Biologically Active Hen Egg Components in Human Health and Disease

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It is widely recognized that eggs are more than a source of dietary nutrients, and extensive studies identifying and characterizing the biologically active components of eggs have been carried out. Numerous biological activities have now been associated with egg components, including antibacterial and antiviral activity, immunomodulatory activity, and anti-cancer activity, indicating the importance of eggs and egg components in human health, and disease prevention and treatment. The potential of some of these biologically active components has already been realized, including egg white lysozyme and avidin, and yolk IgY and lecithin, which are currently produced on an industrial scale, and have been applied for the prevention and treatment of various medical conditions. The information presented here serves to demonstrate the significant potential of biologically active egg components, for medical, nutraceutical, and food-fortification applications.

Key words: Hen eggs, bio-active components, nutraceuticals, health and disease, functional foods

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Introduction

The egg is the largest biological cell originating from one cell division. It is an important source of nutrients, containing all of the proteins, lipids, vitamins, minerals and growth factors required by the developing embryo, as well as defense factors against bacterial and viral infection.

The formation of an egg involves the conversion of the feed into egg constituents through a number of intricate and highly coordinated steps. The reproductive system of the hen, shown in Figure 1, consists of the ovary and oviduct (Romanoff and Romanoff, 1949). The ovary is the site of assembly of the primordial germ cells in the embryo (Burley and Vadehra, 1989), which are later transformed into oocytes. Each oocyte becomes a follicle after being covered with the granulosa and theca layers. Yolk constituents are synthesized in the liver and are transported to the follicular walls via the blood. The follicle undergoes a rapid development during which most of the yolk is deposited prior to ovulation. When sufficient yolk has accumulated, the ovulated

Fig. 1. The reproductive system of the hen (Romanoff and Romanoff, 1949). Reproduced with permission from John Wiley & Sons, Inc., New York.
ovum enters the oviduct (Burley and Vadehra, 1989; Okubo et al., 1997). In laying hens, the oviduct consists of five regions, infundibulum, magnum, isthmus, uterus and vagina. The ovulated ovum is held in the infundibulum, where the yolk probably acquires the outer layer of the vitelline membranes and the chalazal layer of the albumen (Burley and Vadehra, 1989). In the albumen-secreting region (magnum), the egg albumen is secreted to cover the yolk, which is then immediately covered with the shell membranes (Burley and Vadehra, 1989). Egg shell formation occurs in the uterus. The exact mechanism is unclear, but calcium from the blood is deposited to the shell, secreted by shell glands, and assembling as a crystalline-like calcium structure on the shell membranes (Burley and Vadehra, 1989; Okubo et al., 1997). The vagina is the last portion of the oviduct, and the end of the vagina connects with the cloaca.

Eggs consist of about 9.5% eggshell (including shell membrane), 63% albumen, and 27.5% yolk (Cotterill and Geiger, 1977) (Table 1). The main components are water (75%), proteins (12%), lipids (12%), as well as carbohydrates and minerals (Burley and Vadehra, 1989; Sugino et al., 1997). The proteins are distributed throughout the egg, with the majority found in the egg yolk and egg white, and a small proportion in the eggshell and shell membrane (Sugino et al., 1997). The lipids are found almost exclusively in the egg yolk, mainly in the form of lipoproteins, and include phosphorous, nitrogen, and sugar-containing lipids (Burley and Vadehra, 1989; Sugino et al., 1997). Several minerals have also been found in eggs, most of them in the egg shell. Carbohydrates are a minor egg component, present throughout the egg, both as free and conjugated forms, attached to proteins and lipids (Sugino et al., 1997).

Diverse biological activities have now been attributed to egg components, including antimicrobial and antiviral activity, protease inhibitory action, vitamin-binding properties, anti-cancer activity and immunomodulatory activity (Li-Chan et al., 1995). The role of these egg components in human health has been extensively studied, and will be discussed here.

**Egg Shell and Membrane**

Chicken eggshell is a mineralized structure consisting of approximately 95% calcium carbonate by weight, the rest made up of an organic matrix consisting mainly of glycoprotein and proteoglycans (Hincke, 1995; Hincke et al., 1999). Generally considered a waste product, only recently has significant research been carried out to examine the potential biological activity of egg shell components (Suguro et al., 2000; Daengprok et al., 2002). Eggshell calcium has been proposed for pharmaceutical applications for calcium deficiency therapies in humans, and in animals for bone mineralization and growth (Tsugawa et al., 1995). Eggshell powder was shown to have antirachitic effects in rats (Rovensky et al., 1994). In vitro, eggshell powder stimulated the growth of chick embryo cartilage cells (Schafasmas and Pakan, 1999), and in humans the use of the eggshell powder resulted in an increase in bone mineral density (Schafasmas and Beelen, 1999). In piglets, the apparent absorbability of calcium from eggshell powder was found to be at least as good as that from purified CaCO3 (Schafasmas and Beelen, 1999), and it has been suggested as a calcium supplement to
Table 1. Chemical composition of hen eggs (Modified from Mine, 2002)

<table>
<thead>
<tr>
<th>Constituent</th>
<th>% (w/v)</th>
<th>Major components (relative %, w/w)</th>
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</thead>
<tbody>
<tr>
<td><strong>EGG SHELL</strong></td>
<td>9.5</td>
<td>Inorganic salts (91.87):</td>
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<tr>
<td></td>
<td></td>
<td>Calcium carbonate (98.4)</td>
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<td></td>
<td></td>
<td>Magnesium carbonate (0.8)</td>
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<td></td>
<td></td>
<td>Tricalcium phosphate (0.8)</td>
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<tr>
<td></td>
<td></td>
<td>Proteins (6.4)</td>
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<tr>
<td></td>
<td></td>
<td>Water (1.7)</td>
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<tr>
<td></td>
<td></td>
<td>Lipids (0.03)</td>
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<tr>
<td><strong>EGG WHITE</strong></td>
<td>63.0</td>
<td>Proteins (9.7–10.6):</td>
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<tr>
<td></td>
<td></td>
<td>Ovalbumin (54)</td>
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<tr>
<td></td>
<td></td>
<td>Ovotransferrin (12.0)</td>
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<tr>
<td></td>
<td></td>
<td>Ovomucoid (11)</td>
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<tr>
<td></td>
<td></td>
<td>Ovomucin (3.5)</td>
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<tr>
<td></td>
<td></td>
<td>Lysozyme (3.4)</td>
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<td></td>
<td></td>
<td>G2 Globulin (4.0?)</td>
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<td></td>
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<td>G3 Globulin (4.0?)</td>
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<tr>
<td></td>
<td></td>
<td>Avidin (0.05)</td>
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<tr>
<td></td>
<td></td>
<td>Lipids (0.03)</td>
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<tr>
<td></td>
<td></td>
<td>Carbohydrates (0.4–0.9)</td>
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<td></td>
<td></td>
<td>Ash (0.5–0.6)</td>
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<tr>
<td><strong>EGG YOLK</strong></td>
<td>27.5</td>
<td>Proteins (15.7–16.6):</td>
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<tr>
<td></td>
<td></td>
<td>Apovitellenin (I-VI) (37.3)</td>
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<td></td>
<td></td>
<td>Lipovitellin apoproteins (40.0)</td>
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<td></td>
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<td>α-lipovitellin</td>
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<td></td>
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<td>β-lipovitellin</td>
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<tr>
<td></td>
<td></td>
<td>Livetins (9.3)</td>
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<tr>
<td></td>
<td></td>
<td>α-livetin (serum albumin)</td>
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<td></td>
<td></td>
<td>β-livetin (α2 glycoprotein)</td>
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<td></td>
<td></td>
<td>γ-livetin (γ globulin)</td>
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<tr>
<td></td>
<td></td>
<td>Phosvitin (13.4)</td>
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<td></td>
<td></td>
<td>Biotin-binding protein (trace)</td>
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<td></td>
<td></td>
<td>Triglycerol (66)</td>
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<tr>
<td></td>
<td></td>
<td>Phosphatidylcholine (PC) (24)</td>
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<td></td>
<td></td>
<td>Phosphatidylethanolamine</td>
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<tr>
<td></td>
<td></td>
<td>(PE) (2.8)</td>
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<tr>
<td></td>
<td></td>
<td>Lysophosphatidylcholine</td>
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<tr>
<td></td>
<td></td>
<td>(LPC) (0.6)</td>
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<tr>
<td></td>
<td></td>
<td>Sphingomyelin (0.6)</td>
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<tr>
<td></td>
<td></td>
<td>Cholesterol (5.0)</td>
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<tr>
<td></td>
<td></td>
<td>Others (1.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carbohydrate (0.2–1.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ash (1.1)</td>
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</tbody>
</table>
increase bone mineral density in individuals with osteoporosis (Omi and Ezawa, 1998; Schaafsma et al., 2000).

A recent study demonstrated that a 21 000 Da protein present in soluble eggshell matrix proteins may play an important role in increasing calcium transport across intestinal epithelial cells in vitro (Daengprok et al., 2003).

Eggshell membrane is composed of collagen-like proteins, which are largely located in the inner membrane (Wong et al., 1984). Eggshell membrane-derived peptides, prepared by alkaline treatment, were shown to stimulate human skin fibroblasts in vitro (Suguro et al., 2000). Eggshell membranes also contain antimicrobial substances, with lysozyme (Vadehra et al., 1972) and β-N-acetylglucosaminidase (Winn and Ball, 1975) activity reported.

**Egg White**

**Ovalbumin**

Ovalbumin constitutes over half of the total egg white proteins (Ibrahim, 1997). It is a monomeric phosphoglycoprotein, and has been used extensively as a model for studying the structure-function relationships of proteins (Li-Chan and Nakai, 1989; Ibrahim, 1997). Functionally, ovalbumin is important for the gelling, foaming, and emulsifying properties of egg white (Mine, 2002), however, its biological role in the egg remains unknown. It has been suggested that ovalbumin may serve as a source of amino acids for the developing embryo (Ibrahim, 1997).

Ovalbumin may possess some immunomodulatory activity, as it was found to induce the release of tumor necrosis factor (TNF) alpha in a dose-dependant manner in vitro, when modified with dicarbonyl methylglyoxyl (Fan et al., 2003), and immunogenic ovalbumin peptides have been used to enhance immune responses for cancer immunotherapy (Vidovic et al., 2002; Goldberg et al., 2003; He et al., 2003).

A vasorelaxing peptide, ovokinin (OA 358–365), was isolated by the peptic digestion of ovalbumin (Fujita et al., 1995a). Ovokinin (2–7), a peptide produced by chymotrypsin digestion, and corresponding to OA 359–364, was also found to possess vasorelaxing activity (Matoba et al., 1999). Both peptides were found to significantly lower the systolic blood pressure in spontaneously hypertensive rats, when administered orally (Fujita et al., 1995b). The replacement of amino acids in the ovokinin (2–7) peptide has resulted in enhanced anti-hypertensive activity, with the most potent derivative resulting in 100-fold more potent anti-hypertensive activity (Matoba et al., 2001; Yamada et al., 2002).

Two angiotensin I converting enzyme (ACE)-inhibitory peptides were also identified in ovalbumin by peptic (OA 183–184) and tryptic (OA 200–218) digestions (Yoshikawa and Fujita, 1994). Finally, the phagocytic activity of macrophages was increased by the addition of OA 77–84 and OA 126–134 peptides, derived by peptic and chymotryptic digestions of ovalbumin, respectively (Tezuka and Yoshikawa, 1995).

**Lysozyme**

Lysozyme, which plays an important role in the natural defense mechanism (Kijowski et al., 2000), is a ubiquitous enzyme, present in almost all secreted body
fluids and tissues of humans, as well as plants. The most plentiful source is hens’ egg white, containing around 0.3–0.4 g of lysozyme per egg (Losso et al., 2000).

Lysozyme acts as a mucopentptide N-acetylmuramyl hydrolase, exerting bacteriolytic activity by hydrolyzing the β(1–4) linkage between N-acetylmuraminic acid and N-acetylgalosamine of peptidoglycan, the structural component of bacterial cell walls (Salton, 1957). It has demonstrated antimicrobial activity against such organisms as Bacillus stearothermophilus, Clostridium tyrobutyricum, Clostridium thermosaccharolyticum, Clostridium sporogenes and Bacillus spp. (Losso et al., 2000), as well as Enterococcus faecalis, Weissella viridescens (Gill and Holley, 2003), Brochothrix thermosphacta, Lactobacillus sakei, Leuconostoc mesenteroides, Listeria monocytogenes (Gill and Holley, 2000), and Carnobacterium sp. 2. (Nattress et al., 2001) when used in conjunction with other compounds, such as nisin and EDTA.

The enzymatic treatment of lysozyme, exposing amino acids 98–112, which have been found to exert antimicrobial action (Pellegrini et al., 1997; Ibrahim et al., 2001), as well as synthetic peptides corresponding to the C-terminal of lysozyme (During et al., 1999), have been examined. Pellegrini et al. (2000) demonstrated that polypeptides derived from lysozyme were capable not only of damaging the outer membrane of Escherichia coli, but could also inhibit DNA and RNA synthesis. Along with numerous applications as a food antimicrobial agent, lysozyme has been added to oral health care products, such as toothpaste, mouthwash and chewing gum to protect against periodontis-causing bacteria and prevent infections in the oral mucosa (Sava, 1996; Tenuovo, 2002).

Lysozyme has also been shown to exert antiviral activity, reportedly associated with its charge, rather than its lytic ability (Losso et al., 2000). Oral and topical applications of lysozyme were found to be effective in preventing and controlling several viral skin infections, including herpes simplex and chicken pox (Sava, 1996), as well as acting as exerting anti-inflammatory action (Sava, 1996). It has also been shown, when combined with immunotherapy, to be effective in improving chronic sinusitis (Asakura et al., 1990), and to normalize humoral and cellular responses in patients with chronic bronchitis (Sava, 1996). Lee-Huang et al. (1999) found that chicken lysozyme also possessed activity against HIV type 1.

Finally, lysozyme has also been shown to act as an immune-modulating and immune-stimulating agent, enhancing immunoglobulin productivity, and regulating and restoring the immune responses in immune-depressed patients undergoing anti-cancer treatments (Sava, 1996; Sugahara et al., 2000), and as an anti-cancer agent, inhibiting tumor growth in a number of experimental tumors (Sava, 1989; Sava et al., 1989, 1991; Das et al., 1992; Pacor et al., 1996, 1999) and enhancing the efficacy of chemotherapy treatments (Sava et al., 1995).

Ovotransferrin

Ovotransferrin is a monomeric glycoprotein, belonging to the transferrin family, a group of iron-binding proteins widely distributed in various biological fluids, which has the capacity to reversibly bind two iron ions per molecule (Ibrahim, 2000). Its suggested function is as an iron scavenger, to prevent its use by microorganisms, and as
an iron delivery agent (Abdallah et al., 1999).

Ovotransferrin has demonstrated antibacterial activity against a wide spectrum of bacteria, including *Pseudomonas* spp., *Escherichia coli*, *Streptococcus mutans* (Valenti et al., 1983), *Staphylococcus aureus*, *Bacillus cereus* (Abdallah et al., 1999) and *Salmonella enteritidis* (Baron et al., 2000). It has been suggested that ovotransferrin can exert antibacterial activity by permeating bacterial outer membranes, reaching the inner membrane and causing the selective permeation of ions and dissipation of electrical potential (Aguilera et al., 2003). A 92-amino acid ovotransferrin peptide, OTAP-92, was also found to be capable of killing Gram negative bacteria by crossing the bacterial outer membrane by self-promoted uptake, and damaging the cytoplasmic membrane (Ibrahim et al., 2000).

It has also been shown that ovotransferrin possesses both antiviral activity, against Marek’s disease virus in chicken embryo fibroblasts (Giansanti et al., 2002), as well as antifungal activity, against species of *Candida* (Valenti et al., 1985).

Xie et al. (2002) demonstrated that ovotransferrin can act as an immunomodulator, modulating macrophage and heterophil functions in chickens. Further immunomodulating effects of ovotransferrin have also been shown, including the inhibition of proliferation of mouse spleen lymphocytes (Otani and Odashima, 1997) and the enhanced phagocytic response of peripheral blood mononuclear cells and polymorphonuclear cells in dogs (Hirota et al., 1995). Ovotransferrin was also found to facilitate the recovery of chick eyes from induced myopia (Rada et al., 2001).

Finally, when administered with Syn 2190, an inhibitor of AmpC lactamases, lactoferrin increased the activity of various antibiotics against beta-lactamase-producing bacteria (Babini and Livermore, 2000).

**Ovomucoid**

Ovomucoid is a glycoprotein, arranged into three domains which are cross-linked by intradomain disulfide bonds (Kato et al., 1987). It is relatively resistant to treatment with heat or digestive enzymes, and it is this stability that has resulted in its being one of the dominant egg white allergens (Cooke and Sampson, 1997). Ovomucoid is also one of four egg white proteinase inhibitors, belonging to the group of serine proteinase inhibitors, namely inhibiting trypsin (Kato et al., 1987; Saxena and Tayyab, 1997).

The incorporation of ovomucoid into polymeric microparticles, to overcome the degradation of protein drugs by proteolytic enzymes, has been examined. Agarwal et al. (2001 b) found that when ovomucoid was used, the stability of insulin in poly-methacrylate based microparticulates was increased significantly. Inclusion of ovomucoid also resulted in targeting of drugs to the blood, by acting as a biospecific ligand to lectins on the walls of the gastrointestinal tract (Plate et al., 2002). The presence of ovomucoid was found to enhance insulin flux across rat jejunum (Agarwal et al., 2001 a), suggesting the use of ovomucoid to enhance the oral delivery of insulin. Using a rat model of experimental pancreatitis, intravenous ovomucoid was found to decrease the trypsin-like activity to the level of intact rats, and reduce primary pancreas destruction (Valueva et al., 1992).

Synthetic ovomucoid peptides have also demonstrated immunomodulating activity,
inducing T-cell secretion of cytokines interleukin- (IL) 4, IL-10, IL-13, interferon-(IFN) gamma, and IL-6 (Holen et al., 2001).

Because of its allergenic nature, however, ovomucoid has limited biological and medical applications.

**Avidin**

Chicken avidin is a tetrameric glycoprotein, with an extremely high affinity for the water soluble vitamin biotin (Green, 1975). The unique feature of this binding is the strength and specificity of the formation of the avidin-biotin complex, formed when avidin binds four molecules of biotin (Bayer and Wilchek, 1994).

Avidin possesses antimicrobial activity, and has been found to inhibit the growth of biotin-requiring bacteria and yeasts (Green, 1975; Banks et al., 1986). Avidin antimicrobial activity has also been attributed to its ability to bind to various Gram negative and Gram positive bacteria, including *Escherichia coli* K-12, *Klebsiella pneumoniae*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epidermis* (Korpela et al., 1984).

Avidin has been used in cancer treatment, to localize and image cancer cells and to pre-target drugs to tumors. Because of its tight biotin binding and signal amplification due to the tetrameric structure of avidin, it leads to the accumulation of higher effective doses and increased persistence of biotinylated anti-cancer drugs, as compared to other immunotherapeutic procedures (Hytonen et al., 2003). Tumor pre-targeting with avidin has also been found to be effective in increasing the uptake of tumor necrosis factor (TNF) alpha conjugated to biotin *in vitro*, improving the anti-tumor activity of TNF (Moro et al., 1997; Corti et al., 1998; Gasparri et al., 1999). Yao et al. (1998) found that radiolabelled avidin also bound to lectins expressed on the surface of tumor cells, and localized highly and rapidly in various types of tumors in mice, thereby reducing radioactivity accumulation in other organs.

It has also been found to be essential for the activity of adoptively transferred T-cells at tumor sites (Guttinger et al., 2000), and the utilization of avidin in drug delivery through the blood-brain barrier has been demonstrated, facilitating delivery of therapeutics to the brain (Bickel et al., 2001).

**Ovomucin**

Egg white ovomucin is a macromolecular and heavily glycosylated glycoprotein, consisting of a peptide-rich α-subunit and a carbohydrate-rich β-subunit (Itoh et al., 1987). Ovomucin serves physical functions within the egg, such as maintaining the structure and viscosity of the egg white albumen (Tsuge et al., 1997b), thus preventing the spread of microorganisms (Ibrahim, 1997). It has also demonstrated several biological applications.

Ovomucin has shown antiviral activity against Newcastle disease virus, bovine rotavirus, and human influenza virus *in vitro* (Tsuge et al., 1996a, b, 1997a, b; Watanabe et al., 1998a). Ovomucin peptides, produced by treatment with the enzyme pronase, showed increased solubility, compared to its native form, while still retaining virus-binding activity (Tsuge et al., 1997a; Watanabe et al., 1998a).

Pronase-prepared glycopeptides of ovomucin have also demonstrated anti-tumor
effects in a double grafted tumor system in mice (Watanabe et al., 1998b), suggested to be related to the anti-angiogenic activity of ovomucin, inhibiting tumor growth (Oguro et al., 2001).

Ovomucin peptides may also act as immunomodulators, showing macrophage-stimulating activity in vitro (Tanizaki et al., 1997). Finally, ovomucin was found to inhibit cholesterol uptake in vitro by Caco-2 cells, and reduce serum cholesterol in rats, displaying hypocholesterolemic action (Nagaoka et al., 2002).

**Cystatin**

Egg white cystatin, part of a "superfamily" of cystatins, belongs to the Type 2 cystatins (Barrett, 1986), and inhibits most cysteine proteinases, including ficin, papain, and cathepsins B, C, H and L (Li-Chan et al., 1995). It contains two disulphide bonds, but no carbohydrate (Barrett, 1986). Low contents of cystatins in natural resources may limit their applications (Nakai, 2000), however, genetic modification and expression of cystatin has been examined, not only providing a source for increased quantities of cystatin, but also resulting in the production of recombinant cystatin with enhanced proteinase inhibitory activity (Ogawa et al., 2002).

Egg white cystatin has been shown to possess antibacterial activity, preventing the growth of group A streptococcus (Bjorck, 1990), Salmonella typhimurium (Nakai, 2000), and the periodontis-causing Porphyromonas gingivalis (Blankenvoorde et al., 1996). Blankenvoorde et al. (1998) found that peptides derived from cystatin were also capable of inhibiting the growth of P. gingivalis.

Several proteases, including cysteine proteases, are required for tumor progression and metastasis (Krol et al., 2003a). Increased levels of cysteine proteases, and a decrease in cystatin, have been observed in various cancers (Konduri et al., 2002; Nagai et al., 2003). Cystatin has been shown to inhibit tumor invasion in ras-transformed breast epithelial cells (Premzl et al., 2001), and multifunctional inhibitors, composed of chicken cystatin in conjunction with other protease inhibitors, have been suggested for the therapy of solid tumors (Muehlenweg et al., 2000; Krol et al., 2003 a, b). Cystatins also produce less intensive side effects than other synthetic protease inhibitors currently used in medical treatments (Nakai, 2000).

A relationship between cystatins, cytokines, and immune response has also been suggested. It has been observed that cystatin induced the synthesis of various cytokines (Kato et al., 2000), resulting in an up-regulation of nitric oxide release in vitro using mouse peritoneal macrophages (Verdot et al., 1996, 1999), as well as in vivo, greatly reducing parasite numbers in a mouse model of visceral leishmaniasis (Das et al., 2001).

**Ovomacroglobulin (ovostatin)**

Ovomacroglobulin, also referred to as ovostatin, is a glycoprotein composed of four subunits joined in pairs by disulfide bonds (Kitamoto et al., 1982). It has demonstrated broad-spectrum inhibitory activity against various types of proteases, including serine proteases, cysteine proteases, thiol proteases, and metalloproteases (Kitamoto et al., 1982; Molla et al., 1987).

The antimicrobial effects of ovomacroglobulin against Serratia marcescens and Pseudomonas aeruginosa, due to its proteinase inhibitory action, have been
demonstrated both in vitro (Molla et al., 1987; Miyagawa et al., 1991 b, c, 1994), and in vivo, where it was found to reduce corneal destruction in an experimental keratitis model in rabbits, as well as accelerate wound healing (Miyagawa et al., 1991 a; Ijiri et al., 1993; Miyagawa et al., 1994). Ovomacroglobulin was also found to enhance periodontal wound healing in rats, by accelerating fibroblast growth, collagen deposition, and capillary formation in tissue (Ofuji et al. 1992).

The proteinase inhibitory effects of ovomacroglobulin have also demonstrated several other biological effects, including the suppression of P. aeruginosa and Vibrio vulnificus septicemia due to the inhibition of kinin generating proteases (Maeda et al., 1993; Maruo et al., 1998), the in vitro inhibition of the inflammatory proteinase medullasin (Ikai et al., 1989), and the suppression of metalloproteinases and vascular permeability in skin tissues, which play a role in tumor metastasis (Wu et al., 2001).

Ovoinhibitor

Ovoinhibitor, like ovomucoid, is a serine proteinase inhibitor, inhibiting enzymes such as trypsin, chymotrypsin, and elastase, as well as various bacterial and fungal proteinases (Matsushima, 1958; Vered et al., 1981; Tomimatsu et al., 1966).

It has been found to prevent the development of rotavirus gastroenteritis in a mouse model of rotavirus infection (Yolken et al., 1987), and to inhibit the formation of active oxygen species by human polymorphonuclear leukocytes, which are associated with inflammatory diseases, mutagenicity and carcinogenicity (Frenkel et al., 1987). It has also been used to study models of autoimmune arthritis in mice (Terato et al., 1996).

Proteinases are involved in the regulation of a number of biological processes, and have been implicated as contributors in several diseases, including viral diseases, such as HIV (Maliar et al., 2002), and Alzheimer’s (Schimmoller et al., 2002). Proteinase inhibitors, therefore, such as those from egg white, have significant potential for the treatment and prevention of proteinases-mediated diseases.

The biological activities associated with egg white proteins are summarized in Table 2.

Egg Yolk

Immunoglobulin (Ig) Y

Immunoglobulin (Ig) Y is the functional equivalent of IgG, the major serum antibody in mammals (Carlander et al., 2000). It is transferred to the developing embryo, to give acquired immunity to the chick (Carlander et al., 1999; Sim et al., 2000). Specific IgY can be produced by immunization of chickens with the desired protein, and then purified from the egg yolk (Gassmann et al., 1990; Carlander et al., 2000).

One of the primary applications of IgY is immunotherapy, for the passive immunization of immunocompromised individuals, and as an alternative to traditional antibiotic treatment. IgY has been produced against a number of bacteria and viruses, and has been shown to bind to and inhibit the infection and disease symptoms, in vitro and in vivo, of gastrointestinal pathogens such as human and bovine rotavirus (Yolken et al.,
### Table 2. Biological properties of egg white proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Properties</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Ovalbumin</td>
<td>Immunomodulating activity</td>
<td>Tezuka and Yoshikawa, 1995; Vidovic <em>et al.</em>, 2002; Fan <em>et al.</em>, 2003; Goldberg <em>et al.</em>, 2003</td>
</tr>
<tr>
<td></td>
<td>Anti-hypertensive activity of ovo-kinin and ovokinin (2–7)</td>
<td>Fujita <em>et al.</em>, 1995a, b; Matoba <em>et al.</em>, 1999, 2001; Yamada <em>et al.</em>, 2002</td>
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<td></td>
<td>ACE inhibitory activity of ovalbumin-derived peptides</td>
<td>Yoshikawa and Fujita, 1994</td>
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IgY against Streptococcus mutans have been shown to prevent adhesion of the bacteria in

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1988 ; Hatta et al., 1993 ; Kuroki et al., 1994 ; Ebina et al., 1996 ; Kovacs-Nolan et al., 2001), bovine coronavirus (Ikemori et al., 1997), Escherichia coli (Ikemori et al., 1992 ; O’Farrely et al., 1992 ; Yokoyama et al., 1992 ; Imberechts et al., 1997 ; Jin et al., 1998 ; Marquardt et al., 1999 ; Amaral et al., 2002), Salmonella spp. (Peralta et al., 1994 ; Yokoyama et al., 1998 a, b ; Sugita-Konishi et al., 2000 ; Lee et al., 2002), Yersinia ruckeri (Lee et al., 2000), Edwardsiella tarda (Hatta et al., 1994), Helicobacter pylori (Shin et al., 2002), porcine epidemic diarrhea virus (Kweon et al., 2000), and infectious bursal disease virus (Eterradossi et al., 1997), as well as Staphylococcus aureus, (Sugita-Konishi et al., 1996 ; LeClaire et al., 2002), Pseudomonas aeruginosa (Sugita-Konishi et al., 1996 ; Carlander et al., 2002 ; Kollberg et al., 2003). IgY against Streptococcus mutans have been shown to prevent adhesion of the bacteria in

Chicken anti-venom IgY has been produced, for treatment of snake and spider bites, and was found to have a higher bioactivity than anti-venoms raised in horses (Thalley and Carroll, 1990; Almeida *et al.*, 1998).

Due to the structural differences between IgY and IgG (IgY does not fix mammalian complement components or bind human Fc receptors), IgY has been suggested for use to inhibit pig-to-human xenograft rejection (Fryer *et al.*, 1999; Leventhal *et al.*, 2001), and the use of IgY has also been examined to replace the anti-inflammatory drugs used to treat Crohn’s disease and ulcerative colitis. Worledge *et al.* (2000) reported that anti-TNF antibodies produced in chickens were capable of effectively treating acute and chronic phases of colitis in rats, and were also found to neutralize human TNF *in vitro*, indicating its possible use for the treatment of inflammatory bowel disease in humans.

Finally, IgY has been suggested for use in cancer treatment, to act as a carrier for anti-tumor drugs (Yang *et al.*, 1997), and has demonstrated anti-tumor activity, inhibiting the spreading and invasion of glioma cells into adult rat optic nerve cells *in vitro* (Hensel *et al.*, 1998).

**Phosvitin**

Phosvitin is a highly phosphorylated protein, containing 10% phosphorus and 6.5% carbohydrates (Taborsky and Mok, 1967). Ninety-five percent of the iron in eggs is present in the yolk and is bound to phosvitin (Greengard *et al.*, 1964), however its bioavailability is very low (Morris and Greene, 1972). This is due to the strong binding of phosvitin, or its phosphopeptide derivatives, with Fe³⁺, and the formation of phosvitin-iron complexes which promote the precipitation of iron in the small intestine (Sato *et al.*, 1984, 1985, 1987). However, Jiang and Mine (2000, 2001) produced functional phosphopeptides, with a molecular weight of 1000-3000 Da, derived by tryptic hydrolysis following partial alkaline dephosphorylation. These peptides exhibited enhanced calcium-binding capacity and inhibited the formation of insoluble calcium phosphates.

Phosvitin also demonstrated a capacity to inhibit iron catalysis of phospholipid oxidations (Lu and Baker, 1986). Thus, phosvitin could be useful in foods as a natural antioxidant.

**Lipoproteins (Low density lipoproteins-LDL)**

The low density lipoproteins (LDL) fraction of yolk plasma is made up of 89% lipid and 11% protein (Cook and Martin, 1962). The lipid content of LDL contains 70% triacylglycerol, 4% cholesterol, and 26% phospholipids (Martin *et al.*, 1963).

The immunomodulating activity of LDL has been demonstrated using human histocytic lymphoma cells (U-937), human monocytic leukemia cells (THP-1) and U-937-derived macrophage-like cells (U-M) (Suzuki *et al.*, 1994), promoting the growth of all three cell types. LDL was also shown to enhance the production of IgM in human-human hybridomas (Shinohara *et al.*, 1993).
Sialic acid

Sialic acid is a term referring to derivatives of neuraminic acid, which have an acyl group on the amino nitrogen. The most widely distributed sialic acid in nature is N-acetylneuraminic acid (Neu5Ac) (Hartmann and Wilhelmson, 2001). The use of egg yolk as a source of sialic acid has been examined, and was found to be an excellent source for the large scale preparation of Neu5Ac (Koketsu et al., 1992; Juneja et al., 1994).

Sialic acids possess many biological functions, including acting as receptors for microorganisms, toxins, and hormones, and masking receptors and immunological recognition sites of molecules and cells (Schauer, 1987).

Sialic acid may act as an anti-inflammatory agent. Cells carrying a carbohydrate ligand called sialyl-Lewis X were found to bind to endothelial leukocyte adhesion molecule-1 (ELAM-1), which mediates the adhesion of circulating leukocytes to the vascular endothelium during inflammation (Lowe et al., 1990; Phillips et al., 1990). The anti-inflammatory effects of sialyl-Lewis X were also shown in vivo, reducing lung injury in rats (Mulligan et al., 1993) and reducing tissue injury in rabbit ears, caused by a temporary increase in blood supply (Han et al., 1995).

Sialyloligosaccharides

Several sialyloligosaccharides in chalaza, egg yolk membrane and delipided egg yolk have been isolated by acid hydrolysis or protease digestion (Juneja et al., 1991). These sialyloligosaccharides are likely to be naturally present as glycoproteins or glycopeptides (Koketsu et al., 1995b). Sialylglycoconjugates, sialylgangliosides, sialyloligosaccharides and sialylglycoproteins have been reported to play various important roles in animal and human tissue cells.

The oligosaccharide-enriched fraction of sialyloligosaccharide was found to significantly inhibit rotavirus infection both in vitro and in vivo, with the sialic acid moiety of the oligosaccharide playing an important role in viral inhibition (Koketsu et al., 1995a). Sialyloligosaccharides have also been found to inhibit Salmonella infection by inhibiting the entry of bacteria through the gut (Sugita-Konishi et al., 2002).

Yolk lipids

Dry egg yolk contains approximately 60% lipids (Juneja, 1997), made up of around 65% is triglyceride, 28% is phospholipid, and 5% is cholesterol (Li-Chan et al., 1995). The fatty acid composition of the lipid fraction of egg yolk varies, and is influenced by the type of fat in the hen’s diet (Li-Chan et al., 1995). Egg yolk lipids have found numerous applications in the food industry, due to their surfactant (Juneja, 1997) and antioxidant (Yamamoto et al., 1990, 1991; Juneja, 1997) properties, and in the cosmetic industry, to replenish lipid deficiency in the skin (Juneja, 1997). Egg yolk lipids have also demonstrated a number of medical applications.

Rabinowich et al. (1987) noted that the administration of an egg yolk-derived lipid mixture to elderly individuals, formulated for in vivo rectification of rigidified cell membranes in an attempt to restore proper physiological function, resulted in an increase in lymphocyte responsiveness. As well, a common parenteral fat emulsion derived from egg yolk lipids is often used as a carrier of fat soluble drugs (Hartmann
Cholesterol is an important component in cell membranes and is needed for the growth of infants. As well, it is a precursor of bile acids, sex hormones and cortex hormones (Juneja, 1997). The supplementation of infant formulas with egg yolk lipids has been suggested, to more closely resemble the mother’s milk, and it has been found that while providing essential nutrients, the yolk lipids did not result in an increase in plasma cholesterol, indicating that it could safely be included in the infant diet (Makrides, 2002).

Finally, egg yolk cholesterol has also been suggested for use in the treatment of Smith-Lemli-Opitz syndrome, a condition in which the activity of 7-dehydrocholesterol delta(7)-reductase, the final enzyme in cholesterol biosynthesis, is reduced. Patients receiving supplemental dietary cholesterol from egg yolk exhibited a significant increase in plasma cholesterol levels, and a decrease in plasma levels of cholesterol precursors, which may be toxic (Linck et al., 2000).

**Phospholipids**

Phospholipids (PLs) are lipids which contain phosphate, and have a glycerol-phosphate backbone (Juneja, 1997). About 31% of the egg yolk lipids are PLs (234), 73% of which is phosphatidylcholine (PC), followed by phosphatidylethanolamine (PE 15%), lysophosphatidylcholine (LPC 5.8%), and sphingomyelin (SM 2.5%). The remaining 3.7% is made up of lysophosphatidylethanolamine (LPE 2.1%), plasmalogen (0.9%) and inositol phospholipid (IP) (Rhodes and Lea, 1957).

Phospholipids are amphiphilic molecules, having both polar and non-polar groups (Juneja, 1997), making them ideal for studying membranes and preparing liposomes (aqueous compartments enclosed by a lipid bilayer (Hartmann and Wilhelmson, 2001). Egg PC-containing liposomes have been shown to be effective for drug delivery to tumors (Chelvi and Ralhan, 1995; Avrilionis and Boggs, 1996; Harasym et al., 1997; Sharma et al., 1998), and for drug delivery to the brain (Kobayashi et al., 1996), reducing the toxicity and side effects, as well as prolonging the concentration compared to the free drug by itself. Feeding infants formula containing egg PLs was found to reduce the incidence of necrotizing enterocolitis, suggesting that one or more of the compounds of egg PLs may enhance the immature intestinal functions of infants (Carlson et al., 1998).

Egg yolk PLs were shown to decrease serum cholesterol levels in rats (Murata et al., 1982), with PC in particular reducing the intestinal absorption of cholesterol (Jiang et al., 2001), and they have been found to improve memory retention and increase acetylcholine concentrations, a neurotransmitter which decreases in concentration in cases of Alzheimer’s disease (Masuda et al., 1998; Favreliere et al., 2003). A diet including egg PC was also found to enhance “maze-learning” ability and brain functions in old mice (Lim and Suzuki, 2000).

It has been suggested that egg PLs may exert a degree of antiviral activity by interfering with the viral envelope formation, and by protecting or restoring transmembrane signaling functions in host cells (Liu and Watson, 2002). Furthermore, bacterial or viral infections may also be reduced by an activation of immune cells by egg
PLs, as immune cells grown *in vitro* require exogenous fatty acids, which could be provided by egg PLs (Liu and Watson, 2002).

More recently, research has focused on specific PL components, including arachidonic acid (AA), docosahexaenoic acid (DHA), and choline, whose metabolic products may play a role in membrane integrity, modulation of the membrane, and activation of immune cells (Liu and Watson, 2002). Egg yolk PC is a significant source of choline, which is an important nutrient in brain development, liver function, and cancer prevention (Gutierrez *et al.*, 1997). DHA and AA are important in the maintenance of normal neural functions, and have been shown to promote effects that are hypolipidemic, anti-thrombic, vasodilatory, and anti-inflammatory (Sheppard, 2002).

The biological activities associated with egg yolk components are summarized in Table 3.

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<th>Component</th>
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<td>Anti-venom applications</td>
<td>Thalley and Carroll, 1990 ; Almeida <em>et al.</em>, 1998</td>
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<td>Anti-inflammatory action</td>
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<td></td>
<td>Inhibition of xenograft rejection</td>
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<td>Antioxidant activity</td>
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Conclusion

In the last decade, extensive studies have been conducted to characterize the bio-physiological functions of egg components and to seek novel biologically active substances in hen eggs. By compiling these studies into a single focussed work, this review provides evidence that hen eggs contain various biologically active substances with specific benefits for human health, and significant potential for medical, nutraceutical and food-fortification applications.

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