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Some Physiological and Biochemical Aspects of Saponin Fraction of *Panax Ginseng* C.A. Meyer

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It has been demonstrated that ginseng has a wide range of pharmacological properties including antifatigue and antistress actions, mild normalizing effects on blood pressure and carbohydrate metabolism, suggesting central nervous system stimulatory properties and some effect on macromolecular synthesis in the liver. Brekhman and Dardymor (1969) described in their review article that the basic effect of ginseng action is its capacity to increase nonspecific resistance of the organism to various untoward influences from their experimental results.

Oura and his coworkers (1974, 1975) found that aqueous extracts of ginseng stimulated the synthesis of rat liver nuclear RNA *in vitro*. Their further studies suggested that the saponin might be an active principle. They also found that ginseng could stimulated the carbohydrate metabolism in the liver and could increase the lipid content of adipose tissue. They have considered that the action of ginseng has some special feature in its mode of action and suggested ginseng saponin being a kind of metabolic regulator or hormone-like substance.

We (1973, 1977, 1978, 1980) investigated the solubilizing effect of ginseng saponin by determining the critical micellar concentration (CMC) of the saponin and found that the CMC of the saponin in pure solution was about 2.0%. However, when the saponin is present together with cholesterol, the CMC of the saponin lowered down to as small as 0.1%. Again the CMC of Na-Cholate (5 mM) was found lowered down below 1.0 mM in the presence of 0.1% saponin. When the saponin was added to chicken intestinal lumen fluids, the lipids found dispersed effectively. Observations of the effect of ginseng saponin on pancreatic lipase and cholesterol esterase supported the idea that saponin might act as an excellent lipid solubilizer. It was demonstrated that water-insoluble vitamin such as α-tocopherol are more easily absorbed when the vitamin were given orally with the saponins (Joo & Kim, 1984).

Saponins in nature are terpenoides with side chain occur naturally as glycosides in plants. These glycosides (saponins) were long known to lower the surface tension of water and therefore their aqueous solutions froth readily and the saponin causes hemolysis. It is easily understood from their structure that they are amphiphatic having both hydrophobic sapogenin aglycon part and hydrophilic sugar moiety in the molecule, and therefore, they disperse lipids in aqueous medium. The studies of saponin from various sources, however, show that they behave differently from each other. Some are toxic but the others are not: some are hemolytic while the others are protective to hemolysis (Namba *et al.*, 1972). It was realized that HD_{50} of the saponin fraction extracted from *Panax ginseng* C.A. Meyer was over $10^{-3}\%$ (Kim *et al.*, 1984).

We examined the effect of either purified ginsenoside or the saponin fraction of panax ginseng C. A. Meyer on various enzymes such as dehydrogenases, transaminases, lipase and found that moderate amount ($10^{-6}\%$-$10^{-4}\%$) of the saponin stimulated the all enzyme catalyzed reaction so far tested *in vitro* but their higher concentration inhibited the enzyme reactions unexceptionally as shown in Fig. 1. Therefore we have considered that the biphasic and nonspecific action of the saponins might be due to their surface activity and suggested that

	Concentration of ginseng saponin in assay mixture							
	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1 (%)
SDH (chicken liver)			↑		↓			
MDH (chicken liver)		↑			↓			
α -KGDH (chicken liver)					↑			
L-GLDH (rat liver)			↑			↓		
TP (rat liver)					↑			
AP (rat liver)				↑				
GOT (human serum)			↑	↑				
GPT (human serum)				↑				
ALDH (rat liver)	↑			↓				
ADH (rat liver)	↑		↓					
LPL (rat pancrease)				↑		↓		
G6PDH (human blood cell)			↑	↓				
MOase (rat liver)		↑						
MEOS (rat liver)		↑						
	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1 (%)

Fig. 1. The effect of ginseng saponin on enzyme calalyzed reactions. The corresponding enzyme reaction rate reached maximum at the concentrations shown by mark (↑) and inhibitied when the saponin concentration was over those shown by mark (↓).
Abbreviation: Succinate dehydrogenase (SDH), malate dehydrogenase (MDH), α-Keto-glutarate dehydrogenase (α-KGDH), Isocitrate dehydrogenase (ICDH), L-Glutamate dehydrogenase (GLDH), Glutamate-oxaloacetate transminase (GOT), Glutamate-pyruvate dehydrogenase (GPT), Aldehyde dehydrogenase (ALDH), Alcohol dehydrogenase (ADH), Lipoprotein lipase (LPL), Alkaline phosphatase (AP), Tryptophan pyrolase (TP), Glucose 6-phosphate dehydrogenase (G6PDH), Monoamine oxidase (MOase), Microsomal ethanol oxidizing ststem (MEOS).

the surface activity of the saponin might play a significant role on the enzyme catalyzed reactions (Joo *et al.*, 1976; Joo and Han, 1976).

The Michaelis constants (K_m) of various enzymes for their substrates lowered in the presence of moderate amounts of the saponins. UV difference spectra, CD spectra, electrophoretic mobilities, DTNB titration and substrate binding data demonstrated that moderate amounts of the saponins might bring about a slight change of the enzyme conformation which woulc be in favour for the enzyme reactions being proceeded. Other amphiphiles such as Triton X-100, and deoxycholate showed similar behaviour as ginseng saponins do. The effects of several amphiphiles and ginseng saponin on several enzyme such as porcine pancreatic lipase and succinate dehydrogenase were examined and found that the optimal concentrations of the amphiphiles for the maximum enzyme activity were found to almost the same range (10^{-4}%-10^{-3}%) (Kim *et al.*, 1985, Kim and Joo, 1985, Joo, 1978).

Effect of some amphiphiles on membrane have been investigated by using adenylate cyclase of rat plasma membrane. The maximum activity of adenylate cyclase system was at 10^{-5}M of epinephrine, and epinephrine was found to bind rat hepatic plasma membrane selectively. Epinephrine binding to rat hepatic plasma membrane was maximum when the concentrations of ginseng saponin fraction, Lubrol-PX, Triton X-100 and sodium dodecyl sulfate (SDS) were 10^{-4}%, 10^{-2}%, 10^{-1}% and 10^{-3}% respectively. Uptake of ginseng saponin by rat hepatic plasma membrane was found about 27% of the added ginseng saponin fraction. This again suggests that the surface activity of the ginseng saponin might play an important role in physiological function of the cell (Oh and Joo, 1986).

Our ginseng saponin absorption experiment in rats using ^{14}C-labelled saponins prepared from ^{14}C-acetate using ginseng root slices as enzyme source showed that ginsenosides were absorbed partly in the undissociated form and the saponin level in the liver might be maintained at 10^{-6}%-10^{-5}% in ginseng administered rats. The turnover rate of the saponins was relatively fast and half life of ginsenoside Rb_1 was estimated to be about 5 hours (Joo *et al.*, 1986, Lee and Joo, 1983).

From the above considerations it can be expected that ginseng saponins might stimulate unfavoured metabolisms and/or detoxication of toxic substances by raising up the related enzyme activities *in vivo*.

Ethanol is one of the favorite mood-altering drug and its psychic effects, both pleasant and unpleasant, are well known enough but what is less known

Table 1. Subcellular Distribution of aldehyde dehydrogenase (ALDH) in rat liver.

Subcellular fraction	Relative activity (%)
Mitochondria	51.2
Cytosol	17.3
Microsome	34.0

From Joo, C.N. and E.S. Oh (1989), Korean Biochemical J., 22 (3), 312-320

Table 2. The effect of the saponin fraction of Panax ginseng C.M. Meyer on alcohol dehydrogenase (ADH), cytosolic, mitochondrial and microsomal aldehyde dehydrogenase (ALDH) and microsomal ethanol oxidizing system (MEOS) of rat liver. Relative activity (mean of three determinations) was expressed assuming that of control being 100.

Saponin conc. (%)	ADH	Mitochondrial ALDH	Cytoplasmic ALDH	Microsomal ALDH	MEOS
10^{-10}	–	–	–	109	104
10^{-9}	107	–	–	122	114
10^{-8}	101	108	142	109	113
10^{-7}	113	123	166	104	116
10^{-6}	103	113	166	105	140
10^{-5}	93	–	–	106	138
10^{-4}	89	–	–	94	102
10^{-3}	–	–	–	98	112

is that alcohol is a toxic drug; its overconsumption taxes the body's economy, produced a number of pathological changes particularly in the liver and impairs biological functions.

Unlike carbohydrate and fats, alcohol is essentially foreign to the body and it is known that the body get rid of it by oxidizing alcohol mainly in the liver. Present knowledge on alcohol metabolism showed that over-consumption of alcohol causes cirrhosis and death not only because alcoholism promotes malnutrition but also because alcohol and its metabolite effects are reported directly linked to the first two products of its oxidation, hydrogen and acetaldehyde.

In the factual scene, when ginseng extrat was administered to an animal following the medication of ethanol, there had been reports that the consumption rate of ethanol speeds up.

Table 3. The effect of ginseng saponin on alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH), and microsomal ethanol oxidizing system (MEOS) in prolonged ethanol fed rats *in vivo*. The rats were fed with 10% ethanol (Control) and/or 10% ethanol containing 0.1% ginseng saponin (Test) instead of water 6 days.

Group	ADH (unit[a]/mg protein)	ALDH (unit[a]/mg protein)	MEOS (unit[b]/mg protein)
Normal	8.743+0.159	3.076+0.600	3.165+0.472
	(100)	(100)	(100)
Control	10.136+0.221	2.303+0.661	4.443+0.681
	(116)	(75)	(140)
Test	9.242+0.123	2.678+0.015	7.028+0.775
	(106)	(87)	(222)

Acetaldehyde is produced during ethanol metabolism and is known to be oxidized mainly by aldehyde dehydrogenase (ALDH) but is also oxidized by catalase and microsomal ethanol oxidizing system (MEOS). Table 1 showed that the ALDH activity was found mainly in the mitochondrial fraction but a significant ALDH activity was also present in microsomal fraction. A small ALDH activity was in the cytosol fraction (Oh and Joo, 1989). As shown in Table 2, there were optimum concentration of the ginseng saponins for the maximum activity of enzymes such as ADH, ALDH, MEOS respectively.

Wistar rats (150-200g, male) were given freely with 12% ethanol (Control) and/or 12% ethanol containing 0.1% ginseng saponins (Test) instead of water for 6 days and the liver was analyzed. Liver homogenate was used for ADH, ALDH, and MEOS assay. As shown in Table 3, ALDH activities of both control and test group were lower than that of normal group but test ALDH was less inhibited than control. ADH activities of both control and test were slightly higher than that of normal group. We know that ADH activity is usually stimulated by ethanol feeding at initial stage but our previous data showed that it became gradually steady after pro-

longed ethanol feeding. MEOS activities of both control and test group were much higher than that of normal group. MEOS enzymes are inducible but the activity of test group was greatly higher than that of control.

For the determination of $[NAD^+]/[NADH]$ ratio, the rats were killed by dislocation of the neck. The liver was rapidly removed and pressed between metal clamps previouly cooled in liquid N_2. The average time between dislocation of the neck and deep-freezing the tissuse was 10 seconds. The frozen liver was pulverized in a mortar to a fine powder, with frequent addition of liquid N_2. The powder was transferred to a weighed plastic centrifuge tube containing 6 m*l* of ice-cold 0.6N (w/v) $HClO_4$. After a rapid reweighing, the tissue was mixed with the $HClO_4$ and immediately homogenized in the centrifuge tube. Protein was removed by centrifugation in the cold at 30,000×g for 15 min and the supernatant fluid was adjusted to pH 5–6 with 2N (w/v) KOH and, after standing for 30 min in the cold, the precipitate of $KClO_4$ was centrifuged off. The yellow supernatant fluid was used for the analysis, and lactate, pyruvate, α-ketoglutarate, isocitrate, ammonia and glutamate were determined and the $[NAD^+]1[NADH]$ ratio in cytoplasm was calculated using K_{eq} (1.11×10^{-4}) for lactate dehydrogenase reaction (lactate + NAD^+ → pyruvate + NADH + H^+) and that in mitochondria was calculated using K_{eq} (3.78×10^{-6}M) for glutamate dehydrogenase reaction (glutamate + NAD^+ → a-ketoglutarate + NH_4^+ + NADH + H^+) at pH 7.0, 38 °C. As shown in Table 4, $[NAD^+]/[NADH]$ value of test group was recovered close to the normal level.

Table 4. $[NAD^+]/[NADH]$ ratio and $[NADP^+]/[NADH]$ ratio of the liver of rat fed with ordinary diet and 12% ethanol along with (Test) and/or with (Control) 0.1% ginseng saponin instead of water (free access). Normal group was fed only ordinary diet and water. Calculation of $[NAD^+]/[NADH]$ ratio and $[NADP^+]/[NADPH]$ ratio was described in the text.

	Normal	Control	Test
Cytoplasm	886	507	676
Mitochondria	8.58	4.68	6.12

Ethanol containing [^{1}-^{14}C] ethanol (5 uCi) was injected to the above three groups. 30 min later, the distribution of radioactivity of hepatic lipids was investigated. As shown in Table 5, radioactivity of hepatic lipids of both control and test group was higher than that of normal group, but that of test group was much lower than that of control. Analysis of individual lipids such as phospholipids, cholesterol, fatty acid and triglycerides, showed that phospholipid biosynthesis was significantly impaired and fatty acid and triglyceride biosynthesis were greatly stimulated. However, the saponin prevented the phospholipid biosynthesis depression and triglyceride biosynthesis stimulation considerably. It seems that the ginseng saponin might stimulate ADH, ALDH and MEOS in accelerating ethanol oxidation and acetaldehyde removal from

Table 5. Distribution of radioactivity (DPM) of hepatic lipids of rat which received intraperitoneal injection of 1 m*l* of 10% ethanol (containing [1-^{14}C] ethanol, 5 Ci). The rats were killed 30 min. later. Rats were fed with 12% ethanol (control) or 12% ethanol containing 0.1% saponin (test) instead of water for 6 days prior to [1-^{14}C] ethanol injection.

Lipid fraction	Radioactivity (DPM)			Relative	Ratio
	Normal	Control	Test	C/N	T/N
Total lipid	114,089 (100)	176,867 (100)	142,637 (100)	155.0	125.0
Phospholipid fraction	49,045 (43.0)	12,407 (7.0)	34,322 (24.1)	25.3	70.0
Cholesterol fraction	10,528 (9.2)	10,248 (5.8)	10,267 (7.2)	97.3	97.5
Fatty acid fraction	22,895 (20.1)	49,820 (28.2)	47,119 (33.0)	217.6	205.8
Triglyceride fraction	35,817 (31.4)	73,141 (41.4)	59,326 (41.6)	204.2	165.6

Table 6. Electron microscopic observation of the effect of ginseng saponin on hepatocytes of rats dosed with 12% ethanol instead of water for 6 days.

Group		Sweling & disruption of mitochondria	Dilatation & vesiculation of RER	Proliferation of SER	Pyknosis	Fat deposition
Control	1	+ + +	+ + +	+ + +	+	+ +
	2	+ + +	+ + +	+ + +	+	+ + +
	3	+ + +	+ +	+ +	+	+ +
	4	+ +	+ +	+ +	+	+ +
	5	+ +	+ + +	+ +	+	+ +
Test I	1	−	+	+	−	−
	2	−	−	−	−	+
	3	−	−	−	+	−
	4	+	+	−	−	+
	5	+	−	−	−	−

Control group was fed with 12% ethanol only instead of water for 6 days. Test I group was fed with 0.1% ginseng saponin in 12% ethanol instead of water.
−: normal, +: mild, + +: moderate, + + +: severe

the tissue and excess hydrogen can be shunt more quickly into lipid biosynthesis.

Electron microscopic observation showed that the hepatic cell of control group was significantly damaged. Mitochondria were swollen and disrupted severely. The rough endoplasmic reticulum (RER) were dilated and vesiculated and smooth endoplasmic reticulum (SER) were proliferated. Peroxisomes were increased in number and prominant golgi apparatus were seen, and pyknosis occurred and large fat droplets were seen. However, hepatocytes of test group showed that swollen or desrupted mitochondria were not seen, and dilated or vesiculated RER were very few (Table 6).

It was demonstrated in this laboratory that the ginseng saponin has some preventive effect against hypercholesterolemia induced by prolonged high cholesterol diet administration in rabbits (Joo, 1980) and rats (Joo *et al.*, 1987).

It has been reported that more than 93% of all cholesterol of the animal body are in cell membranes, where it performs vital structural functions, while only about 7% circulates in plasma. It is the plasma cholesterol level, however, which is strongly implicated as a cause of atherosclerosis. Consequently, factors regulating the plasma cholesterol level are the subject of intense study. Cholesterol is transported in the plasma in macromolecules called lipoproteins, which consist of varying amounts of specific proteins, cholesterol, cholesterol ester, triglycerides and phospholipids.

The importance of the Low Density Lipoprotein (LDL) receptor is highlighted by studies of familial hypercholesteremia. The total concentration of cholesterol and LDL in the plasma is markedly elevated in this genetic disorder, which results from a mutation at a autosomal locus. Cholesterol is deposited in various tissues because of the high concentration of LDL-cholesterol in the plasma. Nodules of cholesterol called xanthomas are prominant in skin and tendons. More harmful is the deposition of cholesterol in arterial plaques, which produce atherosclerosis. The molecular defect in most cases of familial hypercholesterolemia is an absence or deficiency and functional receptor for LDL (Brown and Goldstein, 1986).

Table 7 showed that the level of liver lipids such as cholesterol and triglycerides of ginseng fed rats (Test II) was greatly lower than that of nonfed group (Control) in high cholesterol administered

Table 7. Lipid composition of liver of rats under different feeding conditions.

Group	Cholesterol	Triglyceride (mg/g wet weight of liver)	Phospholipid	Chol./PL	TG/PL
Normal	3.8±0.2	4.9±0.3	18.3±0.2	0.21	0.27
Test I	3.7±0.7	4.4±0.8	16.7±0.9	0.22	0.26
Control	13.8±1.7	8.4±0.7	24.0±1.8	0.58	0.35
Test II	7.3±1.2	6.2±0.4	23.8±0.8	0.31	0.26

Normal: normal diet fed rats.
Test I: normal diet and ginseng saponin fed rats.
Control: high cholesterol diet fed rats.
Test II: high cholesterol diet and ginseng saponin fed rats.

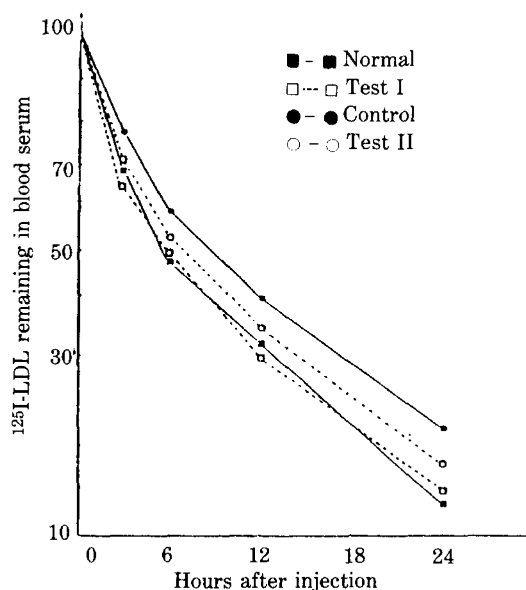

Fig. 2. Effect of ginseng saponin of ^{125}I-LDL removal from the blood serum of normal diet fed rats (normal). normal diet and ginseng saponin fed rats (test I). high cholesterol diet fed rats (control) and high cholesterol diet and ginseng saponin fed rats (test II) from 15 days.

rats while in ordinary fed rat, no significant difference in the above lipid level occured between ginseng fed group (Tast I) and nonfed group (Normal).

^{125}I-LDL was injected intravenously to rats which had been fed with high cholesterol diet with and/or without ginseng saponin for 15 days and the radioactivity disappearance from test group was faster than from control group as shown in Fig. 2.

Table 8. Binding of ^{125}I-LDL to the liver plasma membrane of rabbits fed under different feeding conditions.

Group	Total (ng/mg)	EDTA-resistant (ng/mg)	EDTA-sensitive (ng/mg)
Normal	52.6	26.1	26.5
Test I	55.3	27.0	28.3
Control	38.4	25.8	12.6
Test II	50.8	26.4	24.4

Normal: Normal diet fed rabbits (12 days).
Test I: Normal diet fed and ginseng saponin fed rabbits (12 days).
Control: High cholesterol diet fed rabbits (12 days)
Test II: High cholesterol diet and giseng saponin fed rabbits (12 days).
Assay mixture (150 *l*) contained 100 g of membrane protein and 25 g/m*l* of ^{125}I-LDL (50 cpm/ng) in the absence or presence of 30 mM EDTA.

Table 8 showed that the binding activity of ^{125}I-LDL to rat liver plasma membrane. It was found that the activity was higher in test group than control. It was also observed that LDL receptor activities of other organs such as kidney, adrenal cortex and testis of ginseng saponin fed rats are higher than that of control group. It was reported (Kita *et al.*, 1980) that there are EDTA-sensitive binding site and EDTA-resistant binding site for LDL in rabbit liver and the hypocholesteremic action of cholestryamin and mevinolin is due to increase of the number of EDTA-sensitive binding site. The total LDL receptor activity was lowered in control group but the saponin prevented LDL activity loss

dueing to high cholesterol administration. However the activity of EDTA-resistant binding site did not altered under different feeding conditions but that of EDTA-sensitive binding site did. No significant activity change of both the EDTA-sensitive and resistant binding sites of normal rabbits in the presence of ginseng saponin (Table 9). This indicates that ginseng saponin might not stimulate binding affinity but the population of hepatic LDL receptor.

Table 9. Binding of ^{125}I-LDL to liver membrane of normal rabbit in the presence and/or absence of ginseng (*in vitro*).

Conc. of saponin (%)	Total (ng/mg)	EDTA-resistant (ng/mg)	EDTA-sensitive (ng/mg)
Control	50.2	24.1	26.1
10^{-5}	49.7	23.3	26.4
10^{-4}	50.8	25.2	25.0
10^{-3}	50.5	24.6	25.9
10^{-2}	52.4	25.4	27.1

Assay mixturee (150 *l*) contained 100 g of membrane protein, 5 g/*ml* of ^{125}I-LDL (120 cpm/ng) and various concentrations of saponin in the absence of presence of 30 mM EDTA.

The bile acids into which most of the cholesterol is converted are secreted into the upper intestine, where they emulsify dietary fats. Having done their work, the bile acids are largely reabsorbed from the intestine, taken up by the liver and again secreted into the upper intestine. This enterohepatic circulation of bile salts ordinary limits the liver's need for cholesterol. Therefore, if the recycling coul be interrupted, the liver would be called on to convert more cholesterol into bile acids and this should lead the liver cells to make more LDL receptors. A class of drugs that interrupt the recycling of bile acid such as cholestyramine was already well known. This drug was found to lower the blood LDL level by an average of 10%. On the other hand, HMGCoA reductase inhibitors such as compactin and mevinolin were also known to lower the blood LDL level in animals. The combination of HMGCoA reductase inhibitors and bile acid binding resin was reported to be more effective.

It is easily expected from the nonspecific enzyme stimulation effect of ginseng saponin that the saponins might stimulate the cholesterol conversion to bile acids and *in vitro* experiment showed this

Table 10. The effect of ginsenosides on the hile acid biosynthesis from (4-14C)-cholesterol by rat liver. The values are mean value of three determinations.

Con. of ginsenosides in the reac. mixture (%)		0	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Ginsenoside mixture		115,509±12,761 (100)	126,558±27,855 (110)	147,889± 5,330 (128)*	136,856±25,931 (118)	128,977±10,222 (112)
Giseno side Rb1		115,509±12,761 (100)	150,869± 9,316 (131)*	152,448±57,243 (132)	145,796± 5,332 (126)*	131,368±40,428 (114)
(100)	-Rb2	115,509±12,761 (100)	115,323±15,461 (117)	135,233±36,526 (133)	153,808±34,398 (131)*	151,571±10,109
	Re	115,509±12,761 (100)	142,564±12,616 (123)	143,201±10,644 (124)	156,015± 4,439 (135)*	137,205±20,444 (119)
	Rg1	115,509±12,761 (100)	144,016±12,061 (125)	150,837±14,327 (131)*	126,744±35,509 (110)	136,024± 8,747 (118)

*$p < 0.05$

Reaction mixture (2 *ml*) contained 137 mM NaCl, 5.2 mM $NaHCO_3$, 1% glucose, 0.2% BSA, 10 mM phosphate buffer (pH 7.4), 10 ug cholesterol containing (4-14C)-cholesterol (0.09 uCi), ginsenoside mixture and purified ginsenoside Rb1, -Rb2, -Re, -Rg1 fractions (10^{-2}%-10^{-5}%), and 20% rat liver homogenate.

The figure in brackets are relative percentage assuming that of control group (0% saponin) being 100.

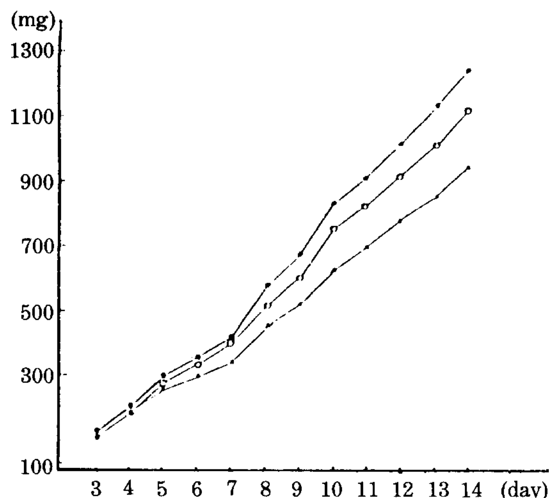

Fig. 3. The accmulative amount of bile acids from feeds of rats fed under various conditions two weeks course. The values are mean value of live determinations.
Control group (▲–▲) were administered normal diet with high cholesterol diet (cholesterol 100 mg olive oil 0.5 m*l*. Na-cholate 10 mg/rat/day). Test I group (○ – ○) were administered under similar diet conditions of control group but either with ginsenoside mixture (1 mg/rat/day). Test II group (● – ●) were administered under similar diet conditions of control group but either with ginsenoside Rb_t (1 mg/rat/day).

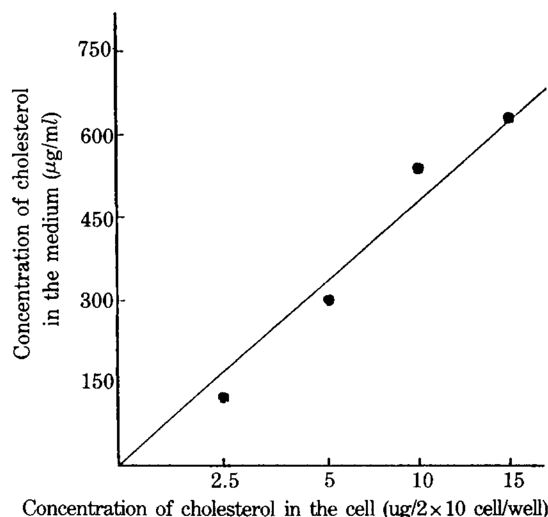

Fig. 4. Uptake of cholesterol by CHO cell cultured in medium containing both cholesterol and (4-14C)-cholesterol.

was the case as shown in Table 10. Furthermore, the secretion of bile acids by ginseng saponin fed group was faster than nonsaponin fed group (Fig. 3). We don't know yet whether the recycling of bile acids is interrupted by the saponin or not which remains to be solved

Effect of total saponin extract and some purified ginsenoside Rb_1 and Rb_2 on LDL receptor biosynthesis of chinese hamster ovary (CHO) cells cultured in a high cholesterol medium was investigated. Cholesterol uptake by CHO cell cultured in a medium containing various amounts of cholesterol was traced and found that the cholesterol uptake was proportional to the concentration of cholesterol in the medium (Fig. 4), and the population of LDL receptors were gradually decreased as the cholesterol concentration in the medium increased (Table 11). However, when the CHO cells were cultured in

Table 11. LDL receptor activity of CHO cells cultured in medium containing various amounts of cholesterol. The values are mean value of three determinations.

Concentration of cholesterol (g/m*l*)	LDL receptor activity ng bound LDL/mg protein	Relative activity (%)
0 (control)	1,158±113	100
2.5	1,026±186	87
5.0	977±106	84
10.0	816± 81	70*
15.0	793±103	68*

Relative activity of LDL receptor was expressed assuming that of control being 100.
*$p<0.05$.

the medium containing ginsenosides, cholesterol concentration was lowered resulting in less loss of LDL receptor activity (Table 12).

It is now known that an excess of cholesterol in the liver reduces transcription of the LDL-receptor gene into messenger RNA, the nucleic acid that is subsequently translated by the cell's protein-synthesizing machinary to make the LDL-receptor. The biosynthesis of protein and RNA of the above cells was higher than that of CHO cells cultured in

Table 12. Cholesterol concentration of CHD cells cultured in medium containing various amounts of cholesterol. The values are mean value of three determinations.

Group	Cholesterol concentration (ug)	Relative (%)
Normal	37±1.51	100
Control	56±2.16	150*
Ginsenoside mixture (10^{-5}%)	34±3.92	91
Ginsenoside Rb1 (10^{-6}%)	32±2.39	86*
-Rb2 (10^{-4}%)	23±1.41	61*
-Re (10^{-7}%)	40±7.32	107
-Rg1 (10^{-6}%)	36-0.82	96

*$p<0.05$

Table 13. The biosynthesis of RNA and protein of CHO cells cultured under various conditions.*

	Radioactivity of 3H-Uridine incorporated into RNA (cpm)	Rodioactivity of 14C-Leucine incorporated into protein (cpm)
Normal	8,239±1,461 (100)	18,383±4,628 (100)
Control	2,730± 194 (33)**	10,473±1,445 (57)
Ginsenoside mixture	4,103± 125 (50)**	12,774±1,862 (69)
Ginsenoside Rb1	4,099±1,296 (50)**	11,438± 969 (62)
-Rb2	4,096±1,391 (50)**	11,274± 996 (61)
-Re	2,821± 53 (34)**	11,391± 216 (62)
-Rg1	3,799±1,140 (46)**	11,439± 614 (62)

Normal group was cultured in standard medium.
Control group was cultured in standard medium containing cholesterol (10 g/m*l*). Test groups were cultured under similar conditions of control group but either with ginseing saponin mixture (10^{-5}%) or with ginsenoside Rb1 (10^{-6}%), -Rb2 (10^{-4}%), -Re (10^{-7}%), -Rg1 (10^{-6}%) fractions.
*The figure in brackets are relative percentage assuming that of normal group being 100.
**$p<0.05$.

the absence of the ginsenosides (Table 13), suggesting that the ginsenosides might stimulate LDL receptor biosynthesis by lowering cholesterol level. It was also observed that the ginsenosides stimulate the biosynthesis of estradiol and progesterone from cholesterol in the CHO cells as shown in Table 14.

Ginseng saponin was administered by stomach tubing prior to intraperitoneal injection of cholesterol containing [4-^{14}C]-cholesterol into adult male rats and the liver, testis and blood serum were analyzed. The first high radioactivity peak of the liver and blood serum of test animal was observed at 6 hours after radioactive cholesterol injection, while that of control appeared at 12 hours after the injection. In the case of testis, the first high radioactivity peak of test group appeared betweem 2 and 6 hours after the radioactive cholesterol injection, while that of control appeared at 10-14 hours. Analysis of radioactivity distribution of cholesterol, androstenedione and testosterone in the testis of rats fed with/without ginseng saponin prior to [4-^{14}C]-cholesterol injection showed that the saponin stimulated the synthesis of androgens from cholesterol. This was confirmed again by *in vitro* experiment using tests homogenate as an enzyme source. Therefore, it was suggested that the ginseng saponin stimulates both cholesterol transport and the biosynthesis of androgens from cholesterol in rat testis (Table 15 & 16).

It seemed that the ginsenosides lower the cholesterol level by stimulating the cholesterol metabolism including bile acids (in liver) and steroid hormone biosynthesis, resulting in the lowering of inhibitory action of cholesterol on LDL receptor biosynthesis.

It is well known that phospholipids play a significant role in the transport of lipids including cholesterol, subsequently, it is expected that the stimulation of phospholipid biosynthesis by the saponin may facilitate the transport of lipids including cholesterol under such condition as prolonged feeding of high cholesterol diet to rabbits. Investigation of

Table 14. The effect of ginsenoside on the biosynthesis of progesterone and esteradiol from (4-14C)-cholesterol in cultured CHO cells.

	Radioactivity (dpm)					
	Cell			Medium		
	Progesterone	Estradiol	Cholesterol	Progesterone	Estradiol	Cholesterol
Control	1,168±421	885± 231	75,472±25,128	412,212	885±340	109,355±21,269
	(1.5)	(1.2)	(100)	(0.4)	(0.8)	(100)
Ginsenoside mixture	2,691±968	962± 57	67,659±19,457	414± 98	855± 32	64,818± 4,659
	(4.0)	(1.4)	(100)	(0.6)	(1.3)	(100)
Ginsenoside Rb1 (3.6)	1,690±279	1,064± 144	47,033±15,854	1,016±203	2,349± 48	64,866±15,717
	(2.2)	(100)	(1.6)	(3.6)	(100)	
-Rb2	1,546±211	1,242±1,089	70,892± 7,398	1,115±585	1,612±361	84,396±19,145
-Re	2,265±719	915± 147	68,704±14,487	1,005±722	1,083±561	64,052±12,635
	(3.3)	(1.3)	(100)	(1.6)	(1.7)	(100)
-Rg1	1,813±113	938± 81	57,544± 6,244	514±121	2,472±510	88,197±21,216
	(3.2)	(1.6)	(100)	(0.6)	(2.8)	(100)

*The figure in brackets are relative percentage assuming that of cholesterol being 100.

Table 15. The effects of ginseng saponin on the biosynthesis of androgens from cholesterol in rat testis

Fraction	Radioactivity (DPM)		Radioactivity of test/ control
	Control (%)	Test (%)	
Cholesterol	870 (57)	1301 (46)	1.5
Androstenedione	310 (20)	720 (26)	2.3
Teastosterone	360 (23)	780 (28)	2.2

The rats were fed with/without ginseng saponin prior to (4-^{14}C)-choldesterol administration were killed 16 hours after the cholesterol injection and the testis was homogenized and analyzed.

Table 16. The effects of ginseng on the biosynthesis of androgens found (4-^{14}C)-cholesterol by rat testis homogenate *in vitro*

Fraction	Radioactivity (DPM)		Radioactivity of test/ control
	Control (%)	Test (%)	
Cholesterol	42301 (60)	36086 (50)	0.9
Androstenedione	12429 (18)	17386 (24)	1.4
Testosterone	15698 (22)	19029 (26)	1.2

The reaction mixture (20 m) contained (final concentration): 60 mM NAD, +60 mM ATP, 4 uM NADPH. 5 mM glucose, 5 mM fumarate, 5 mM fumarate, 5 mM nicotinamide, 5 mM $MgCl_2$, 20 mM Tris buffer (pH 7.4), 2.5 uM cholesterol containing (4-14C)-cholesterol (108, 225 DPM) and 12 *ml* of the testis homogenate. Test group contained 10^{-4}% ginseng saponin. The reaction mixture wsa incubated at 37°C for one hour under the mixture of 95% O_2: 5% CO_2. Following the reaction was terminated, cholesterol, androstendion and testosterone were chromatographed and the radioactivities of corresponding fraction were counted.

the ^{32}P incorporation to the hepatic phospholipids of ginseng saponin administered rats (Test Group) and control group showed that the incorporation was found much greater in the former than the latter suggesting phospholipid synthesis was stimulated by the saponin. The radioactivity of blood plasma lipid fraction of test group was also higher than that of the control group. It appeared that the saponin stimulated the phospholipid biosynthesis of both cytosolic and mitochondrial fractions. Observation of phospholipid biosynthesis *in vitro* using rat liver homogenate again showed that the adequate amount of ginseng saponin greatly stimulated the biosynthesis of phospholipid. It seemed that the increase of phospholipid biosynthesis might come from the sum of stimulation of several enzymes relating to phospholipid biosynthesis by the saponin. Moreover, the *in vivo* stimulation of phospho-

Table 17. Macroscopic occurrence of histological severity of Atheroma in aorta of prolonged cholesterol fed rabbits with and/or without ginseng saponin administration.

Location	Ascending aorta			Thoracic aorta			Adbominal aorta		
Fed period (week)	2	4	6	2	4	6	2	4	6
Cholesterol	+ (x)	+ (xx)	+ + (xx)	−	−	−	−	−	+ (x)
Cholesterol + ginseng saponin	−	−	+ (x)	−	−	−	−	−	−

−; Absence of atheroma formation.
+; Presence of atheroma, mild 5-1%.
+ +; Presence of atheroma, moderate 20-40%.
x; Presence of atheroma involving 1 to 2 days of foam cell intima.
xx; Presence of atheroma involving 3 to 5 days of foam cell intima.
xxx; Presence of atheroma involving 6 to 9 days of foam cell intima.

lipid biosynthesis by the saponin might result in the better transport of lipids including triglyceride and cholesterol in the animal body (Lee *et al.*, 1981).

We examined the atheroma formation in aorta, coronary and renal arteries of prolonged cholesterol administered rabbits (2-6 weeks) with saponin (Test Group) and/or without (Control) microscopically. As shown in Table 17, a slight macroscopical occurence of atheroma in ascending aorta could be seen even within two weeks administered control group and the symptom gradually developed as the cholesterol feeding was continued. However, in the saponin fed group, the occurence of the atheroma was not observed within 4 weeks feeding but a slight symptom of atheroma formation occured after 6 weeks' feeding (Joo, 1980).

From the foregoing discussion, it is likely that the ginseng saponin might stimulate the enzymes relating to the metabolism including cholesterol and phospholipids resulting in the delay of cholesterol level rise in the blood of the cholesterol fed rabbits, consequently the prevension of atheroma formation in such tissues as aorta.

Table 18. The effect of ginseng saponin fraction on ascorbate synthesis during germination of gycine max.

Germination time (day)	Group		
	Control (mg)	Test (mg)	Test control
1	0.61+0.21	1.06+0.19	1.74
2	1.67+0.63	2.67+0.19	1.60
3	3.21+0.90	3.96+0.90	1.23
4	4.06+1.26	5.35+0.55	1.32

Data are mean values of 100 seedlings.

Table 19. The effect of ginseng saponin fraction on amylase activity during the germination of rice seed. The values are mean value of determinations.

Group time	Amylase activity (Units/mg protein)*		
	Control	Test	Relativity** (%)
12 hr	0.67	0.85	127
24 hr	0.53	0.84	158
48 hr	1.06	1.79	169
72 hr	0.84	1.25	149

*One units of enzyme was defined as mg maltose liberated in 50 min at 25°C by 1 m*l* enzyme solution.
**The relative percentage is expressed assuming the activity of control is 100.

Ginseng saponins stimulate plant enzymes too. It was found that ascorbate synthesized during the germination of Glycine max was always much higher than that of control when the soybeans were either rinsed in 10^{-4}% ginseng saponin solution at first for 24 hours or poured afterwards. Using the homogenate of germinating soybean (2nd day) as enzyme source and glucose containing [U-^{14}C]-glucose as substrate, it was confirmed that the saponin fraction stimulated the ascorbate biosynthesis during germination as shoown in Table 18 (Bae *et al.*, 1986).

The effects of ginseng saponin on the germination and early growth of rice seeds (*Oryza sativa* L.) were investigated. The early growth (length) of test rice seeds which were rinsed for 60 hrs in 10^{-4}% saponin solution prior to transplantation to water agar

Table 20. The effect of ginseng saponin fraction ($10^{-4}\%$) on the early growth of rice seed. The values are mean value of the length of 100 seedlings.

Group day	Control (cm)	Test (cm)	Relativity** (%)
2	0.39±0.14	0.40±0.18	103
3	0.93±0.28	1.06±0.54	114*
4	2.34±0.57	2.32±0.64	99
5	4.61±0.73	4.65±0.81	101
6	6.80±0.73	6.94±0.72	102
7	8.16±0.80	8.58±1.01	105*
8	9.53±1.29	10.59±1.43	111*
9	11.47±1.63	12.59±1.68	110*
10	13.40±1.76	14.56±1.77	109*

The relative percentage expressed assuming that of control group being 100.
*$p<0.05$

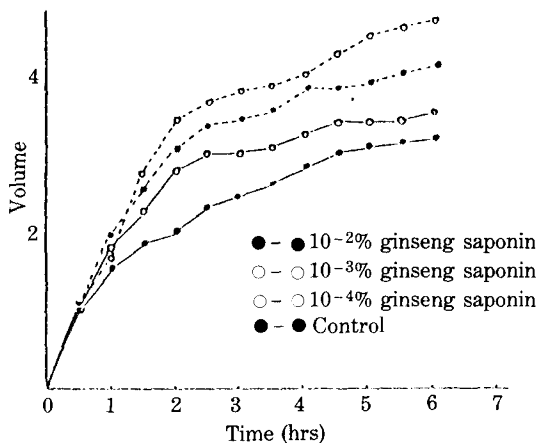

Fig. 5. Generation of CO_2 from yeast cells incubated (37°C) in glucose medium containing 0.1 M phosphate buffer (pH 6.8), 3% glucose, 1g of yeast cake and ginseng saponin fraction ($0\text{-}10^{-3}\%$

bed was about 20% faster than that of control seeds as shown in Table 19. It was also found that the amylase activity of the seeds was most active when the seeds were rinsed in $10^{-4}\%$ saponin solution for 48 hrs (Table 20). *In vitro* investigation showed that the amylase activities were stimulated about 30% by the treatment of $10^{-5}\%$ saponin solution compared with control group as shown in Table 21. From the above results, it seems that the ginseng

Table 21. Effect of ginseng saponin fraction on amylase activity *in vitro*. The values are mean values of three determinations.

Added saponin concentration (%)	Enzyme activity (unit)*	Relativity** (%)
0	0.585	100
10^{-1}	0.320	55
10^{-2}	0.480	82
10^{-3}	0.490	84
10^{-4}	0.510	87
10^{-5}	0.780	113
10^{-6}	0.650	111
10^{-7}	0.625	107

*One units of enzyme was defined as mg maltose liberated in 50 min at 25°C by 1 *ml* enzyme solution.
**The relative percentage is expressed assuming the activity of control is 100.

Table 22. Distribution of radioactivities of glycolytic intermediates of yeast cells incubated in glucose medium containing [U-^{14}C]-glucose

	Control (cpm)	Test (cpm)	Test/control (%)
Initial spotting amount	30,000	30,000	
Glucose	4,440	7,914	178
Glucose 6-phosphate	1,574	1,669	106
Fructose 6-phosphate	3,630	5,767	158
Phosphoenol pyruvate	2,304	2,248	98
Pyruvate	4,564	2,910	63
3-Phosphoglycerate	914	910	100

*The extract was spotted on wattman No. 1 paper and chromatographed by developing solvent (n-Buthanol: Acetate: Water, 74:19:50) for 24 hrs.

saponin might activate amylase of rice seed during germination, resulting in rapid growth of rice (Lee and Joo, 1987).

The effect of ginseng saponin fraction on several glycolytic enzymes of yeast cell was examined. The amount of CO_2 formed during the incubation of yeast cells in medium containing saponin fraction of *Panax ginseng* C.A. Meyer was greater than that of control cells and found that the CO_2 formation was greatest when the uptake of inorganic phosphate

Table 23. The effect of ginseng saponing fraction on yeast glycolitic enzymes *in vitro*. The values are mean value of three determinations.

Saponin conc. (%) in reaction mixture	Relative activity			
	Hexokinase	Phosphoglucoisomerase	Pyruvate kinase	Puruvate decarboxylase
0	100	100	100	100
10^{-7}	102	101	108	99.5
10^{-6}	108	114	113	105.3
10^{-5}	119	170	115	119.2
10^{-4}	115	100	113	127.3
10^{-3}	118	93	92	108.0
10^{-2}	118	96	72	75.3
10^{-1}	54	90	90	45.0

and glucose consumption were increased (Fig. 5). Radioactivity study of several glycolytic intermediates of yeast cells cultured in the medium containing [U-^{14}C]-glucose showed that the radioactivity of fructose 6-phosphate of test cells was as much as 1.6 times that of control group. On the other hand, the radioactivity of pyruvate of test cells was considerably decreased compared to control (Table 22). Investigation of the effect of ginseng saponin on yeast hexokinase, phosphoglucose isomerase, pyruvate kinase and pyruvate decarboxylase *in vitro* showed that the maximum activities of the above enzymes were observed when the concentration of ginseng saponin was 10^{-5}% in the reaction mixture as shown in Table 23.

From the above considerations, I would again suggest that nonspecific enzyme stimulatory effect of the saponin might be play a significant role in biochemical and physiological system in the living body.

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