

Influence of oat saponins on intestinal permeability *in vitro* and *in vivo* in the rat

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The aim of the present study was to investigate whether oat saponins (avenacosides A and B) have any effect on the permeability of the rat intestine to actively and passively transported markers *in vitro* and *in vivo*. Intestinal segments were mounted in modified Ussing chambers, and the passage of the different marker compounds from the mucosal to the serosal side was measured for 120 min. Avenacosides (1 mg/ml) gave a significantly higher passage of the macromolecule ovalbumin and there was a tendency to increased passage of [¹⁴C]D-mannitol and [⁵¹Cr]EDTA. On the other hand, the saponins did not affect the active transport of [³H]methyl glucose. When rats were given saponins (40 mg/kg body weight) together with markers by gastric intubation, the passage of [⁵¹Cr]EDTA into blood and urine was somewhat reduced. For the macromolecule bovine serum albumin, no evident effect on the passage was observed in the presence of saponins. Thus, in contrast to the *in vitro* results, the *in vivo* marker passage seemed to be unaffected or even reduced in the presence of avenacosides. The study shows that saponins can affect the permeability of the rat intestine. However, this effect needs further investigation *in vivo*, especially regarding proteins.

Saponins: Oats: Intestinal permeability

Most of the oats produced are used as animal feed, but several oat-based food products for human consumption have been developed in recent years, mainly due to the plasma-cholesterol-lowering properties of oats. An increased intake of oats may therefore be expected.

Oats have a high nutritional value but also contain antinutrients such as phytic acid and saponins. Saponins are glycosides with triterpenoid or steroid aglycones (Tschesche & Wulff, 1973). The oat saponins were detected in the 1950s (Mohr, 1953) and later on the structure of two saponins, avenacosides A and B, was elucidated (Tschesche *et al.* 1969; Tschesche & Lauven, 1971). Both saponins have a steroid aglycone, nuatigenin, and two sugar chains containing glucose and rhamnose are linked to the aglycone. Avenacoside B has one more glucose residue than avenacoside A. Oats contain 0.2–0.5 g avenacosides A and B/kg DM, depending on the variety (Önning *et al.* 1993).

Saponins are regarded as antinutrients because they can combine irreversibly with membranes in animal cells and increase their permeability (Price *et al.* 1987). Given orally in high doses (300 mg/kg body weight) to rats, saponins cause diarrhoea, restlessness and histopathological changes in liver and kidney, ultimately leading to death (Lalitha *et al.* 1990). Saponins in normal doses are probably not absorbed in mammals (Gestetner *et al.* 1968), but they can affect the absorption of other nutrients in the gut. Several investigations have shown that saponins can reduce the plasma cholesterol concentration (Oakenfull &

Sidhu, 1990). Suggested mechanisms include inhibition of cholesterol absorption and bile acid reabsorption. Saponin-containing diets have therefore been recommended to reduce plasma cholesterol concentration. However, the saponins in oats seem to have only minor effects on lipid metabolism (Önning & Asp, 1995). When oats with up to twice the normal avenacoside A and B content were given to gerbils and rats, there was no influence on plasma cholesterol levels, but lipid deposition in the liver was reduced.

Saponins can affect the enzyme systems and transport mechanisms that are located in the intestinal mucosal cell membranes in mammals. Saponins significantly decreased the active transport of galactose (*Gypsophylla* saponin; Johnson *et al.* 1986) and of glucose (soya saponins; Sidhu *et al.* 1987) in the rat. On the other hand, the saponins seem to increase the uptake of passively transported nutrients. The absorption of L-glucose and polyethylene glycol 4000 (normally not absorbed) by rat intestine *in vitro* increased in the presence of *Gypsophylla* saponin (Johnson *et al.* 1986). Uptake of compounds that are normally not absorbed, especially macromolecules, could possibly enhance allergic responses. In fact, Atkinson *et al.* (1994) gave *Gypsophylla* saponins to rats and this evoked an increased sensitization to oral allergens.

The structure of saponins has an influence on their physiological effects. It was believed previously that saponins with several sugar chains had fewer biological effects than saponins with one sugar chain (Price *et al.* 1987). Thus, bisdesmosidic (two sugar chains) saponins from lucerne (*Medicago sativa*) had a lower activity against the fungus *Trichoderma viride* and also a lower haemolytic activity than monodesmosidic (one sugar chain) lucerne saponins (Oleszek, 1990; Oleszek *et al.* 1992). The effects on the permeability of the intestinal mucosa were similar, however (Oleszek *et al.* 1994). It was suggested that the biological activity depends not only on the number of sugar chains; the structure of the aglycone and the steric orientation of the chains are also important.

The aim of the present study was to investigate whether oat saponins have any effect on the permeability of the rat small intestine regarding an actively transported, but not metabolized, sugar ($[^3\text{H}]$ methyl glucose) as well as passively transported markers of low molecular size [^{14}C]D-mannitol, [^{51}Cr]EDTA) and the macromolecule ovalbumin *in vitro*. This was compared with the effect of the oat saponins on the intestinal uptake of [^{51}Cr]EDTA and the macromolecule bovine serum albumin *in vivo* after oral administration.

MATERIALS AND METHODS

Saponin preparation

Two saponins, avenacosides A and B, were isolated from oatmeal, using silica gel SI-60, Sephadex LH-20 and silica gel RP18 chromatography (Önning & Asp, 1995). The isolated fraction contained 0.695 g avenacoside A and 0.135 g avenacoside B/g DM. This fraction was used in the *in vitro* experiments. In the *in vivo* studies a less purified fraction separated from oatmeal by silica gel SI-60 chromatography and containing 0.300 g avenacoside A/g DM was used.

Determination of haemolytic effect

The haemolytic activity of the oat saponins was measured by the method of Wall *et al.* (1952). An oat saponin extract (a methanol extract from defatted oatmeal) was mixed with a human erythrocyte suspension (2.5 g/l). Two avenacoside concentrations, 1 and 2 mg/ml, were used and the haemolysis was measured after 5 min.

Animals

Male Sprague–Dawley rats (Møllegaard, Skensved, Denmark) weighing 300–400 g, were fed *ad libitum* on a commercial rat chow (Altromin 1324, Brogaarden, Gentofte, Denmark)

and water. They were kept on chopped wood bedding in polycarbonate cages with a 12 h day and night cycle at $20 \pm 2^\circ$ and relative humidity $50 \pm 10\%$.

Intestinal studies in vitro

The effect of saponins on the permeability of the rat small intestine was studied by the method of Pantzar *et al.* (1993). For these experiments the animals were anaesthetized with diethyl ether and two 200 mm segments of the small intestine, a proximal one, taken 30 mm distal to the Treitz's ligament, and a distal one, taken 30 mm proximal to the caecum, were removed and immersed in modified Krebs–Ringer buffer (pH 7.4) oxygenated with O_2 – CO_2 (95:5 v/v). Both segments were divided into six parts, and each part was cut along the mesenteric border and mounted in a modified Ussing chamber. The exposed area was 178 mm^2 and the serosal and mucosal reservoirs were immediately filled with 5 ml Krebs–Ringer buffer. The chambers were held at 37° and the buffer circulated by O_2 – CO_2 gas bubbling. The experiments began ($t = 0$) within 30 min of induction of anaesthesia, by replacing the buffer at the mucosal side with 5 ml marker-containing buffer. In a first experiment using only tissue from the proximal intestine, [^3H]methyl-D-glucose (2.9 MBq/nmol, 15.5 KBq/ml) and [^{14}C]D-mannitol (2.1 MBq/ μmol , 2.2 KBq/ml) were used as markers. In a second experiment using both proximal and distal small-intestinal segments, [^{51}Cr]EDTA (1.85 MBq/ μg , 92.5 KBq/ml; DuPont, Dreieich, Germany) and ovalbumin (25 mg/ml; A-7641, Sigma Chemical Company, St Louis, MO, USA) were used as markers. Saponin preparations were added on the mucosal side to three chambers and to a final avenacoside concentration of 0.5 or 1 mg/ml while three other chambers served as control. Samples were taken from the serosal chambers after 20, 40, 60, 80, 100 and 120 min.

To check whether the saponins could penetrate the proximal rat intestine, the mucosal and serosal contents in chambers with saponins were collected once at the end of the experiment ($n = 3$). The mucosal content was analysed directly for saponins by HPLC, but the serosal content was first concentrated with a Bond Elute C18 column (Önning & Asp, 1993).

Intestinal studies in vivo

A method developed by Wang *et al.* (1994) was used. At 3 d before the start of the experiment the rats were catheterized in the right jugular vein under diethyl ether anaesthesia to permit repeated blood sampling. At $t = 0$ the rats were given a solution (20 ml/kg body weight) containing 0.48 MBq [^{51}Cr]EDTA and 100 mg bovine serum albumin (A-4503 Sigma Chemical Company)/ml saline (9 g NaCl/l). For half of the rats ($n = 7$) the solution also contained 2 mg avenacoside A/ml. The rats were thereafter kept in metabolism cages for 48 h. Urine was sampled from the cages at 1, 2, 4, 8, 24 and 48 h and faeces after 4, 8, 24 and 48 h. Blood (1 ml) was withdrawn after 1, 2, 4, 8, 24 and 48 h and replaced with 2 ml sterile saline (9 g NaCl/l).

The animal studies were approved by the Ethical Review Committee on Animal Experiments at Lund University.

Analytical methods

The radioactivity in the samples containing [^3H]methyl-D-glucose and [^{14}C]D-mannitol was measured using a liquid scintillation counter (1217 Rackbeta, LKB, Bromma, Sweden) after mixture with 10 ml liquid scintillation cocktail (Ready SafeTM, Beckman, Fullerton, CA, USA). A gamma counter (1282 Compugamma, LKB, Bromma, Sweden) was used to measure the radioactivity in the samples containing [^{51}Cr]EDTA. Ovalbumin was quantified

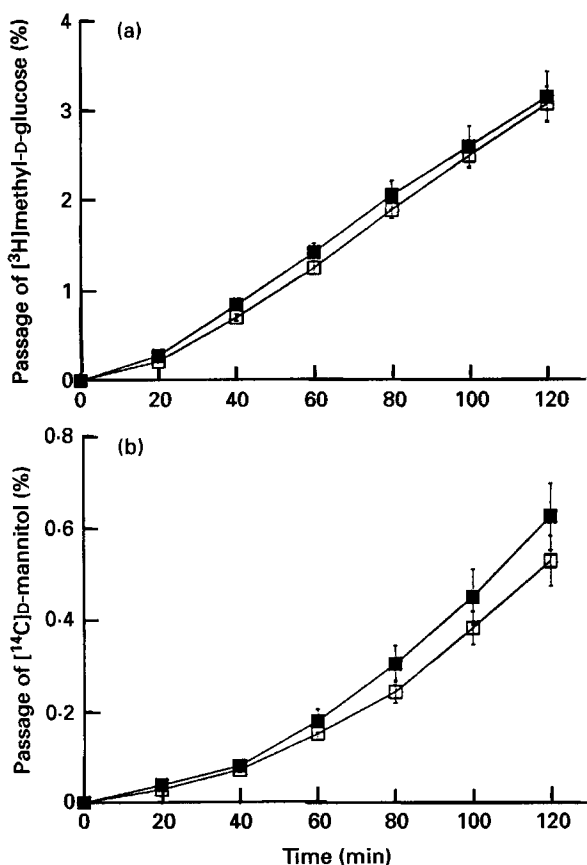


Fig. 1. Passage of (a) [^3H]methyl-D-glucose (n 11) and (b) [^{14}C]D-mannitol (control n 8, saponins n 9) from the mucosal side to the serosal side of the proximal rat intestine *in vitro*, either in the absence (□) or in the presence (■) of saponins (1 mg/ml). Values are means with their standard errors represented by vertical bars.

by electro-immunoassay (Pantzar *et al.* 1993), and bovine serum albumin by a competitive ELISA (Rennard *et al.* 1980).

The collected rat faeces were freeze-dried after the analysis for [^{51}Cr]EDTA and analysed for avenacoside content by the method of Önnings & Asp (1993).

Statistical methods

The *t* test (SPSS for Windows 6.1., SPSS Inc., Chicago, USA) was used to determine whether there was any significant difference in permeability when saponins were present or not, at each time point. To further evaluate if there were any differences in permeability during the entire time period of 120 min, a repeated measures ANOVA with Greenhouse-Geisser adjustment was used.

RESULTS

The oat saponin extract had haemolytic activity. At a concentration of 2 mg/ml it gave complete haemolysis of the erythrocytes in 5 min. When the concentration was halved to 1 mg/ml about 50% of the erythrocytes were lysed. The latter concentration was used in most of the intestinal permeability studies.

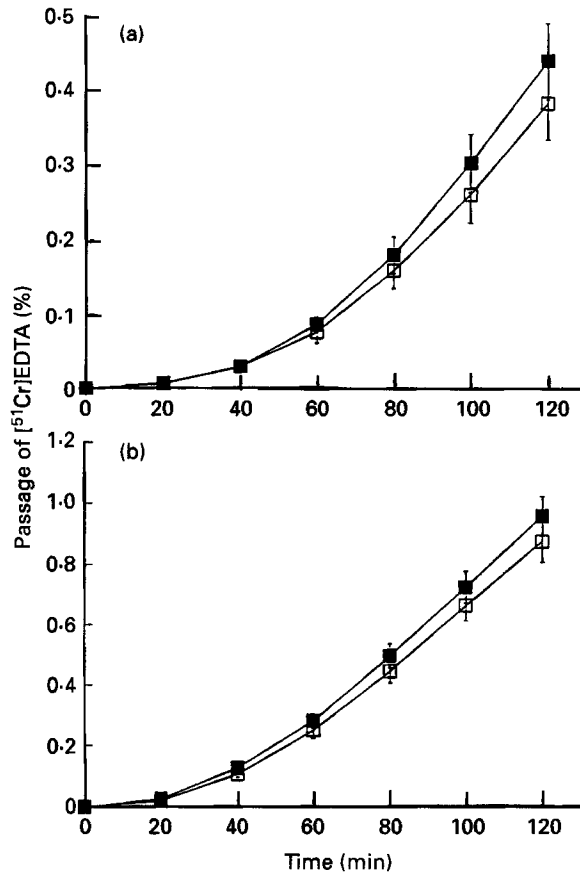


Fig. 2. Passage of $[^{51}\text{Cr}]\text{EDTA}$ from the mucosal to the serosal side of (a) proximal (n 11) and (b) distal (control n 7, saponins n 8) rat intestine *in vitro*, either in the absence (\square) or in the presence (\blacksquare) of saponins (1 mg/ml). Values are means with their standard errors represented by vertical bars.

In vitro studies

The content of avenacosides in the mucosal chamber was not lowered during the 120 min incubation. No avenacosides ($< 0.25 \mu\text{g/ml}$) could be detected in the serosal chamber and thus the saponins did not seem to be able to penetrate the rat's proximal small intestine.

The presence of avenacosides had no effect on the absorption of $[^3\text{H}]\text{methyl-D-glucose}$ in the proximal intestine and thus probably did not affect the active transport of this molecule (Fig. 1(a)). For the passively transported marker $[^{14}\text{C}]\text{D-mannitol}$ there was a tendency to increased passage after 80 min when saponins were added at a concentration of 1 mg/ml (Fig. 1(b)).

A tendency to increased uptake in the presence of saponins was also obtained for $[^{51}\text{Cr}]\text{EDTA}$ in the proximal intestine (Fig. 2(a)), but this tendency disappeared when the avenacoside concentration was lowered from 1 to 0.5 mg/ml. No effect on the passage of $[^{51}\text{Cr}]\text{EDTA}$ in the distal small intestine was found when saponins were added (Fig 2(b)).

At a concentration of 1 mg/ml the saponins increased the passage of ovalbumin significantly in the proximal intestine at 100 min (Fig. 3(a)). The difference was even more pronounced in the distal intestine where the saponins increased the permeability for ovalbumin significantly at all time points after 40 min (Fig. 3(b)). With the lower saponin

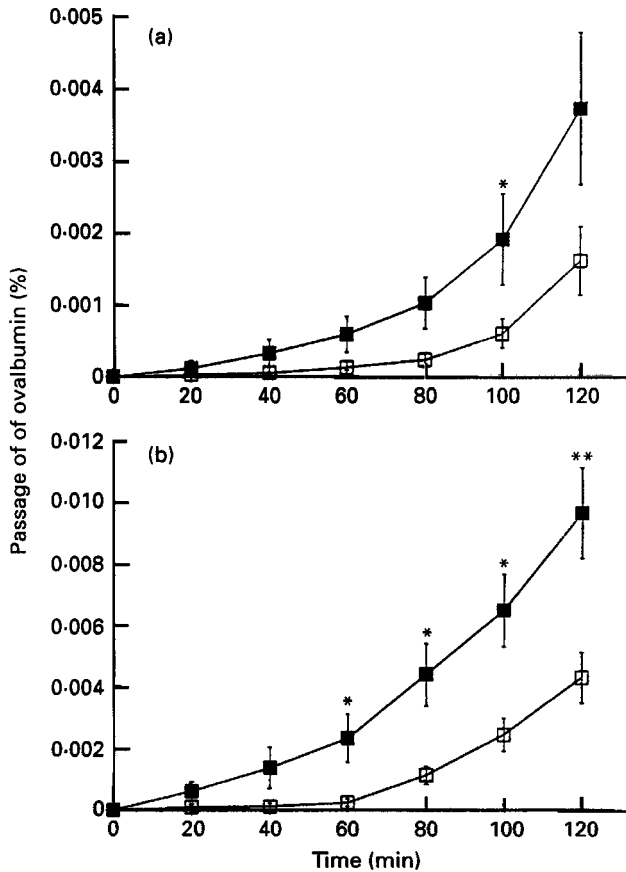


Fig. 3. Passage of ovalbumin from the mucosal to the serosal side of (a) proximal (control n 11, saponins n 10) and (b) distal (n 8) rat intestine *in vitro*, either in the absence (□) or in the presence (■) of saponins (1 mg/ml). Values are means with their standard errors represented by vertical bars. Mean values were significantly different from those in the absence of saponins: * $P < 0.05$, ** $P < 0.01$ (t test).

concentration (0.5 mg/ml) the difference was not significant, indicating a dose-response relationship.

Comparing the passage during the entire time interval up to 120 min using a repeated measures ANOVA test, the saponins had a significant effect on the passage of [^{51}Cr]EDTA (distal and proximal intestine, 1 mg saponins/ml) for each separate rat, but no general effect was obtained.

In vivo studies

The mean concentration of [^{51}Cr]EDTA in the blood was lower for the rats given saponins together with the marker up to 8 h after the feeding than for the controls (Table 1). After 24 and 48 h the blood concentration of [^{51}Cr]EDTA was low and similar for the saponin and control groups. The differences were not significant (t test) at any one point. The smallest P value (0.07) was found at 1 h.

A lower amount of [^{51}Cr]EDTA was excreted in the urine for the animals given saponins (Fig. 4). The difference was significant ($P < 0.05$) in the time interval 8–24 h. However, the total amounts excreted in 48 h, 2.2% of the fed dose for the saponin group and 2.7% for the control group, did not differ significantly.

Table 1. The concentration of [^{51}Cr]EDTA in the blood (% of fed dose/l)
(Mean values with their standard errors)

Time (h)	Saponin (n 7)		Control (n 7)	
	Mean	SE	Mean	SE
1	0.57	0.07	0.73	0.04
2	0.68	0.05	0.74	0.10
4	1.10	0.12	1.21	0.21
8	0.42	0.02	0.58	0.11
24	0.14	0.03	0.15	0.03
48	0.04	0.02	0.02	0.01

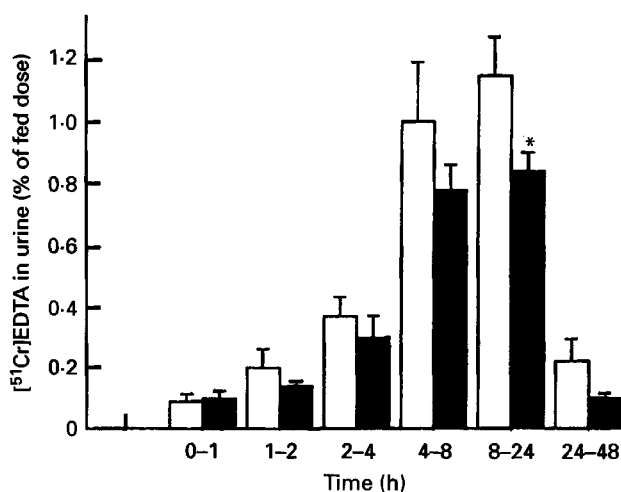


Fig. 4. Excretion of [^{51}Cr]EDTA (% of fed dose) in the urine of rats fed on diets without (□) or with (■) saponins. Values are means for seven rats per dietary group, with their standard errors represented by vertical bars. * Mean value was significantly different from that for the control group, $P < 0.05$ (t test).

No differences in the amount of [^{51}Cr]EDTA excreted in faeces were found at any time interval (Table 2). A large part of the ingested [^{51}Cr]EDTA, 75% for the saponin group and 77% for the control group, was found in the faeces collected during 48 h.

The mean concentration of the macromolecular marker bovine serum albumin (BSA) in the blood for the rats given saponins was 114 ng/ml after 1 h and decreased to 94 ng/ml after 48 h (Fig. 5(a)). Determined mean BSA values for the control rats were higher, at 4, 8, and 24 h more than twice the values found in the saponin-treated rats (210 v. 104, 257 v. 109, 266 v. 110 ng/ml respectively). However, two of the six control rats had high BSA concentrations while the other four had similar values to those of saponin-treated rats (Fig. 5(b)) and further experiments are needed before a conclusion on the passage of BSA can be drawn.

Avenacosides A and B could not be detected in the faeces ($< 0.8 \mu\text{g/g}$). Therefore these compounds seem to have been degraded and/or absorbed in some part of the digestive system.

Table 2. The amount of [^{51}Cr]EDTA recovered in faeces (% of fed dose)
(Mean values with their standard errors)

Time (h)	Saponin (n 7)		Control (n 7)	
	Mean	SE	Mean	SE
0-4	0	—	0	—
4-8	0.3	0.3	0	—
8-24	71.3	3.0	72.8	1.1
24-48	3.4	1.0	4.6	0.5

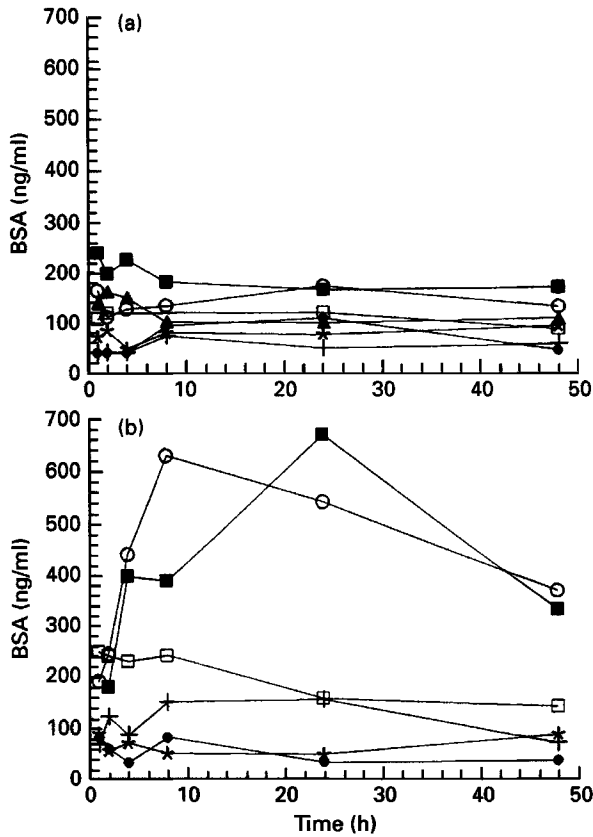


Fig. 5. Concentration of bovine serum albumin (BSA) in the blood of (a) six rats given saponins (40 mg/kg body weight) and (b) six control rats. Values are for individual rats.

DISCUSSION

The haemolytic activity of saponins differs widely. One saponin in lucerne (medicagenic acid 3,28-diglucopyranoside) has no activity at all while another one (medicagenic acid 3-O-glucoside) is highly haemolytic (Oleszek, 1990). The oat saponin extract used in the present study gave complete haemolysis of a 2.5 g/l erythrocyte suspension at a concentration of 2 mg/ml. In an earlier study (Tschesche & Wiemann, 1977) an avenacoside concentration

of > 1 mg/ml was necessary to obtain complete haemolysis of an erythrocyte suspension (1 g/l). Monodesmosidic avenacosides, where the glucose bound to the aglycone is lost, were also investigated and they showed a much higher haemolytic activity; only 9 µg desglucoavenacosides/ml gave complete haemolysis. Generally, the monodesmosidic saponins are more active than their bisdesmosidic analogues. The oat saponins are bisdesmosidic but the biological effects are hard to predict from previous studies and should be investigated specifically.

No avenacosides were found to pass across the intestinal epithelium in the Ussing-chamber experiments on segments of proximal rat small intestine. In the *in vivo* study, however, no saponins were detected in the faeces collected from the rats given avenacosides. The fate of saponins in the intestine is not clear. Gestetner *et al.* (1968) fed soyabean saponins to mice, rats and chickens for 10 d. In all three species saponins could be detected in the small intestine, but in the caecum and colon only sapogenins were found. These results indicate that saponins are probably broken down by the colonic microflora or by enzymes in the lower part of the intestine. No saponins or sapogenins could be detected in the blood, further indicating that the saponins were not absorbed.

The avenacosides, at a concentration of 1 mg/ml, had no effect on the active transport of glucose *in vitro*. Other saponins have been demonstrated to reduce the passage of actively transported compounds in rats. Johnson *et al.* (1986) used *Gypsophylla* saponins (2 mg/ml) and observed a decreased transport of galactose *in vitro*. In an *in vivo* study (Sidhu *et al.* 1987) the transport of glucose was decreased in the presence of soyabean saponins (2 mg/ml). A saponin extract from quinoa (*Chenopodium quinoa*) reduced the passage of glucose *in vitro* at a concentration of 2.5 mg/ml (Gee *et al.* 1993). However, all these studies used higher saponin concentrations than in the present study.

There was a tendency towards increased permeability for the small marker molecules, i.e. mannitol and [⁵¹Cr]EDTA, when saponins were added to the Ussing chambers in the present study. Mannitol probably passes mainly by transcellular diffusion through pores of finite dimensions (Hamilton *et al.* 1987), while [⁵¹Cr]EDTA probably passes paracellularly via the tight junctions. The passage of the macromolecule ovalbumin was increased significantly in the presence of avenacoside (1 mg/ml), in both the proximal and the distal small intestine. Macromolecular marker proteins have been shown to pass both transcellularly and by endocytosis and paracellularly, the latter route especially after intestinal injury or inflammation (Sanderson & Walker, 1993). Johnson *et al.* (1986) and Gee *et al.* (1993), using polyethylene glycol 4000 as a marker molecule, also found that the permeability of the small intestine was increased in the presence of *Gypsophylla* and quinoa saponins respectively. Saponins probably increase the permeability of cells by combining irreversibly with discrete sites within the plasma membrane (Price *et al.* 1987).

In contrast to the *in vitro* results, the *in vivo* passage of [⁵¹Cr]EDTA seemed to be somewhat reduced in the presence of avenacosides. Moreover, the permeability for the macromolecular marker ovalbumin was increased significantly when saponins were added *in vitro* while *in vivo*, the passage of BSA seemed to be unaffected. *In vitro*, a certain saponin concentration (i.e. 1 mg/ml) seemed to be necessary before the permeability was affected, and thus the *in vivo* results could be due to dilution effects in the intestine. Another explanation could be that mucosal cells are damaged by saponins *in vivo*. Such injured cells could have been exfoliated into the lumen more rapidly, explaining a reduced absorption. A third explanation could be that compounds in the intestine influence the membranolytic effect of the saponins. For example, bile salts present *in vivo*, but not *in vitro*, could reduce this effect (Gee & Johnson, 1988).

Nevertheless, it is concluded that the physiological concentrations of oat avenacosides have the potential to affect the intestinal permeability, as previously seen with relatively

high concentrations of other saponins *in vitro*. However, the effects of saponins on the intestinal permeability *in vivo*, especially for proteins, are not clear and need further investigation.

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