# The biological action of saponins in animal systems: a review

George Francis[1], Zohar Kerem[2], Harinder P. S. Makkar[3] and Klaus Becker[1]*

[1]*Department of Aquaculture Systems and Animal Nutrition, Institute for Animal Production in the Tropics and Subtropics, University of Hohenheim (480), D 70593 Stuttgart, Germany*
[2]*Institute of Biochemistry, Food Science and Nutrition, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, P.O.B. 12, Rehovot 76100, Israel*
[3]*Animal Production and Health Section, International Atomic Energy Agency, P.O. Box 100, Wagramerstr. 5, A-1400 Vienna, Austria*

Saponins are steroid or triterpenoid glycosides, common in a large number of plants and plant products that are important in human and animal nutrition. Several biological effects have been ascribed to saponins. Extensive research has been carried out into the membrane-permeabilising, immunostimulant, hypocholesterolaemic and anticarcinogenic properties of saponins and they have also been found to significantly affect growth, feed intake and reproduction in animals. These structurally diverse compounds have also been observed to kill protozoans and molluscs, to be antioxidants, to impair the digestion of protein and the uptake of vitamins and minerals in the gut, to cause hypoglycaemia, and to act as antifungal and antiviral agents. These compounds can thus affect animals in a host of different ways both positive and negative.

The saponins are naturally occurring surface-active glycosides. They are mainly produced by plants, but also by lower marine animals and some bacteria (Riguera, 1997; Yoshiki *et al.* 1998). They derive their name from their ability to form stable, soap-like foams in aqueous solutions. This easily observable character has attracted human interest from ancient times. Saponins consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin) which may be triterpenoid (Fig. 1(a)) or steroid (Fig. 1(b)) in nature. The aglycone may contain one or more unsaturated C–C bonds. The oligosaccharide chain is normally attached at the $C_3$ position (monodesmosidic), but many saponins have an additional sugar moiety at the $C_{26}$ or $C_{28}$ position (bidesmosidic). The great complexity of saponin structure arises from the variability of the aglycone structure, the nature of the side chains and the position of attachment of these moieties on the aglycone. Experiments demonstrating the physiological, immunological and pharmacological properties of saponins have provoked considerable clinical interest in these substances.

There have been several reviews in recent years of published reports about various properties of saponins (Kensil, 1996; Barr *et al.* 1998; Sen *et al.* 1998; Yoshiki *et al.* 1998). Most of them, however, deal with either specific properties or specific sources of saponins. The purpose of the present review is to provide an overview of the extremely diverse biological activities of saponins, relate these to their structure and try to understand the molecular mechanism of their activity, as far as the available literature permits. It is hoped that the information collated here will provide the reader with information regarding the various potential applications of saponins, and stimulate further research into these compounds.

## Occurrence

Saponins occur constitutively in a great many plant species, in both wild plants and cultivated crops. In cultivated crops the triterpenoid saponins are generally predominant, while steroid saponins are common in plants used as herbs or for their health-promoting properties (Fenwick *et al.* 1991). Triterpenoid saponins have been
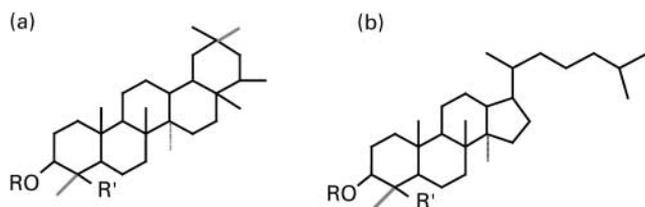
**Fig. 1.** Basic structures of sapogenins: a triterpenoid (a) and a steroid (b).

detected in many legumes such as soyabeans, beans, peas, lucerne, etc. and also in alliums, tea, spinach, sugar beet, quinoa, liquorice, sunflower, horse chestnut, and ginseng. Steroid saponins are found in oats, capsicum peppers, aubergine, tomato seed, alliums, asparagus, yam, fenugreek, yucca and ginseng. One example of an extensively studied group of triterpenoid saponins is produced from *Quillaja saponaria*, a tree native to the Andes region. The bark was peeled off and extracted with water by the indigenous peoples as a shampooing agent, and by the Shamans as an overall curing agent. *Yucca schidigera* is the most common commercial source of steroid saponins.

In general, very little is known about the enzymes and biochemical pathways involved in saponin biosynthesis. Triterpenoid saponins are synthesised via the isoprenoid pathway by cyclisation of 2,3-oxidosqualene to give primarily oleanane (β amyrin) or dammarane triterpenoid skeletons. The genetic machinery required for the elaboration of this important family of plant secondary metabolites is as yet largely uncharacterised, despite the considerable commercial interest in this important group of natural products. This is likely to be due in part to the complexity of the molecules and the lack of commercially available pathway intermediates for biochemical studies. A recent review describes the advances made in the area of 2,3-oxidosqualene cyclisation, the genes that encode enzymes giving rise to the diverse array of plant triterpenoid skeletons, and the characterisation of saponin glucosyltransferases (Haralampidis *et al.* 2002). A number of factors, such as physiological age, environmental and agronomic factors, have been shown to affect the saponin content of plants (for a review, see Yoshiki *et al.* 1998). Reports reviewed here indicate that saponins increase on sprouting in some plants such as soyabeans, lucerne, mung beans, and peas but decrease in others such as moth bean, and that light availability during germination has a profound stimulating effect on the saponin content. Generally, immature plants of a species have been found to have higher saponin contents than more mature plants of the same species.

## Role in plants

The physiological role of saponins in plants is not yet fully understood. While there is a number of publications describing their identification in plants, and their multiple effects in animal cells and on fungi and bacteria, only a few have addressed their function in plant cells. Many saponins are known to be antimicrobial, to inhibit mould,

and to protect plants from insect attack. Saponins may be considered a part of plants' defence systems, and as such have been included in a large group of protective molecules found in plants named 'phytoanticipins' or 'phytoprotectants' (Morrissey & Osbourn, 1999). The first term describes those saponins, such as A and B avenacosides in oat, that are activated by the plant's enzymes in response to tissue damage or pathogen attack (Gus-Mayer *et al.* 1994). The second describes those saponins that have a general anti-microbial or anti-insect activity. A glycosylated triterpenoid saponin from peas (*Pisum sativum*) was purified and characterised as a specific inhibitor of diguanylate cyclase, a key regulatory enzyme in the synthesis of cellulose (Ohana *et al.* 1998). It has also been suggested that saponins could be a source of monosaccharides (see Barr *et al.* 1998).

## Isolation and characterisation of saponins

The unique chemical nature of saponins demands tedious and sophisticated techniques for their isolation, structure elucidation and analysis. The task of isolating saponins from plant material is complicated also by the occurrence of many closely related substances in plant tissues, and by the fact that most of the saponins lack a chromophore. Thus, for many years, the complete characterisation of saponins from even well-known saponin-containing plants was not achieved. However, recently renewed interest in medicinal plants and foods alongside the dramatic evolution of analytical tools has resulted in a burst of publications presenting numerous novel saponins. The modern methods available for the separation and analysis of saponins have been well reviewed by Marston *et al.* (2000), Muir *et al.* (2000) and Schopke (2000). These methods will be only briefly outlined in the present review.

There are several strategies available for the isolation of saponins. As a general rule, they begin with the extraction of the plant material with aqueous methanol or ethanol. Further processing of the extract is carried out after evaporation under reduced pressure, dissolution in a small amount of water and phase separation into n-butanol. It is currently recognised that this step is sometimes undesirable, since only those saponins with short oligosaccharide side chains will eventually be extracted into the butanolic phase. A further purification is then carried out, which involves liquid chromatography over a silica gel column, or a gradient elution from a polymeric support or liquid–liquid partition chromatography, or, as most commonly employed, HPLC separation. In most cases, certain of the above steps have to be repeated with a change of support or eluent to achieve high purity.

Once the saponin has been purified, it may be subjected to analytical methods including MS, proton and carbon NMR, and infrared spectroscopy. Other classical methods are used to ascertain the presence of saponins in a crude plant extract, and to elucidate their composition throughout purification steps. TLC and staining with dehydrating reagents containing aromatic aldehydes (such as anisyl aldehyde in sulfuric acid) are commonly used. The pure saponin may also be hydrolysed to verify the nature of its glycosidic moieties.

## Biological effects in animals

### Effects on cell membranes

*Permeabilisation and effects on other membrane properties.* A large number of the biological effects of saponins have been ascribed to their action on membranes. In fact, their specific ability to form pores in membranes has contributed to their common use in physiological research (El Izzi *et al.* 1992; Authi *et al.* 1988; Choi *et al.* 2001; Menin *et al.* 2001; Plock *et al.* 2001). Saponins have long been known to have a lytic action on erythrocyte membranes and this property has been used for their detection. The haemolytic action of saponins is believed to be the result of the affinity of the aglycone moiety for membrane sterols, particularly cholesterol (Glauert *et al.* 1962), with which they form insoluble complexes (Bangham & Horne, 1962). The amount of glycosides required for permeabilisation is much lower for cholesterol-rich lipid layers than cholesterol-free membranes (Gögelein & Hüby, 1984). Isolated cell membranes from human erythrocytes when treated with saponin developed pores of 40–50 Å diameter as against the 80 Å pores produced in artificial membranes (Seeman *et al.* 1973). Compared with the reversible perforations caused by substances such as vitamin A, the membrane pores or defects produced by saponins were long-lasting and such membranes were then permanently permeable to large molecules like ferritin (Seeman, 1974). The lesions that are caused by saponins are thought to be a micelle-like aggregation of saponins and cholesterol in the plane of the membrane, possibly with saponin molecules arranged in a ring with their hydrophobic moieties combined with cholesterol around the outer perimeter (Bangham & Horne, 1962; Seeman, 1974). Other reports depict the interactions between saponins and biological membranes to be more complex. Brain *et al.* (1990) showed that insertion of the aglycone into the lipid bilayer is independent of the presence of cholesterol. Saponins could induce a permeability change on liposomal membrane without cholesterol when they are glycosylated at both $C_3$ and $C_{28}$ (bidesmosidic) of the oleanolic aglycone (Hu *et al.* 1996). Abe *et al.* (1978*a*) observed no close and direct relationship between the haemolytic activity of saikosaponin (from *Bupleurum falcatum*) and membrane-permeabilising activity, nor was it correlated with either their surface or interfacial tension-lowering properties (Pillion *et al.* 1996; Steurer *et al.* 1999). The efficacy as absorption-enhancing agents across nasal mucosa in rats was greatest in those *Quillaja* saponins with the lowest surfactant strength and haemolytic titres (Pillion *et al.* 1996).

Cholesterol enrichment was shown to have an inhibitory effect on many membrane ATPases, as it may directly interact with the boundary lipids of ATPase and alter the intermolecular hydrogen bonds of the protein. Ginsenosides (from *Panax quinquefolius* and *P. japonicus*) share the steroid backbone and amphipathic nature with cholesterol. The desacyl-jego-saponin (from *Styrax japonica*) and gensenoside-Rd, which had little or no effect on membrane permeability, were capable of stimulating $Na^+$–$Ca^+$ exchange activity in canine cardiac sarcolemmal vesicles (Yamasaki *et al.* 1987; Choi *et al.* 2001). However, not all ginsenosides alter the $Na^+$–$K^+$ ATPase and $Ca^{2+}$–$Mg^{2+}$ ATPase activity in neurones in the same manner (Choi *et al.* 2001). It is possible that some ginsenosides interact with membrane cholesterol and displace it from the immediate environment of ATPases. Since removal of cholesterol will lead to an increase in membrane fluidity, conformational changes that ATPases undergo during their transport cycle may be facilitated. Membrane fluidity controls the enzyme activity of biological membranes and has an important role in ion transport (Ma & Xiao, 1998) and the ability of saponins to affect this parameter may explain their effects on cellular function. The inotropic action of saponins, supposed to be caused by their effect on membrane $Ca^{2+}$ channels, also did not have any simple correlation with membrane permeability effect (Enomoto *et al.* 1986). Saponins such as ophiopogonins and ginsenocides haemagglutinated human, rabbit, and sheep erythrocytes but were not haemolytic (Takechi & Tanaka, 1995*a*). They were thus able to bind to the membrane lipids of erythrocytes and form bridges between the cells. Other studies (Gögelein & Hüby, 1984) explained the increase in electrical conductance caused by saponins in planar lipid bi-layers to be due to fluctuating membrane channels. Soyasaponins I and III, and dehydrosoyasaponin I (isolated from *Desmodium adscendens*) have been shown to be able to open large Ca-dependent K (maxi-K) conductance channels causing membrane hyperpolarization, suppression of electrical activity and relaxation of smooth muscle (McManus *et al.* 1993). On the other hand there are also reports of the ability of saponins to block membrane ion channels on neurons (Kai *et al.* 1998) and human neutrophils (Bei *et al.* 1998). The interactions are therefore complex and may involve different mechanisms.

The side chains present on the aglycone such as sugar chains (Segal *et al.* 1974; Santos *et al.* 1997), acyl recidues (Matsuda *et al.* 1997) or the epoxy-framework system (Abe *et al.* 1978*b*) are also supposed to contribute to the effects on membranes. These effects however were not uniform; for example some saponins with acyl residue (lablaboside D) did not show haemolytic activity (Oda *et al.* 2000), while in another work, only acylated triterpenoid saponins in low concentrations were able to alter membrane activity (Melzig *et al.* 2001). In the latter case, it was suggested that only acetylated saponins might integrate transiently into membranes, thus inducing pore-like structures. It has been suggested that the haemolytic activity of saponins increases with increasing numbers of polar groups in the aglycone moiety (Namba *et al.* 1973). Carbohydrate chain length was shown to influence the manifestation of physiological activity of oligosides (Kuznetzova *et al.* 1982). Steroid and triterpenoid saponins with a single sugar chain (monodesmosides) were found to have strong haemolytic activity, whereas those with two sugar chains (bidesmosides) showed less activity (Fukuda *et al.* 1985; Woldemichael & Wink, 2001). Even though monodesmoside saponins are generally considered to be more active than bidesmosides, there are exceptions. For example, among fifteen synthetic methyl ursolate glycosides, di- and triglycosides showed much higher haemolytic activity than monoglycosides (Takechi & Tanaka, 1995*b*). It was also observed that an increase in sugar moieties enhanced

the effects of saponins on sarcolemmal membrane $Ca^{2+}$ permeability (Yamasaki *et al.* 1987). The permeabilising activity of the native avenacin A-1 was completely abolished after one, two, or all three sugar residues were hydrolysed to yield mono-deglucosyl, bis-deglucosyl, and aglycone derivatives respectively (Armah *et al.* 1999). De-acylated *Quillaja* saponins 1 and 2, which differ only in the absence of one glucose residue, differed significantly in their ability to stimulate absorption of insulin despite having similar surfactant strength and haemolytic potency (Pillion *et al.* 1996). The stereochemistry of the terminal sugar on the saccharide chain also appears to be important in conferring activity on the saponin molecule because of its ability to affect the overall shape of the molecule (Gee *et al.* 1998). The relationship between increased saccharide branching and increased permeabilising activity and the haemolytic property of saponins was not a direct one (Price *et al.* 1994) due to the large number of factors involved. Generally both the aglycone and glycan parts seem to play a role in the haemolytic activity of saponins (Apers *et al.* 2000). The neutral and acidic triterpenoids, and the acyl glycosides are very weakly active, whereas the ester saponins (for example, maesasaponins) are strongly haemolytic.

The interaction between saponins and membrane lipids thus seems to be complicated, with the composition of the target membrane, the type of side chain, and the nature of the aglycone to which these are attached all appearing to be necessary to produce a permeabilising effect (Gee *et al.* 1998; Attele *et al.* 1999). Cellular membranes may exist under conditions of curvature stress, being close to the hexagonal phase transition. Consequently, the physicochemical properties of these membranes are sensitive to changes in membrane components and lipophilic agents, which may modulate curvature stress. Membrane proteins are thought to be localised selectively in cholesterol-rich domains (acetylcholine receptor) or in cholesterol-poor domains (the sarcoplasmic $Ca^{2+}$ ATPase). Therefore, the biophysical properties of the different domains, rather than the bulk lipid, may selectively influence transmembrane protein function and mimic specificity at the effector level. Saponins may interact with the polar heads of membrane phospholipids and the -OH group of cholesterol through OH groups at $C_3$ or $C_{28}$, which will result in their later ability to form micelle-like aggregates. Moreover, their hydrophobic aglycone backbone could intercalate into the hydrophobic interior of the bilayer. Both of these effects may contribute to the alteration of the lipid environment around membrane proteins. It has become increasingly evident that the lipid environment of membrane proteins, including ion channels, transporters, and receptors, plays an important role in their function. The changed-function proteins or glycoproteins in the plasma membranes have been suggested to be the cause of secondary biochemical responses induced by saponins (Abe *et al.* 1978*b*; Rao & Sung, 1995).

The precise details of the interactions between saponins and membranes need more elucidation so that the molecular mechanisms involved could be better understood. It seems likely that different mechanisms such as the formation of the saponin–cholesterol complex, alterations in the organisation of sarcolemmal membrane phospholipids, and the formation of phospholipid breakdown products such as phosphatidic acid (Yamasaki *et al.* 1987) in addition to saponin structure and three-dimentional orientation are all involved in the actions of saponins on membranes.

*Effects on nutrient uptake through the intestinal membrane.* Johnson *et al.* (1986) found that some saponins increase the permeability of intestinal mucosal cells *in vitro*, inhibit active mucosal transport and facilitate uptake of substances that are normally not absorbed. Saponins (from *Gypsophila*, *Quillaja*, clover, guar and lucerne) also lower transmural potential difference (TPD, the electrochemical gradient that acts as a driving force for active nutrient transport across the brush border membrane of the intestine) across the small intestine of the rat (Gee *et al.* 1989). Here the stereo-structure of the saccharide chain appeared to be an important feature in conferring this ability. Soyasaponins with a non-acidic triterpenoid moiety coupled to a straight-chain tri-saccharide caused only a small drop in TPD in Wistar rat intestines *in vitro* whereas saponins from *Gypsophila*, lucerne and guar, which contain acidic triterpenes coupled to branched tetrasaccharide moieties caused significant changes in TPD. Among lucerne saponins (glycosides of medicagenic acid), bidesmosides containing four sugar moieties reduced rat-intestine TPD *in vitro* in a similar fashion to the monodesmoside and to medicagenic acid itself (one exception was 3Glc, 28Glc-medicagenic acid which had no effect on TPD) (Oleszek *et al.* 1994). The tridesmoside of zahnic acid, which is only weakly haemolytic and which neither inhibits fungal growth nor forms insoluble complexes with cholesterol, was the most active compound, giving further evidence of the complexity of the interactions between saponins and membranes.

An increase in the apparent permeability of the brush border observed at sublethal levels of saponins may have important implications for the uptake of macromolecules, such as allergens, whose passage through the epithelium is normally somewhat restricted (Gee *et al.* 1996). The presence of *Gypsophila* saponins enhanced the uptake of β lactoglobulin, a milk allergen in the jejunal loops' tips of brown Norway rats (Gee *et al.* 1997). Histological investigation of the mucosal epithelium exposed to saponin revealed damage, especially of the villi. Rats fed the unwashed bitter quinoa diets (containing saponins that caused rapid depolarisation of the small-intestinal mucosa, even at concentrations of less than 50 μg/ml) demonstrated growth impairment and decreased food conversion efficiency (Gee *et al.* 1993). Avenacosides that were haemolytic in the crude extract significantly increased the passage of ovalbumin in rat intestine *in vitro* at a concentration of 1 mg/ml but did not affect the active transport of glucose (Onning *et al.* 1996). However, when given *in vivo* in the same experiment the saponins did not affect the transport of bovine serum albumin across the intestine wall. *Quillaja* saponins were able to increase uptake of human Γ globulin into the body, when fed to tilapia or administered by anal incubation (Jenkins *et al.* 1991; Anderson, 1992). Rat intestines have been shown to

offset the damage to small-intestinal mucosal cells caused by dietary saponins (up to 1·5 %) by continuous proliferation (Gee & Johnson, 1988).

Some reports describe obstruction of the absorption of micronutrients by dietary saponins. *Gypsophila* saponins in the diet depressed mean liver Fe concentrations and total liver Fe by impairing Fe absorption (Southon *et al.* 1988). Probably the saponin formed complexes with dietary Fe rendering it unavailable for absorption. Lucerne saponins were shown to increase excretion of Fe and Mg when present in rat diets, and reduce plasma Ca and Zn in pigs (for references, see Southon *et al.* 1988). These saponins could complex with Fe and Zn *in vitro* and this complex formation might have hindered their absorption. Triterpenoid saponins from *Gypsophila* and *Quillaja* included in the diet appeared to interfere with the absorption of vitamins A and E in chicks (Jenkins & Atwal, 1994). Feeding a steroid saponin (sarsasaponin) at the same level had no effect on any of these parameters.

The mechanism of action of saponins on the intestinal membranes *in vivo* is not yet clearly understood. Ingested saponins are exposed to many potential ligands in the intestine such as bile salts, dietary cholesterol and membrane sterols of the mucosal cells, and nutrients or antinutrients in food, all of which may reduce or enhance their effectiveness. It also remains to be confirmed whether traces of the compound itself enter the body through the permeabilized membranes, even though all the evidence until now points to their non-absorption (Yoshikoshi *et al.* 1995). Absorption of saponin metabolites produced in the intestine by micro-organisms into the body of ruminants (Flaoyen *et al.* 2001; Meagher *et al.* 2001) and human subjects (Wakabayashi *et al.* 1997; Lee *et al.* 2000c) has, however, been demonstrated.

### Effects on animal growth and feed intake

Animal nutritionists have generally considered saponins to be deleterious compounds. In ruminants and other domestic animals the dietary saponins have significant effects on all phases of metabolism, from the ingestion of feed to the excretion of wastes (Cheeke, 1996). Lucerne and soya beans are the main examples of saponin-rich plants that serve extensively in human, ruminant and poultry diets. Recently, a number of studies have reported both beneficial and adverse effects of these compounds in a variety of animals (for a review, see Sen *et al.* 1998).

*Effects in ruminants.* *Y. schidigera* plant extract (the plant is common in south and central America where it is used as animal feed, and like many other saponin-rich plants, as a herbal medicine) has been found to improve growth, feed efficiency and health in ruminants (Mader & Brumm, 1987). *Quillaja* saponins increased the efficiency of *in vitro* rumen-microbial protein synthesis and decreased degradability of feed protein (Makkar & Becker, 1996). Partially hydrolysed lucerne saponins administered intra-ruminally resulted in a significant reduction in the total protozoa count in the rumen of sheep (Lu & Jorgensen, 1987) which may be the reason for the decrease in feed protein degradability. Saponins are considered to have detrimental effects on protozoa

through their binding with sterols present on the protozoal surface. Sterols are absent on bacterial membranes. *Yucca* extract can also bind $NH_4$ when ruminal $NH_4$ concentrations are high, and release it again when ruminal $NH_4$ is low, providing a continuous and adequate supply of $NH_4$ for microbial protein synthesis (Hussain & Cheeke, 1995). A positive effect of *Yucca* saponins in ruminant nutrition was attributed to the enhancement of the entrapment of $NH_4$-N from urea-supplemented straw (Makkar *et al.* 1999). This increases the availability of nutrients to rumen bacteria and reduces environmental damage by decreasing losses of $NH_4$ to the air. Supplementation of feed with leaves of *Sesbania sesban*, known for its high saponin content, has been found to have the potential to improve protein flow from the rumen by suppressing protozoal action there (Newbold *et al.* 1997) but rumen bacteria were observed to be capable of metabolising the antiprotozoal factor. Saponins may also be degraded by the saliva of sheep fed saponin-rich foods for a long time (Odenyo *et al.* 1997; Teferedegne, 2000). GC–MS analysis of tissue samples (bile, urine, rumen, duodenum, jejunum, colon and rectum, and faeces) from sheep fed *Narthecium ossifragum* (containing mainly sarsasapogenin and smilagenin) for 6 d, followed by 20 mg [20,23,23-H-2(3)]sarsasapogenin/d on the seventh day revealed only negligible levels of $^2$H-labelled sarsasapogenins in these organs (Flaoyen *et al.* 2001). Ingested saponins were quickly hydrolysed in the rumen to free sapogenins and, in part, epimerized at $C_3$ into episapogenins. The absorption of free sapogenins appeared to occur in the jejunum. The concentration of sapogenins in faeces reached a plateau 108 h after dosing started.

The positive effects of saponins were more pronounced when they were directly administered into the rumen rather than added to the feed (Odenyo *et al.* 1997). Killeen *et al.* (1998) proposed that a surfactant or flocculent action of saponins on the feed constituents that alters the rate of digestion would account for the substrate-dependent nature of the effect of *Y. schidigera* on rumen DM and N digestibility. This substrate-dependency might also be due to negative effects of saponins on specific bacterial populations (Hussain & Cheeke, 1995). Wang *et al.* (2000a,b) observed that supplementation with *Yucca* extracts might be beneficial to ruminants fed a high-grain diet. *Yucca* saponins were found to have a direct negative effect on cellulolytic bacteria while being harmless to amylolytic bacteria, suggesting the possibility of using saponins for 'designing' the rumen population. The mechanism of action against bacteriae remains unclear. Other reports point to the fact that some of the effects of dietary saponins in lambs are sex-dependent. Bosler *et al.* (1997) found that both male and female lambs fed up to 40 mg *Quillaja* saponin/kg mixed with a basal diet had significantly higher average daily weight gains than controls but that the difference in weight gain was lower in the females. The dietary saponins reduced the fat deposits around the kidney in females while increasing it in males.

Dietary saponins were often suspected of having a role in causing ruminant bloat (Cheeke, 1996; Sen *et al.* 1998), but clear experimental proof for this is lacking in the literature. The absence of any positive effect in

experiments where *Yucca* saponins were fed to ruminants (for example, Wu *et al.* 1994) might have been because animals with adapted rumen microbial population were used.

*Effects in fish.* Saponins have been reported to be highly toxic to fish because of their damaging effect on the respiratory epithelia (Roy *et al.* 1990). They are also considered to be the active components of many traditionally used fish poisons, like mahua oil cake (see Francis *et al.* 2001a). Fish have also been shown to exhibit stress reactions to the presence of saponins in water. Roy & Munshi (1989) reported that the $O_2$ uptake of perch (*Anabas testudineus*) increased with a concomitant increase in the erythrocyte, haemoglobin and packed cell volume levels, after the fish had been in water containing 5 mg *Quillaja* saponin/l for 24 h. *Penaeus japonicus* that had been previously exposed to concentrations of 20 mg saponin/l for 24 h increased both respiration rate and metabolism (measured as increase in $O_2$ uptake and $NH_4$ excretion) during a 6 h detoxification process (Chen & Chen, 1997). Bureau *et al.* (1998) observed that *Quillaja* saponins damaged the intestinal mucosa in rainbow trout and Chinook salmon at dietary levels above 1·5 g/kg. The condition of the intestines of these fish was similar to that of fish fed a raw soyabean-meal diet indicating the role of saponins in causing the damage. Krogdahl *et al.* (1995), however, did not find any negative effects when saponins were included in the diet of Atlantic salmon at levels similar to those likely to be found in a soyabean meal (300–400 g/kg)-based diet. In the same study, an alcohol extract of soyabean meal caused growth retardation, altered intestinal morphology, and depressed mucosal enzyme activity in the lower intestine. Dietary levels of triterpenoid *Quillaja* saponins of 150 mg/kg showed potential for promoting growth and nutrient utilisation in common carp and tilapia (Francis *et al.* 2001b, 2002a). Growth in common carp was significantly higher than control only when there was a continuous dietary supply of the saponins (Francis *et al.* 2002b). The growth-promoting effect was not pronounced in common carp fed steroid *Y. schidigera* saponins (G Francis, unpublished results). The effects of dietary saponins in fish such as higher growth, reduced $O_2$ consumption and metabolic rate that we observed (Francis *et al.* 2001b; 2002a,b) point to a possible systemic effect. The mechanisms whereby *Quillaja* saponins increased the growth and food conversion efficiency in fish remain to be ascertained.

*Effects in other single-stomached animals.* Legume grains form the staple food in a large part of the world, and serve also as the largest source for plant protein. Surprisingly there are only scarce reports on the effects of soyasaponins (common among legumes other than soyabean as well) on mammals, birds and cold-blooded organisms but for a few reports during the late 60s to the early 70s of the 20th century. Soyabean saponins did not impair growth of chicks when added at five times the concentration in a normal soyabean-supplemented diet (Ishaaya *et al.* 1969). In the same work they observed no effect on the growth response of rats and mice or on the amount of ingested food. *Y. schidigera* extract has also been found to improve growth, feed efficiency and health

in poultry and pigs by mechanisms that are not as yet understood (Johnston *et al.* 1981, 1982; Mader & Brumm, 1987; Anthony *et al.* 1994). Male Wistar rats, given 10 and 100 mg fenugreek extract/300 g body weight mixed with their food had significantly higher feed intake and appetite (Petit *et al.* 1993). The circadian rhythm of feeding behaviour was modified so that the rats fed the fenugreek extract ate continuously during 24 h rather than just at night (Petit *et al.* 1995).

There are also numerous reports of negative effects of dietary saponins. Dietary saponins depressed growth, feed consumption in gerbils and egg production in poultry (Sim *et al.* 1984; Terapunduwat & Tasaki, 1986; Potter *et al.* 1993; Jenkins & Atwal, 1994). These negative effects have been ascribed to several properties of saponins such as reduced feed intake caused by the astringent and irritating taste of saponins (see Oleszek *et al.* 1994), reduction in intestinal motility (Klita *et al.* 1996), reduction in protein digestibility (Shimoyamada *et al.* 1998) and damage to the intestinal membrane and inhibition of nutrient transport described earlier in the present review. Production of active metabolites (see Wakabayashi *et al.* 1998) by hydrolysis of saponins by intestinal bacteria (Bae *et al.* 2000) also needs to be considered. More investigations are required into the fate of ingested saponins in the digestive tract of ruminants and single-stomached animals.

### Effects on protein digestion

Saponins reduce protein digestibility probably by the formation of sparingly digestible saponin–protein complexes (Potter *et al.* 1993). Endogenous saponins affected the chymotrypsic hydrolysis of soyabean protein, particularly glycinin (Shimoyamada *et al.* 1998). The heat stability of bovine serum albumin was increased by the addition of soyasaponin due to electrostatic and hydrophobic interactions. The digestibility of the bovine serum albumin–soyasaponin complex was much lower than that of free bovine serum albumin indicating that complexing with saponin had an obstructing effect. Soyasaponin seemed to slightly activate α-chymotrypsin, but the effect was not great (Ikedo *et al.* 1996). It has also been shown that casein and *Quillaja* saponin form complexes of high molecular weight at elevated temperatures (78°C). Starfish saponin accelerated the thermal aggregation of actomyosin from walleye pollack muscle (Ishisaki *et al.* 1997). This enhancing effect increased concomitantly with increase in starfish saponin:actomyosin. *Quillaja* saponin was also reported to accelerate the thermal aggregation of actomyosin (Ishisaki *et al.* 1997). The same research group showed that tea saponins had the ability to suppress the heat denaturation of salt-soluble proteins from fish, while *Quillaja* saponin accelerated the reaction indicating a lack of uniformity in the way saponins act with regard to these effects.

A large number of foods and feed materials contain both saponins and proteins. The nature of the interactions between them would influence the nutritive value of a diet, and hence these interactions need to be studied to elucidate possible structure–activity relationships.

## Hypoglycaemic activity

Saponins isolated from plants such as fenugreek (Petit *et al.* 1993), *Phellodendron cortex* and *Aralia cortex* (Kim *et al.* 1998*c*), *Pueraria thunbergiana* (Lee *et al.* 2000*a*), and *Calendula officinalis* (Yoshikawa *et al.* 2001) have been shown to have hypoglycaemic effects. Petit *et al.* (1993) found chronically higher plasma insulin levels, probably caused by stimulation of the β-cells in male Wistar rats given 10 and 100 mg fenugreek extract/300 g body weight mixed with food while Matsuda *et al.* (1999*b*) did not find insulin-like or insulin-releasing activity in rats given oleanolic acid glycosides orally. The hypoglycaemic action here was due to suppression of the transfer of glucose from the stomach to the small intestine and the inhibition of glucose transport across the brush border of the small intestine. The saponin momordin Ic was also found to significantly and dose-dependently inhibit gastric emptying (Matsuda *et al.* 1999*a*). The inhibitory activity here was dependent on the level of serum glucose and mediated at least in part by the capsaicin-sensitive sensory nerves and the central nervous system. Yoshikawa *et al.* (2001) showed that the oleanolic acid 3-monodesmosides with hypoglycaemic activity also inhibited gastric emptying showing a correlation between the two effects, while other saponins showing no hypoglycaemic activity in the mixture also did not affect gastric emptying.

## Effects on cholesterol metabolism

A number of studies have shown that saponins from different sources lower serum cholesterol levels in a variety of animals including human subjects (Southon *et al.* 1988; Harwood *et al.* 1993; Potter *et al.* 1993; Matsuura, 2001; also, for a review, see Al-Habori & Raman, 1998). Large mixed micelles formed by the interaction of saponins with bile acids account for their increased excretion when saponin-rich foods such as soyabean, lucerne and chickpea are consumed (Oakenfull, 1986; Oakenfull & Sidhu, 1990). The resulting accelerated metabolism of cholesterol in the liver causes its serum levels to go down. The ethanol extract of de-fatted fenugreek seeds inhibited taurocholate and deoxycholate absorption *in vitro*, in a dose-dependent manner in everted intestinal sacs (Stark & Madar, 1993). Decreased intestinal cholesterol absorption induced by some saponins, however, was seen to be without interference with the entero-hepatic bile acid recirculation (Harwood *et al.* 1993). Saponins also reduced the more harmful LDL-cholesterol selectively in the serum of rats, gerbils and human subjects (Potter *et al.* 1993; Harris *et al.* 1997; Matsuura, 2001). Morehouse *et al.* (1999) found that the mechanism of action of saponins was luminal but did not involve stoichiometric complexation with cholesterol. They also found that the synthetic saponins tiqueside and pamaqueside were much more potent than naturally occurring saponins such as those from lucerne in preventing hypercholesterolaemia and that the *in vivo* potency of pamaquecide was 10-fold that of tiqueside even though it differs from tiqueside only by an additional keto group. Other suggested mechanisms of action of saponins include delaying the intestinal

absorption of dietary fat by inhibiting pancreatic lipase activity (Han *et al.* 2000).

Not all reports, however, agree on the anticholesterolaemic activity of saponins. Soyabean and lucerne saponins were found to be not singularly responsible for the hypocholesterolaemic effect of diets containing them (Calvert & Blight, 1981; Story *et al.* 1984; Sugano *et al.* 1990). Results from our laboratory showed that the level of muscle cholesterol in tilapia fed small amounts of *Quillaja* saponins (up to 300 mg/kg) was higher than that of controls (Francis *et al.* 2001*b*), while the serum levels were not different from control (G Francis, unpublished results). It has also been noted that a saponin-induced reduction of serum cholesterol occurred only when a hypercholesterolaemic diet had been fed (Jenkins & Atwal, 1994).

There is also evidence of increased cholesterol synthesis to compensate for saponin-induced excretion (Jenkins & Atwal, 1994). Dietary sarsasaponin failed to lower the cholesterol content of egg yolk or the serum of laying hens even though it increased the excretion of cholesterol and decreased the transfer of dietary cholesterol to the eggs (Sim *et al.* 1984).

It can be concluded that several dietary saponins do have a hypocholesterolaemic action. Since cholesterol binding takes place in the intestinal lumen, factors such as quantity of saponins and cholesterol, and the presence of other ligands of both these compounds may play a role and these may have caused the observed discrepancies among the various results. Knowledge of the nature of the interaction between the particular saponin and cholesterol, and the nature of the cholesterol moieties and other ligands in the diet are essential to arrive at an effective dietary dose of that particular saponin that could have a significant hypocholesterolaemic effect.

## Effects on animal reproduction

The negative effects of saponins on animal reproduction have long been known and have been ascribed to their abortifacient, antizygotic and anti-implantation properties (Tewary *et al.* 1973; Stolzenberg & Parkhurst, 1976). Saponins from broom weed (*Gutierrezia* sp.) and lechuguilla (*Agave lecheguilla*) or commercial pharmaceutical-grade saponins caused abortion or death or both in rabbits, goats and cows when administered intravenously at concentrations above 2·3 mg/kg body weight (Dollahite *et al.* 1962). Saponins isolated from the crude extract of *Gleditischia horrida*, *Costus speciosus* Sm and *Phytolacca dodecandra* caused sterility in mice (Chou *et al.* 1971; Tewary *et al.* 1973; Stolzenberg & Parkhurst, 1976). Quin & Xu (1998) found that the butanol extract of *Mussaenda pubescens* was capable of terminating pregnancy in rats. Extracts of this plant are used as a contraceptive in the Fujian province of China.

Saponins were found to be extremely strong stimulators of luteinising hormone release from cultured hypophysial cells (El Izzi *et al.* 1989; Benie *et al.* 1990) but their action was neutralised in the presence of serum indicating a passive membrane-permeabilising effect (El Izzi *et al.* 1992) in this case. However, saponin-rich extracts from *Combretodendron africanum* injected into female rats

stimulated uterine growth, lowered luteinizing hormone release, and blocked the oestrous cycle (Benie *et al.* 1990). The steroidal saponin 3β,16β,17α-tri-hydroxy-cholest-5-en-22-one 16-0-(2-0-4-methoxybenzoyl-β-D-xylopyranosyl)-(1 → 3)-(2-0-acetyl-α-L-arabinopyranoside) (or OSW-1) isolated from *Ornithogalum saundersiae* injected into rats on the morning of pro-oestrous at a level of 9 μg/kg inhibited oestrogen production and prolonged the period of dioestrous (Tamura *et al.* 1997). The steroid saponin was found to directly inhibit the genes responsible for steroidogenesis, and also suppress the proliferation of follicle-stimulating hormone-modulated granulosa cells in the ovarian follicle. The mechanism of suppression of cell proliferation here might be through a similar mechanism as saponin-induced proliferation of tumour cells.

Saponins have been shown to have both positive and negative effects on the viability of human sperm cells *in vitro* with some ginseng saponins increasing motility as well as progression of sperm (Chen *et al.* 1998) while *Sesbania sesban* saponins were spermicidal at 1·0–1·3 mg/ml (Dorsaz *et al.* 1988). It is debatable how relevant these *in vitro* effects are for dietary saponins as there are relatively few reports in this regard.

Saponin-rich extracts of *Turnera diffusa* and *Pfaffia paniculata* improved the copulatory performance of sexually sluggish or impotent rats while being ineffective in sexually potent rats (Arletti *et al.* 1999), which the authors ascribed to increased central noradrenergic and dopaminergic tone, and possibly oxytocinergic transmission. We have observed inhibitory effects of dietary *Quillaja* saponins on egg production in Nile tilapia (Francis *et al.* 2001*b*). Saponins also reportedly affect functioning of the male reproductive system. The gonado-somatic index of 6-month-old male tilapia fed a diet containing *Quillaja* saponins during the initial part of the life-cycle was significantly higher than that of the control, whereas male tilapia receiving a continuous supply of dietary saponins tended to have lower gonado-somatic index (G Francis, unpublished results). The mechanism of action in these instances remains to be clarified.

The *in vivo* effects of saponins on the reproductive functioning seem to indicate more than a simple permeabilising effect on secretary cell membranes and could possibly be linked to interactions between saponins and steroid receptors given the similarities between the basic chemical structures of saponins and steroid hormones. Ginseng saponins were found to compete strongly with oestradiol and R5020 (a synthetic progestin) for oestrogen- and progesterone-binding sites in the human myometrial cytosol (Punnonen & Lukola, 1980). *C. africanum* extract was shown to compete with oestradiol and with progesterone on uterine receptors (Benie *et al.* 1990). The spirostanol-steroid saponin, digitonin, stimulates binding of progesterone to bovine luteal membrane and this action is mediated by its ability to specifically complex with membrane sterols rather than by a non-specific detergent effect (Menzies *et al.* 1999). The saponin receptor complexes may then translocate into the nucleus and affect transcription patterns. Ginsenoside Rg3 (20(s) protopanaxadiol type) exhibited a dose-dependent inhibitory activity on the expression of marker genes coding for androgen receptors and 5α-reductases that converts testosterone into the more potent dihydrotestosterone, which in turn binds to androgen receptors for eliciting its actions (Liu *et al.* 2000).

### Effects on the immune system

Saponin-based adjuvants have the unique ability to stimulate the cell-mediated immune system, as well as to enhance antibody production, and have the advantage that only a low dose is needed for adjuvant activity (Oda *et al.* 2000). There have been quite a few reviews on the immunostimulatory activity of saponins (Kensil, 1996; Barr *et al.* 1998; Sjolander *et al.* 1998). Our attempt here would be to look more closely at the mechanisms involved, including from a nutritional point of view, and relationships between structures and activities. Research thus far has concentrated on *Quillaja* saponins and derivatives such as immune-stimulating complex vaccines (immune-stimulating complexes formed by the combination of cholesterol, saponin, phospholipid and amphiphatic proteins). *Quillaja* and other saponins either as crude mixtures or as purified compounds have been reported to increase immune-cell proliferation *in vitro* (Chavali *et al.* 1987; Plohmann *et al.* 1997; Lacaille-Dubois *et al.* 1999). Purified *Quillaja* saponins boosted antibody production without producing any reaginic antibodies (So *et al.* 1997). Immune-stimulating complexes formulated with *Quillaja* saponin preparations induced specific cytotoxic T-lymphocyte responses (Coulter *et al.* 1998) and have been reported to induce antibody responses and/or protective immunity in guinea-pig, turkey, cat, rabbit, dog, seal, sheep, pig, cow, horse and monkeys (Mowat *et al.* 1999). The adjuvant action of saponins was, however, not so pronounced in some of the non-mammalian species tested. For example, saponin adjuvant when injected intraperitoneally had little effect on the humoral immune response in rainbow trout (Cossarni-Dunier, 1985). Neither did the use of *Quillaja* saponins produce any improvement in the immune response to *Yersinia ruckeri*, the causative agent of enteric redmouth disease in rainbow trout, despite some enhancement of the *in vivo* bacterial clearance (Grayson *et al.* 1987).

The mechanisms of immune-stimulating action of saponins have not been clearly understood, but many explanations have been put forward. Saponins reportedly induced production of cytokines such as interleukins and interferons that might mediate their immunostimulant effects (Jie *et al.* 1984; Kensil, 1996). It is likely that they interact with antigen-presenting cells to induce many of these responses (Barr *et al.* 1998). The incorporation of the saponins into cell or endosomal membranes might expose the incorporated antigen to cytosolic proteases.

The effects of saponins at the intestinal level may also need attention, given its presence in some common dietary ingredients. Also several important systemic infections gain access to the body via the intestinal route. Infections of the intestine, along with the respiratory tract, and urogenital tract are the most common cause of mortality and morbidity in man (World Health Organization, 1996). It

has long been considered that a single dose of orally administered sub-unit vaccine would be the most useful means of protecting against these disorders (Bloom, 1989; McGhee *et al.* 1992). Oral vaccines are easy and economical to administer and avoid the hazards of routes involving needles. More importantly, induction of effective immunity at a mucosal site can only be achieved by immunisation via a mucosal route. There is evidence that saponins may increase the immune response by increasing the uptake of antigens from the gut and other membranes. The example of immune-stimulating complexes was mentioned earlier. Oral administration of *Panax ginseng* C. A. Meyer saponins (Jie *et al.* 1984), *Quillaja* saponins (Maharaj *et al.* 1986), and the butanol extract of *Lonicera japonica* (Lee *et al.* 1998), and de-acylated saponin-1 administered on the nasal mucosa (Recchia *et al.* 1995), all stimulated the immune responses *in vivo*. Purified *Quillaja* saponin-21 was able to act as a mucosal adjuvant in DNA vaccination against HIV-1 (Sasaki *et al.* 1998). The saponins may also protect the antigens from digestive degradation by forming complexes with them. The permeation potential of saponins may become important when a systemic response is induced at the intestine. De-acylated saponin-1, for example, was an efficacious intestinal permeation-enhancing agent with low adverse effect on the epithelial viability and barrier function (Chao *et al.* 1998). Human defence strategies, although far from perfect, have evolved since inception. As such, innate reactions that appear to contribute to disease initiation and propagation are likely to have an underlying initial advantageous response for the individual. An alteration in intestinal permeation in response to insult or antigenic challenge is no exception to this rationale. Normally, luminal antigens are sampled in a more controlled fashion by M cells and other antigen-presenting cells in the bowel. Antigens then are presented to undifferentiated immuno-competent cells that mature and hone back to mucosal surfaces, including the gut. This is an essential function because it provides a sensitised line of defence should an insult from a like or similar antigen be mounted (DeMeo *et al.* 2002).

The adjuvant activity of saponins was thought to be related to branched sugar chains or aldehyde groups (Bomford *et al.* 1992) or to an acyl residue bearing the aglycone (Kensil, 1996). Later soyasaponins and lablabosides were found to show strong adjuvant activity despite lacking acyl residues and possessing only unbranched sugar chains (Oda *et al.* 2000). Also most of the escins that have acyl residues and branched sugar chains did not show adjuvant activity. Adjuvant activity and toxicity, but not the cholesterol-binding capacity of QH-B, a *Quillaja* fraction, declined on peroxidate oxidation due to alterations in the structure of the sugars, galactose and xylose. Modification of the apiose moiety may influence adjuvant activity but not toxicity *in vivo* (Ronnberg *et al.* 1997). Oda *et al.* (2000) concluded that the overall juxtaposition of hydrophilic and hydrophobic functional groups, rather than the structures of individual groups, is the essential element that confers adjuvanticity. Soyasaponins, lablabosides and purified *Quillaja* saponin-21, which possess adjuvant action have only two to four O atoms equally distributed around the aglycone, and may retain the typical amphipathic features. On the other hand, escins without adjuvant activity have seven O atoms, with five localised around one side of the aglycone, thus reducing its hydrophobic and adjuvant nature.

As against the stimulatory effects on specific immunity components, saponins have also been shown to be able to prevent some non-specific immune reactions such as inflammation (de Oliveira *et al.* 2001; Haridas *et al.* 2001a) and monocyte proliferation (Delmas *et al.* 2000; Yui *et al.* 2001). Triterpenoid saponins from *Acacia victoriae* having $\alpha$, $\beta$ ester groups at $C_{28}$ and the outer monoterpene chains could have reacted with cysteine residues in the nuclear transcription factor-$\kappa$B (NF-$\kappa$B) molecule and this might have modified this molecule and prevented it from performing its normal function of stimulating genes involved in immune and inflammatory pathways in response to stress signals. One downstream effector substance whose activity has shown to be lowered by saponin is phospholipase $A_2$ (see de Oliveira *et al.* 2001) that causes a decrease in hydrolysis in membrane phospholipids and thereby decreased membrane fluidity.

The varied action of saponins might indicate that the immunostimulatory effects of saponins are the result of specific targeting of physiological intermediaries rather than the result of a non-specific effect on cell membrane permeability.

### Cytostatic effects on malignant cells

Saponins isolated from different plants and animals have been shown to specifically inhibit the growth of cancer cells *in vitro* (Kuznetzova *et al.* 1982; Rao & Sung, 1995; Konoshima *et al.* 1998; Marino *et al.* 1998; Mimaki *et al.* 1998a; Podolak *et al.* 1998). The pursuit of natural substances capable of controlling malignancies has led to considerable research on this property of saponins. Kalopanaxsaponin A (often produced in the intestine from hederagenin glycosides by bacterial action) seems to be the basic saponin structure for cytotoxic activity (Park *et al.* 2001).

The depressive effect of saponins against cancer cells may take place through diverse and complex mechanisms. The destructive activity of saponins against cells such as erythrocytes was, however, not related to cytostatic activity against cancer cells (Mimaki *et al.* 1998b). Triterpenoid saponins (avicins from *Acacia victoriae*) selectively inhibited growth of tumour cell lines by cell cycle arrest in human breast cancer cell line and apoptosis in leukaemia cell line (Mujoo *et al.* 2001) and reduced both tumour incidence and multiplicity in a murine skin carcinogenesis model (Hanausek *et al.* 2001). Ginsenoside Rg3 was found to exert multiple anti-proliferative activity towards human prostate carcinoma cells including cell cycle arrest at G1 phase and stimulation of apoptosis. Saponin (triterpenoid or steroid)-induced apoptosis is primarily caused by stimulating the cytochrome c–caspase 9–caspase 3 pathway in the human cancer and other cell lines (Liu *et al.* 2000; Haridas *et al.* 2001b; Yui *et al.* 2001; Cai *et al.* 2002), a property that is shared by a ginseng saponin derivative (20-*O*-(β-D-glucopyranosyl)-20(S)-protopanaxadiol) produced in

the intestine by bacteria and absorbed into blood (Lee *et al.* 2000*c*). Yui *et al.* (2001) detected specific toxic activity against macrophage colony-stimulating factor-induced growth of macrophages by terpenoids (securiosides) having specific structural features such as presence of dimethoxycinnamoyl group. Apoptosis seemed to be induced by mechanisms similar to those reported for Jurkat cells (Haridas *et al.* 2001*b*) here also, i.e. by stimulating the cytochrome c–caspase pathway. Interestingly the securiosides seemed to have no or considerably weaker effects against other cells including thymocytes, bone marrow cells and tumour cell lines or even macrophages stimulated by growth factors other than macrophage colony-stimulating factor, revealing a high degree of structure-related specificity. Saponin-initiated cytotoxicity thus begins non-specifically with cell aggregation caused by detergent action (Mimaki *et al.* 2001), followed by specific toxicity determined by receptors on the cell surface and saponin structure leading to what seems to be a mechanism of apoptosis that is independent of saponin structure. The tumour-specificity of the cytotoxic action seems to be influenced by the structure of the sugar portion of the saponins (Kuroda *et al.* 2001) after the hydrophobic aglycone core allowed saponins to traverse the mitochondrial membrane. The mechanism of the cytochrome c release was, however, not caused by the anticipated passive permeabilisation of the mitochondrial membrane by saponin. A cell cycle arrest mediated by inhibition of the phosphatidyl-inositol-3-kinase–protein kinase signalling pathway (Mujoo *et al.* 2001) or direct suppression of protein kinase complex genes (Liu *et al.* 2000) is stimulated by saponins either along with the apoptotic pathway (Mujoo *et al.* 2001) or independently (Oh & Sung, 2001), based on the structure of saponin. The complexity of effects and interactions involved could be seen from the fact that the ginsenosides $Rb_1$ and $Rg_1$ are able to inhibit apoptosis in a human neuroblastoma cell line induced by toxins (Rudakewich *et al.* 2001) and the abolishment by orally administered ginseng total saponins of morphine-induced apoptosis of thymocytes (Kim *et al.* 1999). The inhibition of the phosphatidylinositol-3-kinase–protein kinase pathway is considered important in apoptosis, given the role of protein kinases in inactivating the caspases (apoptotic enzymes). Avicins also decreased the release of reactive oxygen species from mitochondria, which along with the inhibition of reactive nitric oxide synthetase (Haridas *et al.* 2001*b*) might be the reason for the prevention of chromosomal damage reported in Hanausek *et al.* (2001). These activities may be considered as antioxidative in nature. But the above saponins lack an H atom-donating group and thus their effect must be at the physiological levels of specific induction. The following examples strengthen this observation. Saponins have been observed to decrease microsomal enzyme activity (Sindambiwe *et al.* 1998; Nguyen *et al.* 2000). Triterpenoid saponins from *Acacia victoriae* decreased expression of NF-κB-regulated proteins such as inducible nitric oxide synthase and cyclo-oxygenase in human leukaemia cells *in vitro*, changes that may help prevent development of malignancies by reducing oxidative and nitrosative stress (Haridas *et al.* 2001*a*). Saponins also reduce occurrence of reactive

oxygen species such as $H_2O_2$ (Haridas *et al.* 2001*a*; Pawar *et al.* 2001), probably by enhancing its breakdown by activation of peroxiredoxins and catalase, and/or glutathione peroxidase (see Deng & Zhang, 1991) as well as by suppressing its production by inhibiting the phosphatidyl-inositol-3-kinase signalling pathway (Haridas *et al.* 2001*a*). Direct effects on genetic material have also been reported. Ginseng saponins have been found to be specific inducers of the superoxide dismutase gene, that codes for one of the major antioxidant enzymes, through transcription factor AP2 binding site and its induction (Kim *et al.* 1996).

Saponins might also exert an anti-cancer effect at the intestinal level. Bile acids are metabolised by intestinal microbes to form secondary bile acids that are implicated as causative agents of colon cancer. The binding of saponins to bile acids in the intestine could reduce the availability of bile acids to the microbial population, thus reducing the formation of carcinogenic substances in the colon (Cheeke, 1996). Saponin metabolites, for example the ginseoside metabolite M1 produced in the intestine by the action of microbes on ingested ginseng saponins, may also have anti-cancer effects (Wakabayashi *et al.* 1998). Hederagenin glycosides from *Kalopanax pinctus* could effectively prevent the metabolic activation of aflatoxin B-1 or scavenge the electrophilic intermediate capable of inducing mutation (Lee *et al.* 2000*b*). Theasaponin E-1 from seeds of the tea plant *Camellia sinensis* L. var. Assamica PIERRE exhibit considerable gastroprotective activity and the whole saponin fraction from teaseed protects gastric mucosal lesions induced by ethanol in rats (Murakami *et al.* 1999).

More clarity as to the structure-related specificity of saponins to different types of cancers would be desirable in designing therapeutic preparations. Interactions between saponins and carcinogens in the intestinal lumen also call for closer studies.

## *Molluscicidal effect*

The molluscicidal properties of saponins were first observed by Lemma (1965) who noticed the toxic effects of extracts of unripe berries of *Phytolacca dodecandra* on river snails in Ethiopia. Efforts were then mounted to utilise this property of saponins to control diseases such as schistosomiasis, which are transmitted by molluscs. Saponins extracted from many other sources were also seen to have similar molluscicidal properties, for example purified *Sesbania sesban* saponins at 3–25 mg/kg (Dorsaz *et al.* 1988) and purified saponin mixtures from *Maesa lanceolata* at above 5 parts per million (Sindambiwe *et al.* 1998) have been found to be active against *Biomphalaria glabrata*. Monodesmodic triglycosides from the pericarp of the fruits of *Sapindus rarak* at 6·25–12·5 mg/kg (Hamburger *et al.* 1992), a spirostanol glycoside from the inflorescence of *Y. aloifolia* (Kishor & Sati, 1990), and some 21,22 diacylated maesasaponins (Apers *et al.* 2000) were also molluscicides. The molluscicidal activity of the saponins may be due to their characteristic detergent effect on the soft body membranes of the molluscs.

## Antifungal activity

Saponins have high toxicity against fungi (Delmas *et al.* 2000; Wang *et al.* 2000*a*). Fungicidal activity against *Trichoderma viride* was previously used as an identification method for saponins. Kalopanaxsaponins A and I isolated from *Kalopanax pinctus* exhibited strong and specific antifungal activity against *Candida albicans* and *Cryptococcus neoformans* (Kim *et al.* 1998*b*). Asparagus saponin-1, from the lower leaves of *Asparagus officinalis*, has antifungal properties in concentrations of 0·5–8·0 μg/ml depending on the type of fungus (Shimoyamada *et al.* 1990). The monodesmosidic spirostanol saponins from *Y. schidigera* destroy certain food-deteriorating yeasts, film-forming yeasts, and dermatophytic yeasts and fungi (Miyakoshi *et al.* 2000). The major mechanism suggested for the antifungal activity of saponins is their interaction with membrane sterols. Synthetic steroid saponins prepared by Takechi *et al.* (1999) were both antifungal and haemolytic but in many cases haemolytic triterpenoid saponins show little antifungal activity. It was observed that those saponins having a branched-chain trisaccharide moiety without any oxygen-containing groups at $C_2$ and $C_{12}$ exhibited the anti-yeast activity, while saponins with 2β-hydroxyl or 12 keto groups showed very weak or no activity (Miyakoshi *et al.* 2000). A saponin with a disaccharide moiety exhibited relatively low activity and the aglycones or bidesmodic furostanol saponins showed no activity.

The antifungal activity of food-originated substances has attracted an applicational research. Some reports describe the anti-yeast activity of saponins as having an anti-food deteriorating effect (Miyakoshi *et al.* 2000).

## Virucidal activity

Some saponins and sapogenins have been shown to be capable of deactivating viruses; for example, purified saponin mixture from *Maesa lanceolata* (Sindambiwe *et al.* 1998). Maesasaponins with 21,22 diacylation had virucidal activity (Apers *et al.* 2000). The triterpenoid sapogenin oleanolic acid inhibits HIV-1 virus replication probably by inhibiting HIV-1 protease activity (Mengoni *et al.* 2002).

## Effects on protozoa

Saponins from different sources have been found to be detrimental to protozoa and have been identified as possible defaunating agents in the rumen (Wallace *et al.* 1994; Newbold *et al.* 1997). This property could be exploited in treatment of protozoal infections in other animals. Triterpenoid and steroid saponins have been found to be detrimental to several infectious protozoans such as *Plasmodium falciparum* (Traore *et al.* 2000), *Giardia* trophozoites (McAllister *et al.* 2001) and *Leishmania* species (Delmas *et al.* 2000; Plock *et al.* 2001). The toxicity of saponins to protozoans seems to be widespread and non-specific and is obviously the result of their detergent effect on the cell membranes. The antiprotozoal property of saponins may be lost upon deglycosylation (Wang *et al.* 2000*a*).

## Antioxidant effects

The importance of the antioxidants contained in foods is well appreciated for both preserving the foods themselves and supplying essential antioxidants *in vivo*. However, the term 'antioxidant' is very loosely used. Often, the term is used to describe chain-breaking inhibitors of lipid peroxidation as free radicals generated *in vivo* damage many targets other than lipids, including proteins, DNA and small molecules. These oxidation reactions might lead to an array of adverse biological effects. Some of the protection mechanisms afforded by saponins against this have already been discussed earlier. Twenty years ago Zilversmit (1979) hypothesised that atherogenesis might result from phenomena that occur immediately after eating, and that it might be affected by chylomicron remnants. Several researchers (Staprans *et al.* 1994; Wolff & Nourooz-Zadeh, 1996; Ursini *et al.* 1998) expanded Zilversmit's hypothesis and suggested that dietary lipid hydroperoxides, which may be partly generated during digestion in the alkaline pH of the intestine, are the source of chylomicron remnants of lipid hydroperoxides, which are elevated in the postprandial state. These findings emphasise the importance of natural antioxidants in food, and throughout the digestive tract. Usually, polyphenols and carotenoid pigments, being the major nutritional antioxidants in food, attract most of the research in this area. Some saponins have also been found to have anti-oxidative or reductive activity.

A group of saponins produced in legumes, namely, group B soyasaponins, contain an antioxidant moiety attached at $C_{23}$ (Yoshiki *et al.* 1998). This unique sugar residue, 2, 3-dihydro-2, 5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP), allows saponins to scavenge superoxides by forming hydroperoxide intermediates, thus preventing bio-molecular damage by free radicals (Yoshiki & Okubo, 1995; Hu *et al.* 2002). Previously it was suggested that the DDMP residue is lost during soyabean processing. However, it has been shown that soya ingredients (soyabean flour, toasted soya hypocotyls, soya protein isolates, textured vegetable protein, soya protein concentrates, and Novasoy) and soya foods (commercial soya milk, tofu, and tempeh) contain the group B soyasaponins from 0·20–114·02 μmol/g (Hu *et al.* 2002). The DDMP moiety is widely distributed among legumes such as kidney beans, peanuts, chickpeas, clover, and Japanese bushclover (Yoshiki *et al.* 1998).

## Effects on nervous system functioning

Ginseng extract has been shown to have neurotrophic and neuroprotective effects (Rudakewich *et al.* 2001). It significantly improves learning ability and cognitive functions in brain-damaged rats in a dose-dependent manner, and enhances the strategic performance of normal rats. These effects are thought to be due to membrane-stabilising effects such as the inhibition of $Na^+$ and $Ca^{2+}$ channels (Zhao & McDaniel, 1998). Ginseng total saponins injected intracerebroventricularly inhibit stress-induced hypothalamo–pituitary–adrenal responses by inducing NO production in the brain (Kim *et al.* 1998*a*). This may be

beneficial in preventing the harmful effects of excessive increases in plasma corticosterone on the target organs under stressful circumstances.

*Panax notoginseng* saponins administered intraperitoneally significantly inhibited abnormal increases in platelet aggregation and platelet adhesiveness in rats subjected to permanent occlusion of the middle cerebral artery. Similar effects were observed with *in vitro* preparations. The anti-cerebral ischemic effect of *Panax notoginseng* saponins is probably due to changes in the rank and structure of functional membrane proteins induced by fluidity of membranes that lead to changes in protein activities (Ma & Xiao, 1998). Platelet membrane glycoproteins play a key role in the adhesion of platelets to the walls of blood vessels, in the accretion of platelets and in the formation of thromboses.

Zhang *et al.* (2001) studied inhibition of gap junction-mediated intercellular communication (GJIC) by ginsenosides. Gap junctions are believed to be involved in the pathogenesis of many inherited and acquired human diseases. Some ginsenosides reduced GJIC by activating tyrosine kinase, while others produced the same effect by activating protein kinase C. Tyrosine kinase and protein kinase C are the two primary kinases that phosphorylate connexins, leading to GJIC down regulation. Still other ginsenosides inhibited GJIC by directly modulating the gap junction channels. They were, for example, found to inhibit striatal dopamine release stimulated by psycho-stimulants (Shim *et al.* 2000) probably due to their effects on receptor-operated $Na^+$ channels in dopaminergic nerve terminals or on presynaptic nicotinic acetylcholine receptors. Even among the chemically related ginsenosides prominent structure–activity relationships were difficult to find, even among chemically-related ginsenosides.

### Glucocorticoid-like effects of saponins

Lee *et al.* (1997) reported that the ginsenoside $Rg_1$ suppressed growth in rat hepatoma cells through transactivation induced by its binding to the glucocorticoid receptor (GR). By using the antiglucocorticoid RU486 it was found that $Rg_1$ induced gene expression through a GR-mediated mechanism. This GR-mediated gene induction by $Rg_1$ was coupled with other signalling pathways, as in the case of glucocorticoids, evidenced by synergistic induction in the presence of increasing doses of a cyclic adenosine monophosphate analogue (Chung *et al.* 1998). Again, $Rg_1$ also caused a down regulation of GR in a manner similar to glucocorticoids. In contrast, chronic infusion of $Rg_1$ restored binding capacity of type 1 corticosterone-preferring receptors in the hippocampal region of aged rats (de Kloet *et al.* 1987). Orally administered ginseng total saponins were shown to restore morphine-induced elevation of plasma corticosterone to the control level (Kim *et al.* 1999), probably through negative feedback exerted by ginseng saponin or metabolite–GR complexes. Other reports point out that saponin ingredients act by mechanisms other than binding to steroid receptors. Glycyrrhetinic acid, the active aglycone of a liquorice saponin, for example, was found to be a highly efficient inhibitor of corticosteroid 11β-dehydrogenase (Monder

*et al.* 1989) and showed mineralocorticoid-like activity. This enzyme catalyses the oxidation of the biologically active steroid cortisol to its inactive metabolite cortisone. (Glycyrrhizic acid and glycyrrhetinic acid glucuronide given orally to rats were reported to inhibit renal 11β-dehydrogenase in the Monder *et al.* (1989) study, providing further evidence of absorption of active saponin derivatives from the intestine.) Oral administration of saponins from *Hernaria glabra*, on the other hand, resulted in a significant decrease in blood pressure in hypertensive rats and increased salt and water transport in the renal tubules (Rhiouani *et al.* 1999).

The glucocorticoid-like action of saponins could possibly explain the diverse effects of saponins in biological systems. Glucocorticoids, acting through GR, are involved in the regulation of numerous physiological processes (Gagliardo *et al.* 2001). Upon ligand binding GR inhibits or stimulates gene transcription. On nuclear translocation GR transactivate DNA elements compatible with glucocorticoid response elements. Activated GR also antagonise transcription factors, particularly NF-κB, directly and indirectly through gene transcription and protein synthesis of the NF-κB inhibitor (Almawi & Melemedjian, 2002). NF-κB regulates a number of genes involved in immune and inflammatory pathways such as various pro-inflammatory cytokines, adhesion molecules, and apoptosis and has been shown to be affected by saponins (see Haridas *et al.* 2001a,b). Higher glucocorticoid activity has been described to have negative effects on female reproductive function (Andersen, 2002). Contradictory results are, however, common when the action of glucocorticoids is described, as several local mechanisms modulate its potency and availability at the tissue level. The end effects of glucocorticoid-like activity of saponins could hence be unpredictable and different from that of glucocorticoids itself as it is not known whether the modulatory machinery works in the same manner for saponins.

The action of saponins that evolved in plants as important defence adaptation against highly conserved key pathways such as the NF-κB pathway in potential rivals may also have evolutionary significance (Haridas *et al.* 2001a).

### Conclusions

Saponin mixtures present in plants and plant products possess diverse biological effects when present in the animal body. With the information presently available, it is difficult to establish clear functionality and structure–activity relationships regarding the effects of saponins in biological systems. This is largely due to the occurrence of a vast number of saponins with similar chemical structures, and to the complexity of cellular physiological reactions, which are often differently influenced by small and subtle differences in stereo-structures of effector ligands. Other factors that could have substantial influence on saponin actions could be their interactions with other dietary constituents.

The membrane-permeabilising activity seems to be possessed by a large number of saponins, whether triterpenoid or steroid. The adjuvant effect of specific saponins and their derivatives on the specific immune system function

have been proved in animal models. Saponins also show wide-ranging cytostatic effects against cancer cells. The ability of saponins to lower the serum cholesterol level of animals has also been reported. These favourable effects point to the potential of saponins, including those present in the diet, as a remedy against two of the major health hazards in many countries; obesity and cancer. Similarly, the sterol-mediated antiprotozoal and antifungal effects, and molluscicidal and antiviral effects, also offer considerable potential in managing various diseases. Other properties ascribed to saponins such as the beneficial effects that some of them are reported to have on the efficiency of feed utilisation and growth in ruminants, single-stomached animals and fish need further experimental proof to be conclusively established, and studies need to be directed towards understanding the factors, dietary- or animal- or saponin-related, for presence or absence of these beneficial effects. Another important area that calls for further investigation relates to the effects of saponins or their derivatives on animal and human reproductive cycles and hormonal balance. In view of their widespread presence in animal and human diets it would also be particularly interesting to further investigate the fate of saponins in the intestine of animals and their mechanisms of action at the intestinal and systemic levels.

Extensive *in vivo* studies are required using purified individual compounds and their well-defined derivatives (chemically altered or naturally occurring). Since the quantities available of such purified saponins are not large, small-animal models or fish could be used to understand structure–activity relationships or the mechanisms of their actions. Other problems worth keeping in mind are the changes in the nature and biological effects of the native compound taking place during the isolation and purification process. In addition, the saponins might produce the desired effects in conjuction or interacting with other moieties. Another approach, therefore, worth considering is the use of a well-defined saponin preparation (not necessarily structurally well-defined saponins) targeted at achieving the desired biological effects in a reproducible manner.

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