61	52.5	42.0	1.25
45	75	50.49	51.7	41.9	1.23
46	76	51.67	52.4	42.0	1.25
47	78	51.81	54.3	41.4	1.31
48	79	55.40	54.3	43.0	1.26
49	81	52.12	52.8	42.3	1.25
50	82	73.68	60.7	46.7	1.30

Strain 2 (24weeks)

	force	stiffness	ND stiff	tot thick	mam thick
1	34.9	154.4	161.3	0.323	0.064
2	33.0	138.1	135.1	0.293	0.059
3	38.8	172.4	147.1	0.304	0.067
4	31.9	153.4	142.9	0.296	0.058
5	29.6	124.9	138.9	0.291	0.062
6	35.7	141.7	135.1	0.296	0.059
7	32.0	144.1	135.1	0.317	0.063
8	38.7	170.5	185.2	0.326	0.069
9	40.5	198.5	138.9	0.344	0.066
10	31.2	150.7	172.4	0.309	0.071
11	36.6	140.8	147.1	0.317	0.057
12	35.0	144.6	128.2	0.304	0.055
13	37.6	155.4	142.9	0.322	0.058
14	22.3	94.9	83.3	0.242	0.073
15	39.7	158.8	156.2	0.342	0.060
16	31.8	155.1	135.1	0.299	0.060
17	29.1	118.8	119.0	0.270	0.057
18	34.2	117.1	122.0	0.276	0.052
19	27.6	123.8	142.9	0.313	0.065
20	24.2	136.7	147.1	0.306	0.066
21	44.6	187.4	161.3	0.311	0.061
22	38.7	162.6	151.5	0.300	0.061
23	29.8	153.6	142.9	0.330	0.063
24	35.0	175.9	151.5	0.314	0.063
25	30.6	126.4	125.0	0.282	0.051
26	32.1	142.7	135.1	0.266	0.060
27	30.1	128.1	125.0	0.289	0.053
28	35.6	148.3	138.9	0.305	0.066
29	37.2	154.4	142.9	0.302	0.065
30	40.4	174.9	156.2	0.327	0.058
31	32.8	132.8	122.0	0.274	0.058
32	32.5	134.3	151.5	0.295	0.056
33	32.3	150.2	151.5	0.296	0.054
34	21.4	123.0	142.9	0.282	0.054
35	31.9	147.7	151.5	0.297	0.073
36	21.8	86.5	76.9	0.296	0.059
37	31.4	166.1	147.1	0.306	0.064
38	29.4	122.5	125.0	0.291	0.060
39	39.1	167.8	156.2	0.317	0.055
40	30.9	135.5	131.6	0.295	0.073
41	39.8	188.6	156.2	0.307	0.063
42	20.3	105.7	104.2	0.267	0.065
43	37.4	174.8	151.5	0.293	0.057
44	33.7	130.6	138.9	0.302	0.064
45	33.0	139.8	131.6	0.331	0.055
46	32.8	139.0	138.9	0.299	0.063
47	29.4	129.5	122.0	0.272	0.057
48	37.4	147.8	166.7	0.309	0.065
49	31.8	153.6	142.9	0.295	0.060
50	36.4	137.4	138.9	0.318	0.066

Strain 2 (24weeks)

	Kc	ND Eshell	t effective
1	681.9	2.85e+4	0.258
2	740.3	2.94e+4	0.234
3	867.4	3.08e+4	0.237
4	705.8	2.93e+4	0.238
5	689.3	3.08e+4	0.229
6	787.6	2.84e+4	0.237
7	638.5	2.52e+4	0.254
8	760.5	3.32e+4	0.257
9	706.9	2.19e+4	0.278
10	684.6	3.58e+4	0.238
11	721.5	2.49e+4	0.259
12	712.2	2.49e+4	0.249
13	712.0	2.44e+4	0.264
14	841.3	3.21e+4	0.169
15	671.4	2.40e+4	0.282
16	694.8	2.80e+4	0.240
17	767.9	3.04e+4	0.213
18	831.4	2.83e+4	0.224
19	568.9	2.81e+4	0.248
20	523.9	3.09e+4	0.240
21	904.5	3.09e+4	0.250
22	850.8	3.10e+4	0.239
23	552.6	2.38e+4	0.267
24	716.1	2.85e+4	0.251
25	702.8	2.78e+4	0.231
26	876.9	3.68e+4	0.206
27	668.3	2.73e+4	0.236
28	776.9	2.82e+4	0.239
29	822.1	2.99e+4	0.237
30	732.8	2.63e+4	0.269
31	839.3	3.05e+4	0.216
32	715.7	3.07e+4	0.239
33	703.8	2.91e+4	0.242
34	507.8	3.13e+4	0.228
35	776.0	3.34e+4	0.225
36	488.9	1.57e+4	0.237
37	682.0	2.91e+4	0.242
38	678.6	2.63e+4	0.231
39	747.1	3.60e+4	0.262
40	746.9	3.16e+4	0.222
41	838.0	3.28e+4	0.243
42	564.4	3.08e+4	0.202
43	827.6	3.32e+4	0.236
44	745.6	2.81e+4	0.238
45	585.1	1.98e+4	0.276
46	734.9	2.85e+4	0.236
47	761.9	3.09e+4	0.215
48	789.9	3.31e+4	0.244
49	715.3	2.98e+4	0.235
50	709.5	2.83e+4	0.252

Strain 2 (47weeks)

	egg no	weight	length	breadth	shape index
1	101	69.96	60.1	45.9	1.31
2	102	62.02	56.5	44.4	1.27
3	103	68.84	61.1	44.9	1.36
4	105	65.45	61.8	43.3	1.43
5	106	67.77	60.0	45.1	1.33
6	107	69.21	59.6	45.7	1.34
7	108	70.21	60.8	45.4	1.30
8	110	68.49	58.8	45.7	1.29
9	111	70.03	60.0	46.0	1.30
10	112	70.42	59.5	45.8	1.30
11	113	66.68	57.4	45.4	1.26
12	114	74.39	62.6	46.2	1.35
13	115	58.54	57.3	42.7	1.34
14	116	65.32	59.6	44.6	1.34
15	118	68.74	60.4	45.0	1.34
16	120	66.78	59.2	45.0	1.32
17	121	67.89	60.1	44.9	1.34
18	122	65.19	56.5	45.2	1.25
19	123	60.89	57.8	43.9	1.32
20	124	69.10	59.8	45.8	1.31
21	125	72.19	62.0	45.8	1.35
22	126	66.81	59.0	44.9	1.31
23	127	62.19	59.0	43.6	1.35
24	128	70.14	57.8	47.3	1.22
25	129	70.60	62.0	45.0	1.38
26	132	66.28	60.1	44.3	1.36
27	133	64.98	59.5	44.6	1.33
28	134	61.47	57.0	44.3	1.29
29	135	61.12	56.4	43.8	1.29
30	136	72.30	60.0	46.8	1.28
31	137	67.54	58.0	46.0	1.26
32	138	57.98	56.3	43.3	1.30
33	139	70.45	58.5	46.4	1.26
34	140	66.63	58.4	45.0	1.30
35	143	64.93	57.8	44.8	1.29
36	145	57.51	57.3	45.0	1.27
37	146	67.76	60.0	41.7	1.44
38	147	60.55	58.2	43.4	1.34
39	148	61.28	58.3	43.9	1.33
40	149	71.53	59.6	46.3	1.29
41	150	61.39	58.4	43.4	1.35
42	151	60.20	57.6	43.9	1.31
43	152	66.72	61.2	44.4	1.38
44	153	68.45	57.2	46.4	1.23
45	154	61.14	56.4	43.9	1.28
46	155	58.34	57.8	42.4	1.36
47	156	62.82	58.0	44.4	1.31
48	157	67.50	58.8	45.0	1.31
49	158	83.04	62.6	49.0	1.28
50	159	62.54	58.0	44.0	1.32

Strain 2 (47weeks)

	force	stiffness	ND stiff	tot thick	mam thick
1	25.5	137.8	147.1	0.308	0.050
2	24.3	147.9	125.0	0.317	0.096
3	27.4	148.5	131.6	0.316	0.073
4	33.2	170.1	172.4	0.339	0.089
5	35.9	169.3	161.3	0.298	0.072
6	31.4	171.7	125.0	0.331	0.067
7	25.0	123.8	166.7	0.329	0.069
8	27.7	125.9	122.0	0.293	0.060
9	23.9	145.6	131.6	0.299	0.073
10	24.6	153.0	151.5	0.306	0.068
11	32.8	236.2	161.3	0.264	0.046
12	24.1	151.8	125.0	0.284	0.063
13	21.4	138.9	142.9	0.331	0.065
14	19.6	109.8	94.3	0.254	0.048
15	25.6	114.9	104.2	0.271	0.058
16	31.8	168.5	142.9	0.314	0.054
17	16.4	99.5	104.2	0.283	0.077
18	25.0	135.1	135.1	0.280	0.063
19	28.7	134.7	135.1	0.270	0.069
20	24.7	124.7	111.1	0.249	0.059
21	26.0	148.6	151.5	0.322	0.061
22	28.2	148.4	142.9	0.312	0.078
23	21.5	130.3	119.0	0.334	0.055
24	25.0	120.2	111.1	0.260	0.040
25	27.0	150.8	131.6	0.346	0.100
26	28.0	120.7	125.0	0.283	0.056
27	22.5	91.1	104.2	0.261	0.037
28	27.0	130.4	119.0	0.338	0.056
29	31.0	180.2	161.3	0.302	0.042
30	33.5	158.0	142.9	0.321	0.041
31	34.4	204.5	166.7	0.308	0.052
32	32.8	161.7	138.9	0.278	0.034
33	34.4	174.4	151.5	0.316	0.044
34	22.8	150.1	151.5	0.305	0.046
35	21.8	99.0	94.3	0.249	0.054
36	24.0	160.1	161.3	0.317	0.030
37	18.6	132.6	131.6	0.281	0.034
38	23.2	139.2	122.0	0.341	0.079
39	22.3	127.9	111.1	0.324	0.078
40	31.8	135.1	122.0	0.292	0.049
41	29.0	182.4	151.5	0.331	0.064
42	28.5	114.9	116.3	0.266	0.041
43	20.8	107.0	111.1	0.329	0.054
44	33.0	201.2	172.4	0.339	0.076
45	24.2	128.0	111.1	0.278	0.046
46	21.0	106.6	108.7	0.272	0.058
47	37.2	178.2	151.5	0.327	0.054
48	34.8	175.5	147.1	0.328	0.050
49	26.0	125.6	122.0	0.284	0.039
50	28.8	137.6	122.0	0.355	0.072

Strain 2 (47weeks)

	Kc	ND Eshell	t effective
1	483.2	2.88e+4	0.258
2	587.9	3.11e+4	0.221
3	572.4	2.88e+4	0.243
4	674.4	3.54e+4	0.250
5	834.8	4.02e+4	0.226
6	575.8	2.31e+4	0.264
7	470.3	3.20e+4	0.260
8	612.6	2.84e+4	0.233
9	551.9	3.30e+4	0.226
10	526.5	3.44e+4	0.238
11	803.7	4.19e+4	0.218
12	574.5	3.36e+4	0.221
13	398.1	2.49e+4	0.266
14	526.0	2.79e+4	0.206
15	651.2	2.92e+4	0.213
16	599.8	2.69e+4	0.260
17	439.0	3.10e+4	0.206
18	617.3	3.53e+4	0.217
19	804.6	4.09e+4	0.201
20	741.2	3.92e+4	0.190
21	484.6	2.93e+4	0.261
22	623.5	3.29e+4	0.234
23	369.5	1.94e+4	0.279
24	594.7	2.87e+4	0.220
25	553.3	2.84e+4	0.246
26	651.2	3.07e+4	0.227
27	532.5	2.62e+4	0.224
28	453.5	1.86e+4	0.282
29	591.2	2.92e+4	0.260
30	557.0	2.38e+4	0.280
31	658.9	3.21e+4	0.256
32	691.0	2.83e+4	0.244
33	599.5	2.60e+4	0.272
34	432.5	2.85e+4	0.259
35	634.1	3.05e+4	0.195
36	390.3	2.46e+4	0.287
37	390.5	2.68e+4	0.247
38	438.9	2.22e+4	0.262
39	461.7	2.29e+4	0.246
40	656.9	2.65e+4	0.243
41	533.3	2.66e+4	0.267
42	674.6	2.82e+4	0.225
43	362.5	1.91e+4	0.275
44	601.4	3.10e+4	0.264
45	547.1	2.51e+4	0.232
46	543.1	2.91e+4	0.214
47	655.5	2.57e+4	0.273
48	593.7	2.43e+4	0.278
49	519.1	2.73e+4	0.245
50	482.9	1.93e+4	0.283

Strain 2 (67weeks)

	egg no	weight	length	breadth	shape index
1	201	84.79	63.2	49.7	1.27
2	202	69.00	61.4	45.0	1.36
3	203	64.18	58.0	44.4	1.31
4	204	64.16	58.5	45.0	1.30
5	205	71.65	62.2	45.4	1.37
6	206	59.43	54.5	44.4	1.23
7	207	55.23	56.0	42.5	1.32
8	208	67.90	59.0	45.1	1.31
9	209	69.69	59.2	46.1	1.28
10	210	62.02	57.6	44.0	1.31
11	211	73.39	61.8	46.5	1.33
12	212	65.01	60.7	43.6	1.39
13	213	69.42	61.4	45.0	1.36
14	214	66.51	62.0	44.0	1.41
15	215	70.30	59.4	46.0	1.29
16	216	59.02	57.4	42.9	1.34
17	217	62.35	58.7	43.8	1.34
18	218	62.80	57.3	44.6	1.28
19	219	75.81	64.4	46.0	1.40
20	220	74.34	61.7	47.0	1.31
21	221	69.13	61.7	45.4	1.36
22	223	63.13	59.4	43.7	1.36
23	225	89.39	64.5	50.5	1.28
24	226	56.38	58.6	42.0	1.40
25	227	69.15	60.7	45.0	1.35
26	228	61.26	56.2	44.0	1.28
27	229	72.91	61.3	47.0	1.30
28	230	70.67	61.6	45.8	1.34
29	231	73.35	63.0	45.7	1.38
30	232	72.74	62.0	46.0	1.35
31	233	73.05	63.0	45.6	1.38
32	234	73.32	62.0	46.2	1.34
33	235	69.55	60.8	45.7	1.33
34	236	70.45	59.3	46.2	1.28
35	237	70.79	60.2	46.5	1.29
36	239	62.24	58.9	43.4	1.36
37	240	56.56	56.1	42.5	1.32
38	241	73.28	60.2	46.7	1.29
39	242	69.76	60.4	46.5	1.30
40	243	64.49	59.4	44.5	1.33
41	244	62.62	59.6	43.6	1.37
42	245	73.96	63.4	45.2	1.40
43	246	78.73	62.0	48.3	1.28
44	247	63.17	60.1	43.2	1.39
45	248	69.21	60.4	45.2	1.34
46	250	71.86	63.4	45.5	1.39
47	251	67.85	61.9	44.6	1.39
48	252	69.80	58.6	46.2	1.27
49	253	67.44	58.6	46.0	1.27
50	254	68.67	59.7	45.3	1.32

Strain 2 (67weeks)

	force	stiffness	ND stiff	tot thick	mam thick
1	19.2	139.5	90.9	0.269	0.060
2	16.5	114.6	102.0	0.267	0.061
3	19.8	131.7	122.0	0.279	0.069
4	37.2	186.2	156.2	0.316	0.052
5	13.2	72.8	82.0	0.310	0.044
6	27.7	149.2	156.2	0.286	0.058
7	21.2	126.5	119.0	0.273	0.055
8	21.5	116.8	102.0	0.268	0.044
9	24.0	127.7	116.3	0.265	0.066
10	19.8	133.4	122.0	0.299	0.064
11	20.2	177.6	111.1	0.293	0.053
12	17.5	125.0	108.7	0.287	0.061
13	24.5	127.6	138.9	0.265	0.052
14	16.2	112.8	102.0	0.261	0.047
15	23.2	116.2	128.2	0.294	0.058
16	23.0	188.5	166.7	0.314	0.070
17	22.3	125.0	131.6	0.303	0.053
18	21.2	138.0	131.6	0.286	0.062
19	14.8	86.8	128.2	0.293	0.059
20	19.8	108.5	92.6	0.273	0.052
21	21.5	126.5	116.3	0.261	0.049
22	18.8	120.2	98.0	0.262	0.050
23	25.0	131.6	111.1	0.278	0.096
24	18.5	107.6	108.7	0.273	0.064
25	35.0	210.8	178.6	0.353	0.055
26	32.5	193.4	172.4	0.311	0.079
27	31.2	138.3	156.2	0.313	0.083
28	32.5	178.6	151.5	0.313	0.047
29	26.2	156.2	151.5	0.310	0.061
30	24.0	144.6	128.2	0.299	0.072
31	19.2	135.6	128.2	0.295	0.074
32	24.2	126.3	108.7	0.274	0.076
33	21.0	152.2	131.6	0.297	0.070
34	33.0	242.7	166.7	0.336	0.065
35	23.0	95.0	96.2	0.344	0.061
36	22.5	150.0	131.6	0.293	0.088
37	20.5	111.4	102.0	0.261	0.039
38	24.5	131.7	128.2	0.278	0.071
39	15.7	121.2	69.4	0.225	0.049
40	20.2	115.1	90.9	0.267	0.066
41	23.0	135.3	128.2	0.289	0.074
42	25.2	242.8	116.3	0.286	0.071
43	19.2	102.4	111.1	0.281	0.081
44	19.2	155.2	122.0	0.302	0.060
45	25.0	147.1	138.9	0.324	0.076
46	24.0	126.3	108.7	0.275	0.055
47	17.5	91.2	87.7	0.250	0.063
48	33.2	173.2	178.6	0.325	0.066
49	29.5	153.7	142.9	0.288	0.057
50	29.5	184.4	172.4	0.336	0.055

Strain 2 (67weeks)

	Kc	ND Eshell	t effective
1	483.8	2.80e+4	0.209
2	441.3	3.08e+4	0.206
3	517.1	3.41e+4	0.210
4	685.7	2.84e+4	0.264
5	240.0	1.53e+4	0.266
6	639.5	3.57e+4	0.228
7	532.8	2.99e+4	0.218
8	506.7	2.56e+4	0.224
9	670.1	3.70e+4	0.199
10	438.7	2.73e+4	0.235
11	424.3	2.53e+4	0.240
12	412.5	2.69e+4	0.226
13	614.5	3.86e+4	0.215
14	413.1	2.85e+4	0.214
15	502.0	2.94e+4	0.236
16	486.2	3.43e+4	0.244
17	451.1	2.64e+4	0.250
18	501.7	3.23e+4	0.224
19	324.4	1.37e+4	0.234
20	469.0	2.48e+4	0.221
21	549.3	3.34e+4	0.212
22	487.4	2.72e+4	0.212
23	770.9	4.56e+4	0.182
24	497.4	3.03e+4	0.209
25	538.0	2.63e+4	0.298
26	734.1	3.89e+4	0.232
27	696.1	3.63e+4	0.230
28	588.7	2.82e+4	0.266
29	524.5	3.24e+4	0.249
30	550.5	3.25e+4	0.227
31	460.1	3.44e+4	0.221
32	680.2	3.60e+4	0.198
33	483.0	3.29e+4	0.227
34	579.3	2.92e+4	0.271
35	377.3	1.57e+4	0.283
36	615.0	3.86e+4	0.205
37	501.4	2.48e+4	0.222
38	641.4	3.83e+4	0.207
39	525.2	2.85e+4	0.176
40	562.9	2.81e+4	0.201
41	584.3	3.46e+4	0.215
42	631.0	3.29e+4	0.215
43	522.6	3.65e+4	0.200
44	409.9	2.63e+4	0.242
45	505.3	2.91e+4	0.248
46	579.5	2.95e+4	0.220
47	530.2	3.10e+4	0.190
48	623.8	3.39e+4	0.259
49	659.2	3.38e+4	0.231
50	494.3	2.82e+4	0.281

APPENDIX 5

SEM Structural Scores for Strains 1 and 2: Beginning, Middle, End of Lay.

Strain 1 24weeks

	egg no	confluence	basal caps	early fusion	late fusion	mam align
1	1	4	6	4	3	2
2	5	6	3	4	1	4
3	6	4	6	4	3	4
4	8	6	6	4	1	4
5	9	4	8	4	3	4
6	10	4	6	4	3	4
7	11	6	3	4	1	4
8	12	6	6	4	3	4
9	13	4	6	2	1	4
10	14	6	6	4	1	2
11	15	4	3	4	1	2
12	16	6	3	4	1	2
13	18	4	8	4	1	4
14	19	4	6	4	3	4
15	20	4	3	4	1	2
16	23	4	6	4	1	4
17	24	6	8	4	3	2
18	25	4	6	4	1	4
19	26	6	6	4	3	2
20	29	4	6	2	1	4
21	30	4	6	2	1	2
22	31	4	6	4	1	4
23	34	6	6	4	1	4
24	36	3	3	4	1	4
25	38	4	6	4	1	4
26	39	4	3	4	3	2
27	41	4	6	4	1	4
28	43	4	8	4	1	4
29	45	4	6	4	1	4
30	47	6	6	4	6	2
31	48	4	6	4	1	7
32	51	4	6	4	1	4
33	54	4	3	4	1	2
34	55	6	6	4	1	2
35	56	4	3	4	1	2
36	59	4	3	4	1	4
37	62	4	8	4	3	4
38	63	4	6	4	1	2
39	66	4	3	4	1	4
40	67	4	6	4	3	2
41	69	4	6	4	1	2
42	72	6	6	2	1	2
43	74	4	6	4	3	4
44	76	4	3	4	3	2
45	78	4	6	4	1	4
46	80	4	3	2	1	4
47	81	6	6	4	1	4
48	86	3	8	4	3	4
49	88	4	6	2	1	2
50	90	4	6	2	1	2

Strain 1 24weeks

	type B	pitted	aragonite	type A	cubics	cuffing
1	1	1	1	2	1	4
2	1	1	1	2	1	4
3	5	1	2	2	2	5
4	2	1	1	2	1	5
5	2	1	1	2	1	5
6	2	5	1	2	1	5
7	2	1	1	2	1	4
8	2	1	1	1	1	5
9	2	5	2	2	1	4
10	2	1	2	2	1	5
11	2	1	1	2	1	5
12	2	1	1	2	1	4
13	1	1	1	1	1	4
14	2	1	1	1	1	5
15	2	1	1	2	1	4
16	2	1	1	1	1	4
17	2	1	1	1	1	5
18	2	1	1	2	1	5
19	5	1	2	1	1	5
20	2	1	1	1	1	4
21	2	5	1	2	2	5
22	1	5	1	2	1	4
23	2	1	1	2	1	4
24	2	1	1	2	1	4
25	1	1	2	2	2	5
26	2	1	1	2	1	5
27	2	7	1	2	1	5
28	2	1	1	2	1	5
29	2	1	1	1	1	5
30	2	1	1	2	1	5
31	1	5	1	2	1	5
32	2	1	1	1	2	5
33	2	1	1	2	1	4
34	2	1	1	1	1	4
35	2	1	1	1	1	5
36	2	1	1	2	2	5
37	5	1	1	2	1	5
38	2	1	1	1	1	4
39	2	1	1	2	1	5
40	2	1	1	2	1	4
41	2	5	1	1	1	4
42	2	1	1	2	1	4
43	2	1	1	2	1	5
44	2	1	1	2	1	5
45	2	5	1	1	1	5
46	1	5	1	1	1	4
47	1	1	1	1	1	4
48	2	1	1	2	1	5
49	2	1	1	2	1	5
50	1	1	1	1	1	4

Strain 1 24weeks

	c' memb	total score	mam density	st dev
1	1	30.0	104.7	2.1
2	4	32.0	74.0	3.4
3	4	42.0	103.0	2.0
4	4	37.0	81.7	3.7
5	1	36.0	91.0	8.3
6	4	41.0	75.3	2.1
7	4	33.0	77.8	2.6
8	1	35.0	77.0	5.5
9	4	37.0	89.0	6.4
10	4	36.0	71.2	6.5
11	4	30.0	77.0	1.7
12	4	31.0	74.5	8.3
13	8	38.0	78.0	5.6
14	4	36.0	57.2	6.7
15	4	29.0	85.2	2.7
16	1	30.0	97.6	4.3
17	1	35.0	81.8	3.4
18	4	35.0	98.7	6.5
19	1	37.0	63.0	9.5
20	1	28.0	71.7	5.1
21	1	33.0	97.8	4.8
22	1	34.0	66.8	5.6
23	4	36.0	99.5	4.6
24	1	27.0	63.2	3.4
25	1	33.0	75.0	5.5
26	1	29.0	78.5	2.4
27	4	41.0	78.2	4.6
28	1	34.0	96.2	2.9
29	4	34.0	93.0	14.7
30	1	37.0	72.0	5.0
31	1	38.0	67.8	6.1
32	1	32.0	75.0	2.6
33	1	26.0	81.0	7.3
34	1	30.0	55.4	6.8
35	1	26.0	70.2	5.3
36	1	30.0	60.0	9.1
37	1	39.0	78.8	1.3
38	1	28.0	94.5	8.2
39	1	29.0	101.5	7.6
40	1	31.0	83.7	5.1
41	4	35.0	70.5	3.1
42	1	29.0	73.0	4.1
43	4	37.0	110.3	6.6
44	1	29.0	69.8	6.3
45	1	35.0	75.1	13.0
46	1	28.0	87.7	2.1
47	4	34.0	55.2	2.2
48	1	35.0	98.6	4.4
49	4	31.0	82.7	9.8
50	1	25.0	83.8	5.4

Strain 1 47weeks

	egg no	conf	cap	early fuion	late fusion	alignment
1	101	3	6	2	1	4
2	102	3	3	4	1	4
3	103	4	6	4	6	2
4	104	6	6	4	3	1
5	105	3	6	4	1	2
6	106	3	6	2	1	4
7	107	3	1	2	1	4
8	108	4	6	4	3	4
9	110	6	6	4	1	2
10	111	3	8	1	1	1
11	112	3	8	4	3	2
12	114	4	3	2	1	2
13	116	6	3	2	3	4
14	117	6	3	2	3	2
15	119	3	6	4	3	4
16	120	4	8	1	3	7
17	121	4	6	4	3	2
18	123	3	3	4	1	4
19	124	4	8	4	3	2
20	125	3	6	4	1	4
21	126	4	6	4	6	2
22	129	6	8	2	1	4
23	130	4	6	2	3	4
24	131	3	3	4	1	4
25	132	4	6	4	1	2
26	133	3	3	4	3	2
27	134	4	3	4	6	4
28	136	6	8	4	3	2
29	137	3	3	4	1	2
30	138	4	6	2	1	2
31	139	3	6	2	1	2
32	140	4	1	1	1	4
33	141	3	6	4	1	4
34	143	4	6	4	1	2
35	144	3	6	4	3	4
36	145	4	6	4	1	2
37	146	3	6	2	1	2
38	147	3	3	2	3	4
39	148	4	6	4	3	2
40	149	6	6	4	6	1
41	150	4	8	4	6	1
42	152	3	3	1	3	2
43	154	4	6	2	1	2
44	156	4	8	4	3	4
45	157	4	3	4	3	2
46	158	4	6	4	1	2
47	159	3	6	1	6	2
48	160	6	6	2	3	2
49	161	3	3	4	3	2
50	162	3	3	4	3	4
51						

Strain 1 47weeks

	type B's	Pitting	aragonite	type A	cubics	cuffing
1	2	1	1	2	1	5
2	2	5	1	2	1	5
3	5	7	1	2	5	5
4	1	1	1	2	1	5
5	1	7	1	2	1	5
6	2	1	1	2	1	5
7	1	1	1	2	1	5
8	1	1	1	1	1	4
9	2	1	1	2	1	4
10	2	7	1	2	1	4
11	1	1	1	1	1	4
12	2	1	1	1	1	4
13	1	5	1	1	1	1
14	2	1	1	2	1	4
15	2	7	1	2	1	5
16	1	1	1	1	1	4
17	2	7	1	1	1	5
18	2	1	1	2	1	5
19	2	5	1	2	1	5
20	2	7	1	1	1	5
21	2	1	1	2	1	5
22	2	5	1	2	1	5
23	2	5	1	1	1	4
24	2	5	1	2	1	5
25	2	7	2	2	2	4
26	2	5	1	2	1	5
27	5	1	1	1	1	5
28	2	1	2	2	1	5
29	2	5	1	1	1	5
30	1	5	1	2	1	5
31	2	7	1	2	1	5
32	2	1	1	2	2	5
33	2	5	1	2	1	5
34	2	1	1	1	1	5
35	2	5	1	2	1	4
36	2	5	1	2	1	5
37	1	7	1	2	1	5
38	2	1	1	1	1	5
39	2	5	1	2	1	5
40	2	7	2	1	1	4
41	5	7	1	2	1	5
42	1	1	1	1	1	5
43	2	5	1	2	1	4
44	2	1	1	1	1	5
45	2	1	2	1	1	4
46	2	5	1	1	1	4
47	5	7	1	1	1	5
48	1	5	1	1	1	5
49	1	7	1	2	1	1
50	2	1	1	1	1	5
51						

Strain 1 47weeks

	c'memb	total score	mam density	st dev
1	4	32.0	117.0	4.0
2	1	32.0	106.7	7.6
3	4	51.0	85.0	9.5
4	4	35.0	77.3	2.1
5	1	34.0	103.7	6.4
6	1	29.0	77.3	8.1
7	1	23.0	92.0	6.1
8	4	34.0	65.7	5.9
9	4	34.0	81.0	13.1
10	8	39.0	73.7	9.5
11	4	33.0	79.0	3.6
12	4	26.0	92.0	4.4
13	4	32.0	76.7	12.0
14	4	31.0	80.3	3.8
15	1	39.0	101.0	1.4
16	8	40.0	81.5	11.9
17	1	37.0	99.3	1.2
18	1	28.0	65.3	4.5
19	1	38.0	97.7	7.1
20	1	36.0	89.0	6.1
21	1	35.0	94.0	9.5
22	8	45.0	93.0	13.0
23	4	37.0	83.0	3.0
24	1	32.0	78.0	3.6
25	1	37.0	90.3	5.0
26	4	35.0	111.7	7.2
27	4	39.0	112.3	8.1
28	4	40.0	132.7	4.2
29	1	29.0	91.0	1.0
30	4	34.0	93.0	5.0
31	1	33.0	96.7	5.5
32	4	28.0	68.3	15.2
33	1	35.0	90.3	5.5
34	4	32.0	88.0	5.6
35	4	39.0	65.0	8.2
36	4	37.0	58.0	4.0
37	1	32.0	90.3	5.7
38	4	30.0	84.0	8.2
39	1	36.0	86.3	6.8
40	1	41.0	118.7	8.7
41	4	48.0	104.7	4.2
42	4	26.0	89.3	5.7
43	1	31.0	68.3	8.6
44	1	35.0	103.0	7.8
45	4	31.0	62.7	11.6
46	4	35.0	81.0	6.5
47	1	39.0	103.3	7.5
48	1	34.0	97.3	10.1
49	4	32.0	122.3	12.8
50	1	29.0	93.7	8.0
51				

Strain 1 69weeks

	egg no	confluence	basal cap	e fusion	l fusion	alignment
1	201	3	6	4	1	2
2	207	4	6	4	6	2
3	208	3	6	4	6	2
4	210	6	6	4	1	2
5	216	3	6	2	1	4
6	221	6	10	2	6	1
7	222	3	3	4	3	4
8	225	3	6	4	3	4
9	226	3	6	4	3	4
10	236	4	3	4	3	4
11	239	4	3	4	3	4
12	242	4	6	1	3	2
13	247	3	6	4	6	2
14	248	4	6	4	3	2
15	249	4	3	4	6	2
16	250	6	6	2	3	2
17	255	3	6	2	3	4
18	256	3	3	2	1	4
19	257	3	3	4	3	2
20	260	3	3	4	3	4
21	261	3	3	4	6	4
22	202	3	6	4	1	2
23	204	4	8	4	3	2
24	205	3	10	4	6	1
25	206	4	6	2	1	2
26	209	3	1	1	1	2
27	211	6	6	4	3	2
28	212	3	8	4	3	2
29	214	3	1	4	3	4
30	215	6	3	4	3	2
31	217	3	1	4	6	2
32	223	4	6	4	3	4
33	227	3	6	4	3	2
34	228	3	6	4	3	2
35	230	3	3	4	3	4
36	234	4	8	4	3	2
37	235	4	8	4	3	2
38	237	3	6	4	3	2
39	238	4	6	4	3	4
40	240	4	3	4	1	4
41	241	3	6	2	1	4
42	243	6	6	1	1	2
43	244	4	6	4	3	2
44	245	3	3	4	6	2
45	246	4	6	1	1	4
46	251	4	6	4	3	4
47	252	6	8	4	3	2
48	253	4	8	1	1	1
49	258	4	6	4	3	4
50	259	3	10	4	6	1

Strain 1 69weeks

	type B	pitting	aragonite	type A	cubic	cuffing
1	2	5	2	2	1	4
2	8	7	1	1	1	5
3	5	7	1	2	1	5
4	2	7	1	1	1	5
5	2	1	1	2	1	4
6	8	7	5	2	1	4
7	1	7	1	2	1	5
8	1	7	1	1	1	5
9	2	5	1	2	1	5
10	1	1	1	1	1	5
11	2	1	1	2	1	4
12	5	1	5	2	1	5
13	2	7	1	2	1	5
14	2	5	2	2	1	5
15	2	1	2	2	2	4
16	1	5	1	1	1	4
17	1	1	1	1	1	5
18	1	1	1	1	1	1
19	2	5	1	1	1	5
20	2	1	2	1	2	5
21	2	1	1	1	1	5
22	2	7	1	2	2	5
23	8	7	1	2	1	4
24	8	7	1	2	2	5
25	2	7	1	2	1	1
26	2	7	1	2	1	4
27	2	7	1	1	1	5
28	8	5	1	2	1	5
29	2	7	1	2	1	5
30	2	1	1	2	1	4
31	5	1	5	1	1	5
32	2	5	1	2	1	5
33	2	7	2	2	2	5
34	2	7	2	2	2	4
35	5	1	2	2	2	4
36	2	7	1	2	1	5
37	5	1	5	2	1	5
38	5	12	1	2	1	4
39	2	1	1	1	1	4
40	2	7	2	2	1	5
41	2	7	2	2	1	4
42	2	5	1	1	1	4
43	5	7	1	2	1	5
44	2	1	5	2	2	4
45	5	7	1	2	1	5
46	5	7	1	2	1	5
47	5	1	2	2	1	4
48	2	1	1	2	1	4
49	2	7	1	2	2	5
50	8	7	1	2	1	5

Strain 1 69weeks

	c'memb	total score	mam density	st dev
1	1	33.0	77.67	8.02
2	4	49.0	76.33	8.62
3	1	43.0	91.00	4.36
4	1	37.0	109.3	7.02
5	1	28.0	89.67	4.04
6	4	56.0	79.67	6.6
7	4	38.0	77.33	3.06
8	1	37.0	76.00	6.00
9	1	37.0	83.66	1.53
10	4	32.0	66.33	8.88
11	1	30.0	45.0	6.55
12	1	36.0	98.3	4.04
13	4	43.0	92.00	3.61
14	4	40.0	59.00	10.40
15	1	33.0	92.00	4.58
16	1	33.0	74.00	6.56
17	4	32.0	101.67	5.13
18	4	23.0	96.33	3.51
19	4	34.0	66.0	4.4
20	4	34.0	103.3	7.63
21	4	35.0	86.33	11.24
22	4	39.0	104.7	6.03
23	4	48.0	53.3	7.63
24	8	57.0	66.0	6.24
25	1	30.0	103.00	7.54
26	4	29.0	90.33	12.06
27	1	39.0	102.00	3.6
28	4	46.0	58.3	2.52
29	4	37.0	97.3	4.16
30	4	33.0	69.67	3.51
31	1	35.0	83.67	13.6
32	1	38.0	86.3	7.02
33	1	39.0	63.00	13.74
34	4	41.0	96.33	6.43
35	4	37.0	85.00	10.58
36	1	40.0	43.00	5.29
37	4	44.0	97.33	9.71
38	4	47.0	97.3	6.8
39	4	35.0	100.67	6.11
40	1	36.0	61.67	3.21
41	1	35.0	85.67	16.65
42	1	31.0	54.67	2.89
43	1	41.0	59.67	5.86
44	1	35.0	71.67	4.51
45	1	38.0	68.3	11.59
46	4	46.0	74.3	7.57
47	4	42.0	86.3	6.66
48	4	30.0		
49	1	41.0	77.67	6.43
50	4	52.0	73.33	4.16

Strain 2   24weeks

	egg no	confluence	basal caps	early fusion	late fusion	alignment
1	1	6	6	2	1	2
2	3	4	8	2	1	2
3	4	6	6	2	1	4
4	8	4	6	2	1	4
5	10	4	3	1	1	4
6	12	4	6	4	1	4
7	13	6	6	4	1	2
8	15	4	3	4	3	4
9	16	6	6	2	1	2
10	17	6	6	4	1	4
11	18	6	8	2	1	2
12	19	6	6	2	1	2
13	20	4	6	2	1	4
14	22	6	3	1	1	4
15	27	6	6	4	3	4
16	28	4	6	1	1	2
17	29	4	6	2	1	7
18	30	6	6	1	1	2
19	31	4	6	4	1	2
20	33	4	6	2	1	2
21	35	3	8	4	1	2
22	37	4	6	4	1	2
23	38	4	6	4	1	2
24	39	3	6	4	1	2
25	40	3	8	4	3	2
26	41	4	8	2	1	4
27	42	4	6	4	1	2
28	43	6	6	2	1	2
29	45	4	6	4	1	4
30	46	4	6	4	1	2
31	47	4	3	2	1	4
32	50	4	6	4	1	7
33	51	3	3	4	1	7
34	53	6	8	4	3	2
35	57	4	6	4	1	4
36	59	4	3	4	1	7
37	60	4	6	2	1	4
38	61	4	6	2	3	2
39	62	3	6	4	1	4
40	63	3	3	4	3	4
41	66	6	6	4	3	2
42	71	6	6	4	1	4
43	72	4	6	4	3	4
44	74	3	6	4	3	4
45	75	6	6	1	1	2
46	76	6	6	2	3	4
47	78	4	6	2	3	2
48	79	4	8	2	3	2
49	81	4	6	2	1	4
50	82	4	6	4	1	4

Strain 2 24weeks

	type B	Pitting	aragonite	type A	cubics	cuffing
1	2	1	1	2	1	5
2	2	5	1	2	1	4
3	2	1	1	1	1	4
4	1	1	1	1	1	4
5	1	1	1	2	1	5
6	2	1	1	2	1	5
7	1	1	1	2	1	4
8	2	1	1	2	1	4
9	2	5	1	2	1	4
10	2	1	1	2	1	5
11	1	1	1	1	1	4
12	1	1	1	1	1	1
13	1	1	1	2	1	4
14	2	1	1	1	1	4
15	2	1	1	1	1	4
16	2	1	1	2	1	1
17	2	1	1	2	1	4
18	1	7	1	2	1	1
19	2	1	1	1	1	1
20	2	5	1	2	1	4
21	2	5	1	2	1	4
22	2	1	1	2	2	4
23	2	5	1	2	1	4
24	2	5	1	2	1	4
25	2	5	1	2	1	4
26	2	1	1	2	1	1
27	2	1	1	2	1	4
28	2	5	1	1	1	4
29	2	1	1	1	1	4
30	2	5	1	2	1	4
31	2	1	1	1	1	1
32	2	1	1	1	1	4
33	2	1	1	2	1	5
34	5	5	1	2	1	5
35	2	1	1	1	1	5
36	2	1	1	2	1	5
37	1	5	1	2	1	4
38	2	1	1	2	1	4
39	2	1	1	2	1	5
40	2	1	1	2	1	5
41	1	5	1	2	1	4
42	2	1	1	1	1	5
43	2	1	1	1	1	5
44	2	7	1	2	1	5
45	2	1	1	1	1	1
46	2	1	1	2	1	5
47	2	1	1	2	1	1
48	2	1	1	2	1	4
49	2	1	1	2	1	4
50	2	5	1	2	1	5

Strain 2 24weeks

	c' memb	total score	mam density	stdev
1	8	38.0	93.5	7.9
2	1	34.0	103.0	8.9
3	1	31.0	109.0	8.5
4	1	28.0	89.0	7.0
5	1	26.0	104.0	14.6
6	1	33.0	84.0	2.2
7	1	31.0	90.5	6.5
8	1	31.0	91.6	8.9
9	1	34.0	89.5	3.11
10	1	35.0	80.0	9.4
11	1	30.0	86.5	2.89
12	1	25.0	92.0	4.32
13	4	32.0	89.5	3.42
14	1	27.0	71.5	4.4
15	4	38.0	88.1	6.0
16	4	27.0	87.0	4.47
17	1	33.0	92.5	5.45
18	1	31.0	107.8	11.2
19	4	29.0	112.7	8.32
20	1	32.0	87.6	4.34
21	1	35.0	113.6	8.3
22	4	34.0	89.5	3.11
23	1	34.0	105.25	2.5
24	1	33.0	100.2	4.6
25	1	37.0	104.5	4.2
26	4	32.0	98.8	6.5
27	4	33.0	103.5	5.7
28	1	33.0	100.0	7.9
29	1	31.0	84.2	4.9
30	1	34.0	100.5	6.6
31	1	23.0	69.8	4.1
32	1	34.0	95.8	5.4
33	1	32.0	121.0	4.36
34	1	44.0	110.0	7.5
35	1	32.0	88.8	2.4
36	1	33.0	86.8	5.4
37	4	36.0	111.7	11.5
38	1	30.0	73.25	2.87
39	1	32.0	90.25	8.18
40	1	31.0	113.5	3.11
41	1	37.0	83.0	6.08
42	1	34.0	108.25	7.36
43	1	34.0	100.0	5.66
44	1	40.0	96.0	6.5
45	1	25.0	100.2	18.0
46	4	38.0	73.6	2.9
47	1	27.0	87.2	7.85
48	1	32.0	95.0	4.97
49	1	30.0	112.7	2.52
50	1	37.0	115.3	4.04

Strain 2 47weeks

	egg no	confluence	basal cap	early fusion	late fusion	alignment
1	101	4	6	1	1	4
2	102	4	6	1	3	2
3	103	4	6	2	1	4
4	105	4	6	1	1	2
5	106	4	3	1	1	4
6	107	4	6	4	3	2
7	108	4	6	2	1	2
8	110	3	3	4	3	2
9	111	4	3	4	6	1
10	112	4	6	2	1	2
11	113	3	6	4	1	2
12	114	4	3	1	1	7
13	115	6	3	4	1	2
14	116	3	3	2	1	2
15	118	4	6	2	3	2
16	120	4	3	4	1	2
17	121	4	8	4	3	2
18	122	6	3	4	3	2
19	123	4	3	2	1	2
20	124	4	3	2	3	7
21	125	4	3	2	1	2
22	126	3	6	4	1	1
23	127	4	6	4	3	2
24	128	4	3	1	1	2
25	129	3	3	4	1	2
26	132	3	3	4	1	2
27	133	4	6	2	3	2
28	134	6	6	2	1	2
29	135	6	6	2	3	2
30	136	6	8	4	6	2
31	137	6	8	2	3	2
32	138	4	6	2	1	2
33	139	4	3	4	6	4
34	140	4	6	1	1	4
35	143	6	6	2	1	2
36	145	4	6	2	1	2
37	146	4	6	4	3	2
38	147	4	6	4	3	2
39	148	4	6	4	1	2
40	149	6	6	2	6	2
41	150	3	6	4	3	2
42	151	4	6	1	1	2
43	152	3	6	2	1	4
44	153	4	6	2	1	2
45	154	3	3	4	1	2
46	155	3	6	2	1	2
47	156	4	3	4	3	4
48	157	3	6	4	3	4
49	158	4	3	2	1	1
50	159	4	6	4	3	2

Strain 2 47weeks

	type B's	Pitting	arag	a's	cubics	cuffing
1	2	1	1	2	1	4
2	2	7	1	2	1	1
3	1	5	1	1	1	4
4	1	1	1	1	1	4
5	2	7	1	1	2	1
6	2	1	1	1	1	1
7	2	1	1	2	1	5
8	1	5	1	1	1	5
9	2	1	1	2	1	5
10	2	5	2	2	1	1
11	2	5	1	2	1	1
12	1	1	1	1	1	4
13	5	1	1	2	1	5
14	2	1	1	2	1	4
15	1	5	1	1	1	4
16	2	5	1	2	1	4
17	5	1	1	2	1	5
18	1	5	1	1	1	5
19	2	1	1	1	1	4
20	2	1	1	2	1	1
21	1	1	1	1	1	4
22	1	7	1	2	1	4
23	5	7	1	2	1	5
24	1	5	1	1	1	5
25	2	1	1	1	1	5
26	2	1	1	1	1	4
27	1	1	1	1	1	5
28	1	7	1	1	1	4
29	2	1	1	2	1	5
30	8	1	1	2	1	5
31	2	1	1	1	1	4
32	1	5	1	1	1	4
33	1	1	1	2	1	4
34	1	1	1	1	1	4
35	2	7	1	2	1	5
36	1	5	1	1	1	1
37	2	1	1	1	1	5
38	2	1	1	1	1	4
39	2	1	1	2	1	5
40	2	1	1	1	1	1
41	2	5	1	2	1	4
42	2	5	1	1	1	4
43	2	5	1	1	1	1
44	2	1	1	1	1	4
45	2	1	1	2	1	4
46	2	1	1	1	1	5
47	2	7	1	1	1	5
48	2	7	2	2	1	4
49	2	5	1	1	1	4
50	1	5	1	1	1	4

Strain 2 47weeks

	c'memb	total	mam density	stdev
1	4	31.0	99.0	2.6
2	1	31.0	107.0	3.0
3	4	34.0	134.0	5.3
4	14	37.0	91.3	5.6
5	1	28.0	109.0	5.0
6	1	27.0	93.3	3.5
7	1	28.0	108.7	8.1
8	1	30.0	133.1	27.9
9	4	34.0	140.3	8.7
10	1	29.0	96.7	11.0
11	1	29.0	133.0	6.6
12	1	26.0	102.7	3.8
13	1	32.0	114.7	5.7
14	1	23.0	117.7	4.0
15	4	34.0	129.0	8.2
16	1	30.0	118.0	11.5
17	8	44.0	73.3	9.6
18	1	33.0	106.0	10.8
19	8	30.0	104.3	8.5
20	1	28.0	99.0	1.6
21	1	22.0	103.3	10.0
22	1	32.0	115.0	4.6
23	1	41.0	115.7	5.5
24	1	26.0	121.3	8.3
25	1	25.0	111.3	6.0
26	4	27.0	123.7	10.4
27	4	31.0	134.0	10.4
28	1	33.0	115.3	11.0
29	1	32.0	126.7	18.1
30	1	45.0	108.3	11.1
31	1	32.0	75.0	11.3
32	4	32.0	117.0	14.8
33	8	39.0	105.0	14.7
34	4	29.0	96.3	19.0
35	1	36.0	121.3	8.6
36	4	29.0	100.3	7.5
37	4	34.0	131.0	5.3
38	8	37.0	121.0	7.0
39	1	30.0	76.3	10.4
40	4	33.0	89.3	4.2
41	1	34.0	119.3	4.0
42	4	32.0	118.7	4.9
43	1	28.0	10.0	8.9
44	1	26.0	118.3	17.9
45	4	28.0	124.0	6.2
46	1	26.0	126.3	11.7
47	4	39.0	10.3	3.2
48	4	42.0	101.3	2.5
49	4	29.0	116.0	7.5
50	4	36.0	132.0	7.0

Strain 2 69weeks

	egg no	confluence	basal cap	early fusion	late fusion	alignment
1	201	4	8	4	6	7
2	202	3	6	2	1	4
3	203	6	6	2	3	2
4	204	3	8	2	1	4
5	205	4	8	2	3	2
6	206	1	8	1	1	1
7	207	3	3	4	6	2
8	209	6	6	2	1	2
9	210	3	6	4	3	4
10	211	3	8	4	6	2
11	212	4	3	2	1	2
12	213	3	6	4	3	4
13	214	4	8	4	3	2
14	215	4	8	4	1	2
15	217	3	6	2	3	4
16	218	4	6	2	1	2
17	219	6	8	4	6	7
18	220	6	6	4	1	2
19	221	3	6	4	6	4
20	222	4	6	1	1	2
21	223	3	6	2	3	2
22	224	4	8	2	1	2
23	225	6	6	4	3	4
24	226	4	6	4	3	4
25	227	4	6	2	1	4
26	228	6	6	2	1	1
27	229	6	8	4	3	2
28	230	6	6	4	6	4
29	231	3	6	1	1	2
30	233	3	6	4	3	4
31	234	3	1	4	3	4
32	235	4	6	1	1	1
33	236	4	6	4	3	2
34	237	4	8	4	3	2
35	238	6	8	1	3	1
36	239	6	6	1	1	2
37	240	4	3	4	3	2
38	241	4	8	2	3	2
39	242	4	6	2	1	4
40	243	6	6	2	3	1
41	244	4	6	2	1	2
42	245	6	6	4	6	2
43	246	3	3	4	3	2
44	247	4	6	1	1	2
45	248	4	6	2	1	2
46	249	3	6	4	1	2
47	250	3	6	4	3	2
48	251	4	8	1	6	7
49	252	6	8	4	3	2
50	253	3	8	1	1	2

Strain 2 69weeks

	type B's	Pitting	aragonite	type A	cubics	cuffing
1	5	1	2	1	1	4
2	2	1	1	2	1	4
3	2	5	1	1	1	1
4	2	7	1	2	1	1
5	2	5	1	2	1	4
6	2	7	1	1	1	1
7	2	1	1	2	1	4
8	2	5	1	1	1	1
9	2	7	2	1	1	5
10	5	7	1	2	1	5
11	2	7	1	2	1	4
12	2	7	1	1	1	1
13	2	7	1	2	1	5
14	5	1	2	2	1	5
15	2	7	1	2	1	4
16	2	7	2	2	1	4
17	2	7	2	2	1	1
18	1	5	1	1	1	5
19	2	5	1	1	1	5
20	1	1	1	1	1	4
21	2	1	1	2	1	5
22	2	5	1	2	1	4
23	2	5	1	2	1	4
24	5	7	1	2	1	4
25	2	1	1	1	1	4
26	1	1	2	1	1	1
27	2	5	1	2	1	5
28	5	5	1	2	1	4
29	2	7	1	1	1	4
30	1	7	1	2	1	5
31	2	7	1	2	1	5
32	2	7	2	2	2	1
33	2	5	1	2	1	4
34	2	5	2	2	1	1
35	2	7	2	1	1	5
36	1	5	1	1	1	1
37	2	7	1	1	1	5
38	1	5	2	1	1	1
39	2	5	1	2	1	5
40	2	1	1	1	1	4
41	1	5	1	1	1	5
42	2	1	1	1	1	4
43	2	5	1	1	1	5
44	2	5	1	1	1	4
45	2	7	1	2	1	5
46	1	1	1	2	1	4
47	5	5	2	2	1	5
48	1	7	1	1	1	1
49	2	1	2	1	1	5
50	2	5	1	1	1	1

Strain 2 69weeks

	c'memb	total score	mam density	stdev
1	8	51.0	102.0	5.0
2	1	28.0	89.3	9.45
3	4	34.0	135.3	7.6
4	4	36.0	98.3	21.77
5	4	38.0	147.3	6.1
6	1	26.0	128.0	2.0
7	8	37.0	121.7	9.5
8	8	36.0	109.3	11.2
9	4	42.0	104.7	2.08
10	4	48.0	140.7	6.8
11	4	33.0	110.7	7.8
12	8	41.0	136.7	5.5
13	1	40.0	132.7	6.5
14	4	39.0	99.0	7.0
15	4	39.0	137.3	8.02
16	4	37.0	114.0	1.0
17	1	47.0	115.7	11.0
18	4	37.0	99.33	11.15
19	4	42.0	177.7	5.1
20	1	24.0	108.3	9.71
21	8	36.0	108.7	11.50
22	1	33.0	125.0	10.5
23	8	46.0	88.7	7.0
24	4	45.0	126.9	7.2
25	4	31.0	101.7	3.05
26	4	27.0	93.00	7.21
27	1	40.0	80.00	8.18
28	1	45.0	87.67	13.6
29	8	37.0	113.0	13.0
30	8	45.0	114.00	12.12
31	4	37.0	108.67	13.01
32	4	33.0	90.33	10.69
33	4	38.0	75.33	14.6
34	8	42.0	129.67	9.07
35	1	38.0	111.00	5.29
36	1	27.0	70.67	4.16
37	8	41.0	129.0	7.94
38	4	34.0	122.33	14.29
39	4	37.0	118.33	18.58
40	8	36.0	105.00	6.9
41	4	33.0	88.00	6.24
42	1	35.0	136.3	8.6
43	4	34.0	85.0	7.5
44	1	29.0	144.0	9.5
45	4	37.0	99.0	1.00
46	1	27.0	97.7	5.0
47	8	46.0	127.7	11.6
48	4	42.0	98.7	7.6
49	8	43.0	100.3	5.9
50	8	34.0	77.3	14.2