THE EFFECT OF GINSENG SAPONINS ON SEVERAL ENZYMES

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Saponins are plant glycosides which were long known to lower the surface tension of water and therefore their aqueous solutions froth readily and the saponins cause hemolysis. It is easily understood from their structure that they are amphipathic having both hydrophobic sapogenin part and hydrophilic sugar part in the molecule, and therefore they disperse lipids in aqueous medium.

The studies of saponins 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. It has been reported that the saponins of Korean ginseng root are mainly triterpenoidal dammarane glycosides (Elyakov *et al.*, 1964; Hujita *et al.*, 1962) and they have no hemolytic activity but rather protective against hemolysis (Namba *et al.*, 1974).

It has been observed in this laboratory (Joo *et al.*, 1973) that ginseng saponins, one of the major components of *Panax ginseng* C. A. Meyer root, are surface active and good solubilizer of nonpolar lipids such as triglycerides and cholesterol. A significant portion of cholesterol palmitate and olive oil were found hydrolyzed *in vitro* by cholesterol esterase and pancreatic lipase respectively in the presence of the saponin, suggesting that the lipids were effectively dispersed and were brought to better contact with the enzyme resulting in a better hydrolysis of the lipids.

It has also been observed during the past few years that moderate amounts of the saponins stimulated the reactions catalyzed by enzymes such as mitochondrial dehydrogenases, alcohol dehydrogenase, aldehyde dehydrogenase and transaminases *in vitro* so far examined in this laboratory unexceptionally, suggesting that the detergent action of the saponin might give rise to better situation for the reactions being proceeded.

This paper described the general action of the saponin on lipid dispersion and its effects on several enzyme catalyzed reactions *in vitro*. In connection with the above experimental results, the saponin effect on pyruvate oxidation, alcohol detoxication and on some metabolism in the body has been discussed.

Extraction and Purification of Ginseng Saponins

Powdered Korean white ginseng roots (150 g Keumsan, 5 years) were placed in 760 ml of chloroform-methanol-water mixture (1:2:0.8, v/v/v) and the mixture was continuously shaken for two hours at room temperature. Following filtration by suction, the insoluble precipitates were placed in 190 ml of the above chloroform-methanol-water

mixture and shaking was continued for another one hour and filtered. The combined filtrate was then diluted with 250 ml of water to make the volume ratio of chloroform-methanol-water in the mixture being 1:1:0.9 so that a good separation into two phases (chloroform phase and methanol-water phase) might be obtained.

The methanol-water phase was concentrated under reduced pressure to remove the methanol and then lyophilized, yielding 35 g of the crude saponin preparation. The crude preparation was then dissolved in methanol with warming and the methanol-insoluble fraction was removed by centrifugation.

To the above methanol-soluble fraction (50 ml), 150 ml of chloroform were added and the mixture was cooled and centrifuged. The supernatant was then passed through the silica gel column (dia. 2 cm, height 15 cm) to remove the remaining sugars. The filtrate was then concentrated under reduced pressure and dried *in vacuo*, obtaining 2.31g of saponin preparation. The chromatogram of the saponin preparation showed that it contained several saponins with R_f values of 0.66, 0.59, 0.50, 0.43, 0.33 and 0.25 on silica gel plate using chloroform-methanol-water (14:6:1, v/v/v) as a developing solvent. It appeared that the saponin with Rf value of 0.59 was the most abundant, the saponins with R_f values of 0.43, 0.33 were less abundant and the saponins with R_f values of 0.66, 0.50, 0.25 were the least. The above saponin mixture was used without further purification in this study.

The Effect of the Saponins on Lipid Dispersion and its Absorption from Small Intestine

Previous studies in this laboratory showed that ginseng saponin lowered surface tension of water significantly (surface tension of pure water: 72.75 dyne/cm^2; that of 0.5 % saponin: 35.27 dyne/cm^2 at 20°C) (Joo *et al.*, 1973).

It has been believed that dietary fats and sterols must become colloidally dispersed in intestinal lumen to form particles of at least micellar size to account for the specificity of the absorption system. During digestion, nonpolar dietary lipids such as triglycerides and cholesterol are mixed with bile. Following emulsification, they are partly hydrolyzed into more polar substances, the result being a highly complex mixture of nonpolar (triglycerides and cholesterol esters), slightly polar (diglycerides, cholesterol and unionized fatty acids), and polar substances (bile salts, lecithins, monoglycerides, and ionized fatty acids). It has been well realized that at very low concentrations of bile salts they behave as ordinary electrolytes. Above a critical micellar concentration(CMC), both ions and micelles are present but as the concentration of the bile salts rises, an increasing proportion enters the micellar phase. It is also known that the CMC of bile salt may depend not only on its own concentration, but also on the presence of other solubilizer and substance to be solubilized.

The osmotic pressure measurement of saponin solutions of different concentrations showed that the osmotic pressure of the solution increased as the concentration of the saponin rose until the concentration of saponin reached 1.9 % but when the saponin concentration was over the above concentration, the osmotic pressure of the solution dropped and then again started to increase as shown in Figure 1. This suggested that molecular aggrega-

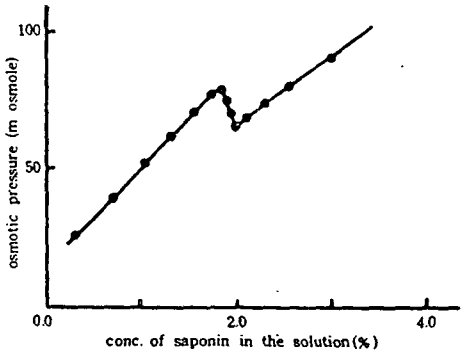

Fig. 1. Osmotic pressure of ginseng saponin solution concentrations. The osmotic pressure was measured using Precision Osmometer, Framingham, Mass. at 25°C|and the plots were the mean values of three determinations. The saponin used in this determination was the saponin mixture extracted from Korean white ginseng roots (Keumsan, 5 years) as described in the experimental part of this paper.

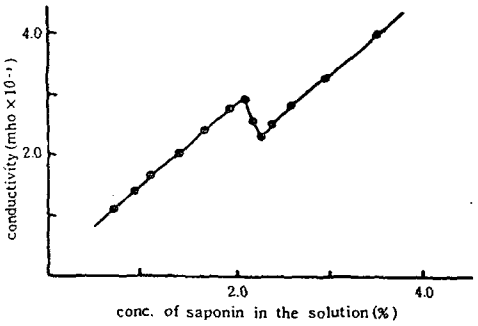

Fig. 2. Electric conductivity of ginseng saponin solutions. The electric conductivities were measured using conductivity meter, WTW, Messgeraet at 25°C and the plots were the mean values of three determinations. The saponin used in this measurement was the saponin mixture extracted from Korean white ginseng roots (Keumsan, 5 years) as described in the experimental part of this paper.

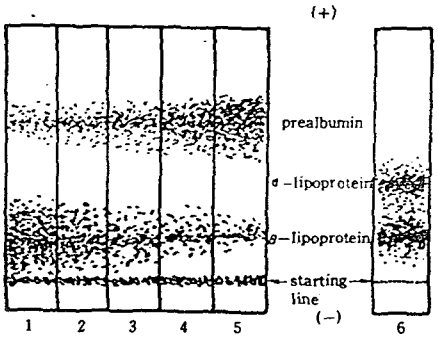

Fig. 3. Electrophoretogram of chicken intestinal lumen fluid containing various amounts of ginseng saponin. 1 is control: 2,3,4 and 5 are tests containing 0.6, 1.2, 1.8 and 2.4% saponin respectively: 6 is blood serum.

tion occured at the concentration of saponin around 1.9 %. A similar phenomenon was observed when the electric conductivity of the solution was measured as shown in Figure 2. The conductivity of the solution increased as the concentration of the saponin increased but dropped again when the concentration of the saponin was over 2.2 %. From the above results, it was concluded that the CMC of the saponin might be around 2 %. Assuming the average molecular weight of the saponins being 1,000, the CMC of the saponin would be about 20 mM. However, it was observed that the CMC of the ginseng saponin was greatly lowered by the presence of cholesterol to be solubilized. Furthermore, the CMC of Na-cholate with cholesterol were significantly lowered by the co-presence of the ginseng saponin. It is very interesting that even in the presence of saponin under 0.1 %, the CMC of Na-cholate lowered below 1 mM.

It was also found that when the ginseng saponin was added to the intestinal lumen fluid so that the concentration of the saponin in the mixture being 0.6 %, 1.2 %, 1.8 % and 2.0 %, respectively, the electrophoretogram showed that fast moving lipid zone appeared significantly dark when the paper was stained with Sudan Black B and the darkness of the zone increased as the saponin concentration increased as shown in Figure 3, suggesting again that the saponin greatly favored the lipid micellar formation. Observations of the effect of ginseng saponin on serum lipoproteins, pancreatic lipase and cholesterol esterase catalyzed reactions (Joo *et al.*, 1973) and the lipid extraction effect of the ginseng saponin from intestinal lumen fluid again supported the idea that the saponin might act as an excellent lipid solubilizing agent.

The effect of ginseng saponin on the absorption of water-insoluble vitamins such as vitamins A and E has been studied (Tables 1 and 2). When the vitamin A and ginseng saponin were fed by stomach tubing, vit. A concentration of blood serum of test group was found as much as twice that of control, but the vitamin A content started to decrease after three hours. The vitamin A content of the liver of test animal was also found about 1.5 times that of control and its value returned to normal value three hours later. The vitamin E contents of the blood and liver of test animals were found as much as 1.4-1.5 times that of control 40 minutes after feeding. These results suggested that saponin stimulated the absorption of water-insoluble vitamins A and E. On the other hand, the contents of the above vitamins in the kidneys of test animals were significantly higher, suggesting that the ginseng saponin might also affect the transport of the above water-insoluble vitamins (Tables 1 and 2).

The above results led us to suggest that under

Table 1. Absorption of vitamin A-acetate in albino rat

Tissue	Group	Time(min)				
		0	40	60	120	180
Serum(μg/ml)	Control	1.015	1.545	2.258	3.682	2.227
	Test	1.105	2.924	5.955	7.576	1.742
Liver(μg/rat)	Control	209.508	216.761	286.364	339.394	212.784
	Test	214.110	280.398	310.890	510.417	231.345
Kidney(μg/rat)	Control	—	—	3.394	3.394	8.909
	Test	—	—	5.818	15.152	15.758

Twelve mg of vitamin A-acetate and 50mg of ginseng saponin in 2 ml of 50% ethanol was fed by stomach tubing and the amounts of vitamin A in blood serum, liver and kidney were analyzed at 0,40 min, 60 min, 120 min later and 180 min according to Carr-price method.

Table 2. Absorption of vitamin E

Tissue	Group	Time(min)			
		40	60	120	180
Serum(μg/ml)	Control	5.58	6.20	6.71	7.74
	Test	7.74	7.64	7.15	10.00
Liver(μg/rat)	Control	80.24	114.98-	160.47	217.94
	Test	117.10	114.93	173.43	203.84
Kidney(μg/rat)	Control	—	3.47	—	3.47
	Test	—	8.92	20.07	18.09

Twelve mg of vitamin E and 60 mg of ginseng saponin in 2 ml of 50% ethanol was fed by stomach tubing and the amounts of vitamin A in blood serum, liver and kidney were analyzed at 40 min, 50 min, 120 min and 180 min later according to Dipyridyl-ferrichloride method.

the physiological conditions, a small amount of the saponin would affect sufficiently the lipid micellar formation in the intestinal lumen so that the lipids might be absorbed easily in the small intestine.

The Effect of Ginseng Saponin on Enzyme-Catalyzed Reactions

It was found that adequate amounts of the saponin stimulated the reactions catalyzed by enzymes so far tested in this laboratory such as mitochondrial dehydrogenases (SDH, MDH, α-KGDH ICDH(NAD^+), GLDH), transaminases (GPT & GOT) and ICDH ($NADP^+$), ADH, ALDH while the reactions were inhibited at the excess amounts of saponin unexceptionally.

The activity of succinate dehydrogenase (SDH, EC 1. 3. 99. 1) was found highest when the concentration of the saponins in the assay mixture was 8.3×10^{-2}% as shown in Table 3. However, SDH was inhibited when the concentration of the saponins in the reaction mixture was over 1.7×10^{-1}%. On the other hand, malonate inhibition of SDH was found recovered little by the addition of the saponins, suggesting that a recovery of malonate inhibition of SDH can not be expected by the saponins.

The ginseng saponins were also found to activate malate dehydrogenase (MDH, EC 1. 1. 1. 37). When the concentration of the saponins in the assay mixture was 1.4×10^{-5}%, the activity of MDH was highest, 1.32 times that of the control. Again when the concentration of the saponins was over 1.4×10^{-4}%, the MDH was inhibited (Table 4).

α-KGDH was also activated in the presence of the saponins (Fig. 4). It was found that the activity of α-KGDH was highest when the concentration of the saponins in the assay mixture was 1.5×10^{-3}%.

It is known that ADP is one of the cofactors for ICDH (NAD^+)-catalyzed reaction and ATP inhibited this reaction. It was found that the saponin stimulated this enzyme but

Table 3. The effect of ginseng saponin on malonate inhibition of chicken hepatic mitochondrial succinate dehydrogenase. Test volume was 3.0 ml. It contained (final concentrations): phosphatic buffer (pH 7.4) 0.017M, succinate 0.01 M, DICPIP 0.28×10^{-4} M, malate 6.7×10^{-3} M, KCN 6.7×10^{-4} M, saponin, malonate and mitochindrial preparation*** 0.3 ml.

Conc. of saponin in assay mixture (%)	Conc. of malonate in assay mixture (M)	Enzyme activity* (units)	Relative activity**
0	—	4.15	100
2.5×10^{-2}	—	5.06	122
5.8×10^{-2}	—	5.48	132
8.3×10^{-2}	—	5.69	137
16.7×10^{-2}	—	3.61	87
0	1.67×10^{-3}	1.83	44
2.5×10^{-2}	1.67×10^{-3}	1.78	43
5.8×10^{-2}	1.67×10^{-3}	1.91	46
8.3×10^{-2}	1.67×10^{-3}	2.08	50
16.7×10^{-2}	1.67×10^{-3}	1.99	48
23.4×10^{-2}	1.67×10^{-3}	1.99	48
0	1.67×10^{-2}	1.08	26
2.5×10^{-2}	1.67×10^{-2}	1.04	25
5.8×10^{-2}	1.67×10^{-2}	1.20	29
8.3×10^{-2}	1.67×10^{-2}	1.41	34
16.7×10^{-2}	1.67×10^{-2}	1.33	32
23.4×10^{-2}	1.67×10^{-2}	1.29	32

* One unit of enzyme was defined as an optical density decrement of 0.01 per minute under the above conditions.

** Relative activities are expressed assuming the activity of control is 100.

*** See the experimental part.

Table 4. The effect of ginseng saponin on chicken hepatic mitochondrial malate dehydrogenase. The test volume was 3.5 ml. It contained (final concentrations): phosphate buffer (pH 7.4) 0.014 M, NAD^+ 4.3×10^{-4} M, nicotinamide 0.036 M, KCN 6.6×10^{-4} M, DICPIP 0.34×10^{-4} M, malate 7.1×10^{-3} M, saponin and mitochondrial preparation*** 0.5 ml.

Conc. of saponin in assay mixture (%)	Enzyme activity* (unit)	Relative activity**
0	2.11	100
1.4×10^{-7}	2.07	98
1.4×10^{-6}	2.19	104
1.4×10^{-5}	2.79	132
1.4×10^{-4}	2.55	121
1.4×10^{-3}	2.07	98
1.4×10^{-2}	1.91	91
1.4×10^{-1}	1.01	48

* One unit of enzyme was defined as an optical density decrement of 0.01 per minute under the above conditions.

** Relative activities are expressed assuming the activity of control is 100.

was not able to recover the ATP inhibition (Table 5).

The activity of purified bovine hepatic glutamate dehydrogenase in the presence of the saponins was studied and found that the activity of GLDH was the highest when the concentration of the saponins in the assay mixture was $10^{-5} \sim 10^{-6}$ %, the activity being about 1.5 times that of control. In the presence of the excess saponins above 10^{-6}% in the assay mixture the GLDH was inhibited gradually (Table 6).

It was also found that the ginseng saponins

Table 5. The effect of ginseng saponin on pig cardiac NAD^+ specific isocitrate dehydrogenase

Enzyme activity condition	Reaction rate		
	Units*	Relative activity**	Relative activity***
Control	2.8	100	—
AMP	2.8	100	—
ADP	3.5	125	100
ADP + ATP	2.6	93	74
ADP + saponin	4.2	150	120
ADP + ATP + saponin	2.6	93	74

The test volume was 4 ml. It contained (final concentrations): PIPES buffer (pH 6.5) 1.25×10^{-2} M, NAD^+ 5.0×10^{-4} M, $MgSO_4$ 2.5×10^{-3} M, DICPIP 1.27×10^{-5} M, threo-Ds(+)—isocitrate 1.25×10^{-5} M, nucleotides(AMP, ADP and ATP) 5.0×10^{-5} M, saponin 1.0×10^{-5} % and enzyme preparation 0.5 ml.

* One unit of the reaction rate was defined as an optical density decrement at 620 nm of 0.01 per 10 minutes under the experimental conditions.

** Relative activities were shown assuming the activity of control being 100.

*** Relative activities were shown assuming the activity of ICDH (NAD^+) in the presence of ADP was 100.

Table 6. The effect of ginseng saponin on bovine hepatic glutamate dehydrogenase in the presence of effector. Enzyme activities were determined by oxidation of glutamate in the following reaction mixture. The test volume was 3.0 ml. It contained (final concentrations) phosphate buffer (pH 7.6) 0.02 M, NAD^+ 0.25 mM, glutamate 5 mM, effector, saponin and enzyme preparation 0.3 ml.

Conc. of saponin / Conc. of effector	Control	Enzyme activity*				
		8.3×10^{-5} %	1.7×10^{-5} %	8.3×10^{-5} %	1.7×10^{-4} %	8.3×10^{-4} %
No effector	100	175	180	153	113	90
GTP 5×10^{-5}M	33	43	38	38	35	31
ADP 5×10^{-5}M	238	235	240	228	233	229
ADP 5×10^{-5}M +GTP 5×10^{-5}M	128	105	125	127	113	113

*Enzyme activities were shown as relative activities assuming the activity of control was 100.

**The final concentration of saponin and effectors were shown.

did not affect ADP activation of GLDH or GTP inhibition of GLDH signifying that the saponins did not interfere with GLDH regulation of the metabolism involved.

Furthermore, the ginseng saponins were realized to activate both the glutamate-pyruvate transaminase and glutamate-oxaloacetate transaminase but the degree of activation was not significant when compared with that of GLDH (Table 7).

In the presence of Mg^{++}, ICDH($NADP^+$)-catalyzed reaction rate lowered in the presence of the saponin when the concentration of isocitrate was higher (3.3×10^{-5} M) than its Km (4.5×10^{-5} M). However, when the isocitrate concentration was low (below 1.0×10^{-5} M), moderate amounts of the saponin (8.3×10^{-5} %—8.3×10^{-4} %) were found to stimulate the reaction significantly (Table 8). A similar effect of the saponin was also observed at the ICDH($NADP^+$)-catalyzed reaction in the presence of Mn^{++}.

It was observed that ADP(8.3×10^{-5} M) inhibited the ICDH($NADP^+$) activity but its inhibition effect was not significant when the isocitrate concentration in the reaction mixture was high (3.3×10^{-4} M). The saponin (8.3×10^{-5} %) was found to lower the ADP inhibition at the low isocitrate concentration. AMP, ATP and NADPH

Table 7-(a). The effect of ginseng saponin on glutamate-pyruvate transaminase (GTP). Enzyme activities were determined by assaying the pyruvate produced using LDH. The test volume was 3.0 ml. It contained (final concentrations): L-alanine 1.56 mM, α-ketoglutarate 1 mM, pyridoxal phosphate 0.027 mM, GPT (4.2 units/ml.) 0.4 ml, NADH 0.1 mM, LDH (1.3 units/ml.) 0.2 ml, phosphate buffer (pH 7.4) 0.06 M and saponin.

Conc. of saponin in assay mixture (%)	Enzyme activity* (units)	Relative activity**
Control	1.20	100
5×10^{-5}	1.39	116
5×10^{-4}	1.36	113
5×10^{-3}	1.37	114
5×10^{-2}	1.23	103

*One unit of enzyme was defined as a density decrement of 0.01 per min. under the above conditions.

**The relative activities are expressed assuming the activity of control is 100.

Table 7-(b). The effect of ginseng saponin on glutamate-oxaloacetate tansaminase (GOT). Enzyme activities were determined by assaying oxaloacetate according to Bergmeyer and Bernt (1974). The test volume was 1.5 ml. It contained (final concentrations): phosphate buffer (pH 7.4) 0.072 M, α-ketoglutarate 1.33 mM, L-aspartate 6.67 mM, pyridoxal phosphate 0.054 mM, saponin (various concentration) and enzyme solution 0.1 ml.** At the termination of reaction at room temperature for 10 min, 0.1 ml of 6.2 M trichloroacetic acid and 0.2 ml of aniline citrate were added to the test solution followed by the addition of 1 ml of 2 mM chromogen. The above mixture was allowed to stand for 20 min. Added 10 ml of 0.4 N NaOH and the optical density at 540 mm was read.

Conc. of saponin in assay mixture (%)	Conc. of pyruvate formed (mM)	Relative activity*
Control	2.41	100
6.7×10^{-6}	2.79	115.8
6.7×10^{-5}	2.89	120.1
6.7×10^{-4}	2.69	116.6

*Relative activities were expressed assuming the activity of control was 100.

**See the experimental part.

Table 8. The effect of ginseng saponin on pig cardiac NADP specific isocitrate dehydrogenase in the presence of Mg^{++}*

Conc. of isocitrate in the assay mixture (M)	Saponin concentration			
	Control	8.3×10^{-5} %	8.3×10^{-4} %	1.7×10^{-2} %
3.3×10^{-6}	0.60**	1.27**	1.23**	0.93**
1.0×10^{-5}	1.73	1.87	2.10	2.10
3.3×10^{-5}	3.83	2.47	2.97	3.20
1.0×10^{-4}	4.87	3.10	3.33	3.87
3.3×10^{-4}	5.43	3.47	3.70	4.50
6.7×10^{-4}	5.83	3.57	3.90	4.60
Km(M)	4.5×10^{-5}	5.7×10^{-5}	6.9×10^{-4}	1.3×10^{-4}

*The test volume was 3.0 ml. It contained (final concentrations): HEPES buffer (pH 7.4) 3.3×10^{-2} M, NADP 6.7×10^{-5} M, $MgSO_4$ 6.7×10^{-4} M, threo-Ds(+) -isocitrate, saponin and enzyme preparation 0.4 ml.

**One unit of enzyme was defined as an optical density increment of 0.01 per minute.

Table 9. The effect of ginseng saponin on ADP, AMP, and NADPH inhibition of pig cardiac $NADP^+$ specific isocitrate dehydrogenase

Conc. of effectors \ Conc. of isocit.	Reaction rate(in units*)			
	1.7×10^{-5} M	3.3×10^{-5} M	1.7×10^{-4} M	$3.3 \times 10^{4-}$M
Control	3.57*(100)	5.70*(100)	8.27*(100)	8.97*(100)
ADP (8.3×10^{-5}M)	2.77 (77.6)	4.05 (71.1)	6.93 (83.8)	8.64 (95.3)
ADP (8.3×10^{-5}M) + saiponn**	3.13 (87.7)	4.28 (75.1)	5.91 (71.5)	8.15 (90.9)
AMP (8.3×10^{-5}M)	3.27 (91.3)	4.40 (77.2)	7.70 (93.1)	7.57 (84.4)
AMP (8.3×10^{-5}M) + saponin**	3.10 (86.8)	4.33 (76.0)	6.43 (77.8)	6.87 (76.6)
ATP (8.3×10^{-5}M)	—	5.39 (94.6)	7.73 (93.5)	8.27 (92.2)
ATP (8.3×10^{-5}M) + saponin**	—	4.91 (86.1)	7.60 (91.1)	7.91 (88.2)
NADPH (8.3×10^{-5}M)	—	4.67 (8i.9)	6.31 (76.3)	7.83 (87.3)
NADPH (8.3×10^{-5}M) + saponin**	—	4.00 (70.2)	6.23 (75.3)	7.54 (84.1)

The test volume was 3.0 ml. It contained (final concentrations): HEPES buffer (pH7.4) 3.3×10^{-2} M, $NADP^+$ 6.7×10^{-5} M, $MnSO_4$ 6.7×10^{-4} M, 0.4 ml of the enzyme preparation and various amounts of threo-Ds(+)—isocitrate, effector and saponin. Figures in the blackets show relative rate assuming those of the corresponding control being 100.

*One unit of the reaction rate was defined as an optical density increment at 340 mn of 0.01 per minute under the experimental condition.

**The concentration of the saponin in the assay mixture was 8.3×10^{-5} %.

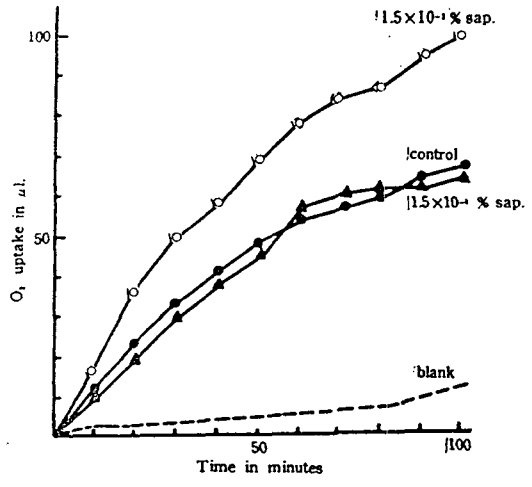

Fig. 4. The effect of ginseng saponin on chicken hepatic mitochondrial α-ketoglutarate dehydrogenase. The test volume was 3.0 ml. It contained(final concentrations): phosphate buffer (pH 7.4) 0.017 M, α-ketoglutarate 0.01 M, MAD^+ 0.001 M, $MgSO_4$ 6.7×10^{-4} M, saponin 1.5×10^{-4} % (△—△), 1.5×10^{-3} % (○—○), control (• — •) and enzyme preparation (see experimental part) 0.4 ml. Blank (. . .) contained all but substrate and saponin.

inhibition of the ICDH($NADP^+$) was also observed but the saponin was found again unable to recover their inhibition of the enzyme activity(Table 9).

The effect of ginseng saponins on equine and chicken hepatic alcohol dehydrogenases was investigated. The Km values of equine and chicken hepatic ADH were found to be 8×10^{-4} M and 5.1×10^{-4} M respectively. When the concentration of ginseng saponins of the assay mixture in the presence of known amounts of ADH and ethanol, the activity of ADH increased gradually as the concentration of the saponins increased. It was found, however, that the activity of the hepatic ADH decreased when the concentration of the saponins was over 0.53×10^{-3} %(horse) and 0.27×10^{-3} % (chicken), respectively. The velocity-concentration curves of the ADH reactions *in vitro* in the presence of the saponins over 1.33×10^{-5} % in the assay mixture were found to be sigmoid. It was realized that the effect of the saponins on ADH activity was dependent not only upon its concentration

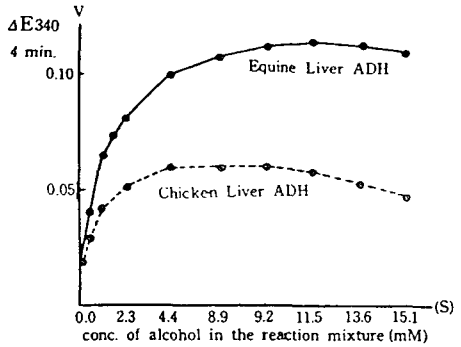

Fig. 5. Michaelis-Menten curves of equine and chicken hepatic alcohol dehydrogenases.

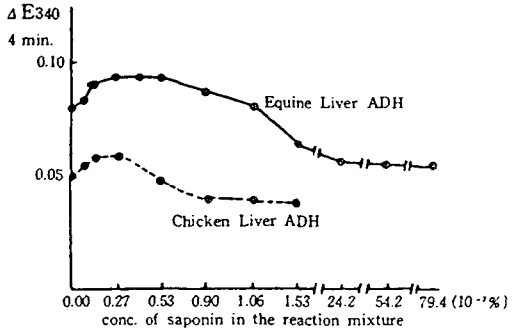

Fig. 6. The effect of ginseng saponins on equine and chicken hepatic ADH.

but also upon the concentration of substrate and that the optimal concentrations of saponins and ethanol for the maximum activity of horse ADH were 1.33×10^{-5} % and 2—4 mM, respectively. (Figures 5, 6 and 7)

The ADH activation effect of the saponin was

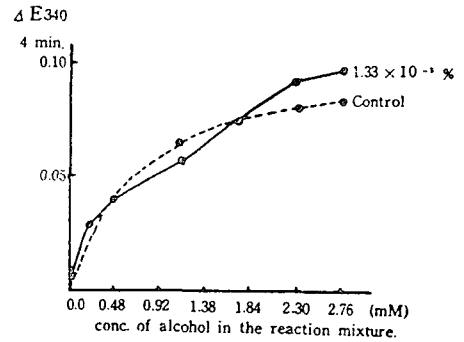

Fig. 7. Reaction velocity-substrate concentration relationship of equine hepatic ADH in the presence of ginseng saponins (conc. of the saponins in the reaction mixture is $1.0 \times 10^{-4} \times 0.4/3.0 = 1.33 \times 10^{-4}$ %).

confirmed using radioactive ethanol-1-^{14}C *in vitro* as shown in Table 10.

It was realized that mitochondrial ALDH was stimulated by the saponin, the concentration of which were around 1×10^{-8} % — 1×10^{-6} % in the assay mixture but inhibited when the saponin concentration was over 1×10^{-4} %. Km of mitochondrial ALDH for acetaldehyde reached the lowest value at 1×10^{-7} % of the saponin suggesting that the saponin stimulation of the ALDH might be brought by Km lowering for acetaldehyde of ALDH. Cytosolic ALDH was also found stimulated by the saponin(Tables 11 and 12).

It seemed that such a common amphoteric effect of the saponin on the above enzyme catalyzed reactions suggest that the stimulation might be not

Table 10. The effect of ginseng saponin on equine hepatic alcohol dehydrogenase *in vitro*

Radioactivity (cpm)				
Concentration of saponin in assay mixture	Total count of assay mixture following centrifugation	RCHO frac.	CPM in RCHO / CPM in Total	Relative* activity
0	4758456	8868	0.187	100
1.4×10^{-5}	4695252	8220	0.175	
1.4×10^{-4}	4247514	11997	0.283	135
1.4×10^{-5}	4268094	8505	0.200	

The test volume was 5.0 ml. The reaction mixture contained (final concentrations): glycine buffer (pH 9.0) 0.044 M, NAD^+ 4.2×10^{-4}M, ethanol 3×10^{-3} M containing 1-^{14}C-ethanol (1.42 μg) 1.67 μCi, saponin and 1ml of equine ADH preparation. After 5min incubation at 25°C, the reaction were terminated by the addition of 1ml of 20% TCA solution and the radioactivities of the supernatant and acetaldehyde were assayed using Tricarb Scintillation spectrophotometer.
*The activity of control was assumed 100.

Table 11. The effect of ginseng saponin on rat hepatic mitochondrial aldehyde dehydrogenase *in vitro*

Substrate mM**	Saponin (%)**					
	0	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}
1.0	0.016*	0.017*	0.023*	0.017*	0.016*	0.013*
	(100)	(106)	(144)	(106)	(100)	(81)
2.0	0.028	0.027	0.034	0.027	0.027	0.023
	(100)	(96)	(121)	(96)	(96)	(82)
5.0	0.041	0.010	0.050	0.045	0.044	0.036
	(100)	(98)	(122)	(110)	(107)	(88)
Km (mM)	2.74	2.13	1.67	2.63	3.08	4.08

Reaction mixture was 3 ml. It contained (final concentrations): pyrophosphate buffer (pH 9.2) 16.7 mM, acetadehyde (various concentration), NAD^+ 0.3 mM, nicotinamide 20 mM, saponin (various concentration) and mitochondrial preparation. Figures in brackets are relative activities assuming those of corresponding control were 100.

*Enzyme activities were shown by the increase of optical density at 340 nm during 9 minutes' incubation at room temperature (15°C) from 1 minute to 10 minutes after the initiation of the enzyme reactions.

**Final concentration in the assay mixture.

Table 12. The effect of ginseng saponin on rat hepatic cytosolic aldehyde dehydregense *in vitro*

Concentration of saponin in assay mixture** (%)	Exp. 1 CO_2 liberation*	Exp. 2 CO_2 liberation*	Km(M)
0	101.5(100)	44.8(100)	6.09×10^{-2}
1.7×10^{-7}	120.7(137)	86.2(192)	1.82×10^{-2}
1.7×10^{-6}	116.9(115)	81.0(181)	1.91×10^{-2}
1.7×10^{-5}	122.1(120)	82.1(183)	2.17×10^{-2}
1.7×10^{-4}	103.3(107)	59.1(132)	4.46×10^{-2}
1.7×10^{-3}	109.1(107)	56.4(126)	4.55×10^{-2}

The test solution was 3.0 ml. It contained (final concentrations): bicarbonate buffer (pH 9.3) 0.04 M, NAD^+ 0.5 mM, nicotinamide 20 mM, acetaldehyde 302.4 mM (Exp.1) and 35.7 mM (Exp 2), saponin (various concentration) and cytosolic fraction 0.5 ml. Figures in brackets are relative CO_2 liberation assuming corresponding those of control being 100.

*The volume of CO_2 liberation was calculated at standard temperature (0°C) and pressure (1 atm).

**Final concentration in the assay mixture.

brought about by direct binding of the saponin to specific site or sites of specific enzyme but rather by such common nature as surface activity of the saponin. Studies of the saponin effect on the reactions catalyzed by regulatory enzymes such as glutamate dehydrogenase (Joo *et al.*, 1976), isocitrate dehydrogenase (Joo and Han, 1976) and succinate dehydrogenase (Joo and Han, 1976) showed that the saponin was unable to recover the inhibitory action of the inhibitors on the above enzymes. This suggested that the saponin would not compete with the inhibitors.

The Effect of the Ginseng Saponin on Pyruvate Oxidation

The effect of ginseng saponin on pyruvate oxidation by rat hepatic mitochondrial preparation was observed *in vitro* by measuring oxygen uptake using Warburg manometric analysis. As shown in Table 13, the rate of pyruvate oxidation increased gradually as the concentration of sapo-

Table 13. The effect of ginseng saponin on pyruvate oxidation by rat's hepatic mitochondrial preparation

Incubation time (min.)	Concentration of saponin in assay mixture (%)			
	0	10^{-7}	10^{-6}	10^{-5}
20	31.78	33.21	40.75	47.30
30	45.40	44.91	45.95	54.98

Incubation time (min.)	Concentration of saponin in assay mixture (%)			
	10^{-4}	10^{-3}	10^{-2}	10^{-1}
20	65.44	27.57	21.05	3.98
30	68.10	41.12	27.78	3.11

The test volume was 3.0 ml. It contained (final concentrations): phosphate buffer (pH 7.4) 4×10^{-2} M, fumarate 3.3×10^{-3} M, $MgSO_4$ 1.3×10^{-3} M, ATP 6.7×10^{-4} M, NAD^+ 2×10^{-3} M, nicotinamide 1.3×10^{-2} M, sucrose 1.3×10^{-1} M, cytochrome c 6.7×10^{-5} M, enzyme preparation 0.5 ml and various concentrations of ginseng saponin. Oxygen uptake was measured at the intervals of 20 and 30 minutes incubation at 37°C. Values are the mean value for three determinations in μl.

Table 14. Radioactivity of CO_2 absorbed in KOH of the center well of Warburg manometric vessel during 20 min. and 30 minutes incubation at 37°C

Incubation time (min.)		Total** radioactivity of CO_2 absorbed in KOH (DPM)	Percent*** isotope recovered (%)	Relative**** radioactivity
20	Control	52.150	9.66	100
	Test	117.196	21.73	225
30	Control	97.489	18.08	100
	Test	141.128	26.7	145

The reaction mixture contained 0.3 M phsophate buffer (pH7.4) 0.4 ml, 0.1 M fumarate 0.1 ml, 0.1 M pyruvate 0.1 ml, Na-pyruvate-3-^{14}C 0.25 μCi, 0.02 M $MgSO_4$ 0.2 ml, 0.01 M ATP 0.2ml, 0.03 M NAD^+ in 0.2 M nicotinamide 0.2 ml, 0.5 M sucrose 0.8 ml, 0.001 M cytochrome c 0.2 ml, mitochondrial preparation* 0.5 ml and 10^{-3}% saponin 0.3 ml (final concentration: 1×10^{-4} %).

*See the text (experimental part).

**The radioactivities were counted using liquid scintillation spectrometer (ALOKA LSC-60). The counting efficiency was 40 %.

***Initially added Na-pyruvate-3-^{14}C was 539328 DPM.

****Relative activities were shown assuming those of the corresponding control being 100.

nin in the reaction mixture increased and the maximum oxidation occured at the concentration of saponin of 1×10^{-4} %. When the saponin concentration increased further, however, it was found that the oxidation was rather inhibited. The stimulating effect of the saponin on pyruvate oxidation might be considered as the sum of the saponin stimulation of individual enzymes participating TCA cycles.

In experiment using radioactive Na-pyruvate-3-^{14}C as substrate, the CO_2 production was indirectly measured by measuring the radioactivity of CO_2 absorbed in KOH of the center well of the manometric vessel at 20 min and 30 min incubation at 37°C. As shown in Table 14, percent isotope recovered in CO_2 of the test (20 minutes incubation) was found as much as 2.25 times that of the control supporting again that the pyruvate oxidation was greatly stimulated by the moderate amounts of saponin.

The stimulation effect of saponin on pyruvate oxidation was further confirmed by the experiment of measuring the radioactivity decrement of pyruvate-3-^{14}C in the reaction mixture. As shown in

Table 15. The effect of ginseng saponin on pyruvate oxidation by rat hepatic mitochondrial preparation

	Radioacitivy (DPM)**		Relative***
	Control	Test	radioactivity
Ether-soluble fraction	863,609(55.0)	712,141(45.3)	82.5
Pyruvate****	800,495(51.0)	678,483(43.2)	84.8
α-ketoglutarate****	52,382(3.3)	25,937 (1.7)	49.5
Remained aq. fraction	670,810(42.7)	850,370(54.4)	126.8
Pyruvate-3-^{14}C initially added	1,570,584(100)		

The test volume was 3.0ml. It contained (final concentrations): phosphate buffer (pH 7.4) 9×10^{-3} M, fumarate 3×10^{-3} M, Na-pyruvate 3×10^{-3} M, Na-pyruvate-3-^{14}C (0.70μCi), $MgSO_4$ 2.4×10^{-2} M, ATP 9×10^{-1} M, sucrose 0.15M, saponin 1.5×10^{-5} %, and the mitochondrial preparation* 0.5ml. At the termination of 15 minutes' incubation, 1ml of 0.66N H_2SO_4, 1ml of 10% sodium tungstate and 4.7ml of H_2O were added and centrifuged. The supernatant was then mixed with 2ml of 0.05% dinitrophenylhydrazine in 1 N HCl and left to stand for 30 min at room temperature. The mixture was then extracted with ethyl ether and the extract was chromatographed and the radioactivities of individual fractions were measured using Tri-Carb Liquid Scintillation spectrometer. Figures in brackets show the percent isotope recovered.

*See the text (Experimental part).

**Counting efficiency of the counter was 32.7%.

***Relative activities were shown assuming the activity of corresponding fractions of control was 100.

****These were separated as 2,4-dinitrophenylhydrazones.

Table 15, radioactivity of unreacted pyruvate fraction of the reaction mixture containing saponin (Test) was found only 85 % of that of control signifying that the pyruvate oxidation was stimulated by the saponin. Much lower radioactivity of α-ketoglutarate of the test (49.5 % that of control) again supported that the saponin accelerated the TCA cycle enzymes resulting in the stimulation of pyruvate oxidation. Furthermore, a higher radioactivity remained in water phase of the test solution following ethyl ether extraction suggested that more water-soluble substances such as amino acids might be formed from pyruvate in the presence of saponin compared with control.

The Effect of Ginseng Saponin on Lipid, Protein and Nucleic Acid Biosynthesis

Oura, Hiai and their coworkers found that *in vitro* synthesis of protein was increased by microsome and polysome fractions obtained from ginseng treated rat liver and that *in vitro* synthesis of serum protein was also increased by the ginseng extract. They also found that ginseng could stimulate carbohydrate metabolism in the liver and could increase the lipid content of adipose tissue and their further studies suggested that ginseng saponin might be an active principle. They have considered that the action of ginseng has some special feature in its mode of action and suggested the ginseng saponin being a kind of metabolic regulator or hormone-like substance.

It has been found in this laboratory that the ginseng saponins stimulate significantly both fatty acid oxidation by rat hepatic mitochondrial preparation and synthesis by the cytosol fraction *in vitro* (Joo *et al.*, 1977).

Distribution of radioactivity of hepatic lipids of albino rats on time course of acetate-1,2-^{14}C administration has also been investigated and found that acetate-1,2-^{14}C was incorporated quickly into lipids at a very early phase, but an analysis of these fractions showed the relatively low specific radioactivities of cholesterol and fatty acids suggested that its high radioactivities might be due to fat-soluble small molecules synthesized from acetate-1,2-^{14}C As shown in Table 16, the highest

Table 16. Distribution of radioactivities of lipid fractions of liver, brain, adipose tissue and blood serum of rats being administered with ginseng saponin prior to acetate-1, 2-^{14}C injection

	No. of sample	Time course of acetate 1,2-^{14}C administration (min.)	Liver		Brain		Adipose tissue		Blood serum
			DPM in total lipid	DPM in lipid per g. wet tissue	DPM in total lipid	DPM in lipid per g. wet tissue	DPM in total lipid	DPM in lipid per g. wet tissue	DPM in lipid per ml
Control	1	6	18,824	3,552	1,340	838	28,505	19,003	90
	2	18	27,544	5,197	265	165	28,415	9,798	662
	3	35	2,649	481	290	193	1,165	1,059	175
	4	58	4,480	747	435	272	4,025	3,096	180
	5	71	4,371	911	645	403	5,515	3,579	350
	6	94	2,420	457	515	286	2,130	2,130	208
	7	124	23,664	4,640	700	438	33,615	16,808	413
	8	156	13,254	2,173	365	215	11,915	5,416	115
	9	216	2,409	482	820	456	3,350	1,457	1,051
	10	240	1,951	331	230	192	670	419	743
Test	11	9	7,935	1,259	8,800	8,800	22,250	11,125	153
	12	15	22,443	4,156	455	569	19,305	11,356	60
	13	30	12,840	2,176	18,570	18,570	1,425	838	260
	14	61	33,845	6,636	280	187	12,515	9,627	520
	15	73	4,829	966	460	511	3,200	1,610	911
	16	79	6,300	1,105	330	254	1,965	1,228	1,858
	17	115	4,709	798	1,135	1,032	2,385	1,403	141
	18	180	16,644	2,685	200	143	10,495	4,998	—*
	19	220	3,739	656	235	138	1,425	570	150
	20	239	4,306	673	175	117	12,265	6,133	231

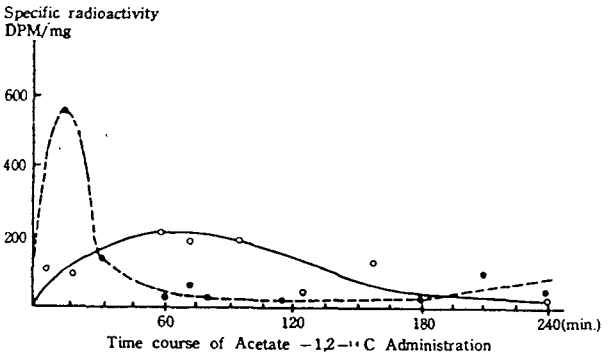

Fig. 8. Specific radioactivity-time course of acetate-1,2-^{14}C administration curve of rat hepatic cholesterol. ○—○ was control group and ●—● was test group.

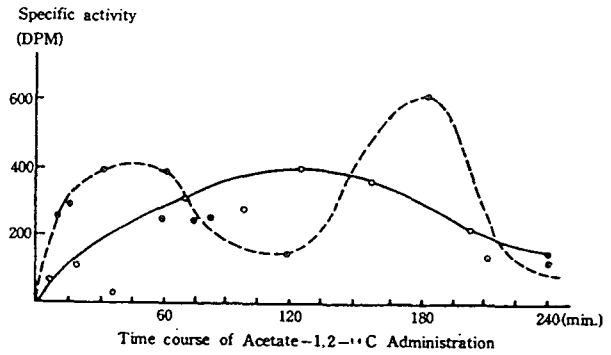

Fig. 9. Specific ratioactivity-time course of acetate-1,2-^{14}C administration curve of rat hepatic fatty acid. ○—○ was control group and ●—● was test group.

incorporation of the isotope (13.44%) into hepatic lipids of test animal was observed at 61min. after acetate-1,2-^{14}C injection while that of control animal was 9.4% at 124min. after the injection.

Specific radioactivity-time course of acetate-1, 2-^{14}C administration curves of cholesterol and fatty acid as shown in Figures 8 and 9, showed that turnover rates of the above lipids of saponin ad-

Table 17. Growth of *E. coli* (ADO1) in BHI broth medium. The total volume of the culture was 10.0 ml. It contained 8.8 ml of BHI broth medium, 0.2 ml of *E. coli* suspension and 1.0 ml of saponin solution of various concentration. The mixture was incubated at 37°C and the absorbance at 570 nm on time course was monitored.

Incubation time	Saponin concentration in the mixture(%)					
	0	10^{-2}	10^{-3}	10^{-4}	10^{-6}	10^{-8}
30 min	0.050	0.060	0.051	0.051	0.047	0.046
1:00	0.080	0.085	0.080	0.085	0.076	0.080
1:30	0.110	0.118	0.110	0.115	0.108	0.108
2:00	0.186	0.194	0.187	0.186	0.184	0.186
2:30	0.270	0.278	0.269	0.271	0.267	0.272
3:00	0.355	0.360	0.360	0.358	0.352	0.352
3:30	0.437	0.440	0.439	0.439	0.437	0.438
4:00	0.520	0.530	0.525	0.523	0.520	0.525
4:30	0.585	0.590	0.586	0.590	0.581	0.590
5:00	0.610	0.610	0.611	0.615	0.608	0.610
5:30	0.615	0.618	0.618	0.618	0.614	0.615

Table 18. Growth of *E. coli* (ADO1) in basic medium. The total volume of the culture was 10.0 ml. It contained 8.8 ml of basic medium, 0.2 ml of *E. coli* suspension and 1.0 ml of saponin solution of various concentration. The mixture was incubated at 37°C and the absorbance at 660 nm on time course was monitored.

Incubation time	Saponin concentration in the mixture(%)					
	Control	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-6}
2:00	0.027	0.025	0.030	0.030	0.028	0.026
2:30	0.040	0.035	0.045	0.050	0.040	0.039
3:00	0.065	0.058	0.080	0.082	0.062	0.062
3:30	0.094	0.088	0.104	0.108	0.092	0.090
4:00	0.128	0.124	0.133	0.136	0.129	0.125
4:30	0.170	0.165	0.179	0.180	0.172	0.170
5:00	0.257	0.248	0.262	0.261	0.250	0.249
6:00	0.342	0.340	0.344	0.344	0.340	0.339
6:30	0.345	0.344	0.346	0.346	0.340	0.342
7:00	0.345	0.344	0.347	0.347	0.342	0.343

ministered rat liver were all found much faster than those of control animals.

It was found in this laboratory that when *E. coli*(ADO1) cells were grown in a nutrient-rich medium such as BHI broth, no effects of ginseng saponin were observed (Table 17); however, when *E. coli* cells were grown in basic medium, the ginseng saponin (10^{-2}%–10^{-3}%) stimulated the growth of *E. coli* as shown in Table 18 suggesting that ginseng saponin stimulated the metabolism particularly under unfavourable conditions.

Table 19. The effect of ginseng saponin on lipids, proteins, nucleic acids (DNA + RNA) and some enzyme(GOT) synthesis of *E. coli* (ADO1) grown in basic medium at 37°C for 4.5 hours. The figures are relative values assuming those of the corresponding control being 100.

concentration of saponin in culture midium components	0 (control)	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-6}
Total lipids	100	104.5	114.9	113.1	101.4	102.7
Proteins	100	95.4	107.6	110.0	101.5	91.0
DNA	100	95.4	110.4	111.1	98.8	96.9
RNA	100	92.8	111.7	118.1	95.2	93.9
GOT	100	82.1	146.4	157.2	125.0	121.4

Table 20. The effect of ginseng saponin on glucose oxidation by *E. coli* (w 3110). The test volume was 3.0 ml. It contained (final concentration): phosphate buffer (pH 7.4) 4×10^{-2}M, glucose 3.3×10^{-2}M, furmarate 3.3×10^{-3}M, Mg SO_4 1.3×10^{-3}M, ATP 6.7×10^{-4}M, NAD^+ 2×10^{-3}M, nicotinamide 1.5×10^{-2}M, sucrose 1.3×10^{-1}M, cytochrome C 6.7×10^{-5}M, *E. coli* preparation 0.5 ml and various concentration of ginseng saponin.

Oxygen uptake was measured manometrically at the intervals of 30 and 60 minutes' incubation at 37°C. Values are the mean value for three determination in μl.

	Oxygen uptake (μl)			
Conc. of saponin (%) / Incubation time(min)	0	10^{-1}	10^{-3}	10^{-5}
30	23.10	6.72	33.32	27.70
60	27.72	8.35	45.08	38.78

Analysis of *E. coli* cells grown in basic medium in the presence of ginseng saponin showed that when the concentration of ginseng saponin was 10^{-3}–10^{-2}%, the synthesis of lipid, protein, nucleic acid and some enzymes (GOT) of the cell was stimulated but at more or less than the above concentration of saponin, no significant effect can be observed (Table 19).

Glucose oxidation by *E. coli* (w 3110) was also examined manometrically and found that when the concentration of the saponin was 10^{-3}%, oxygen uptake was as many as 1.4–1.6 times that of control but when the saponin concentration was 10^{-1} %, oxygen uptake was greatly lowered as shown in Table 20. This suggested again that

Table 21. Distribution of radioactivities of methanol-water phase, chloroform phase and insoluble fraction. *E. coli* (ADO1) suspension (0.45 ml) kept at 4°C was allowed to stand at room temperature for 20 minutes and incubated in the mixture of basic medium (3 ml), 0.45 ml of saponin solution of various concentrations (0–1%), and 0.6 ml (7920 DPM) of glucose-1-^{14}C solution (0.006 μCi/ml) at 37°C for 3.5 hours. The radioactivities were expressed in DPM.

Fractions / Conc. of saponin (%)	MeOH-H_2O phase	% recovered	Chloroform phase	% recovered	Insoluble fraction	% recovered
0	6,158	77.8	451	5.7	619	7.8
10^{-1}	6,438	81.3	268	3.9	1,217	15.4
10^{-2}	6,195	78.2	389	4.8	1,251	15.8
10^{-3}	5,495	69.4	522	6.6	1,427	18.0
10^{-4}	6,067	76.5	463	5.9	982	12.4

moderate amounts of ginseng saponin stimulated glucose oxidation.

Distribution of radioactivities of methanol-water phase, chloroform phase separated by Blye-Dyer technique and insoluble fraction of the extract of *E. coli* (ADO1) grown in basic medium containing glucose-1-^{14}C in the presence of saponin of various concentration at 37°C for 3.5 hours showed that the highest degree of incorporation into lipids, proteins and nucleic acids occured when the concentration of saponin was 10^{-3}% as shown in Table 21. Radioactivity of insoluble fraction (protein and nucleic acid) increased as the concentration of ginseng saponin increased in the culture medium and the activity reached its highest when the saponin concentration was 10^{-3}% and the activity lowered when the concentration of saponin became larger. The radioactivity of chloroform phase (lipid fraction) also reached the highest activity when the saponin concentration was 10^{-3}% in the medium and its radioactivity was even lower than control when the concentration became over 10^{-2}%. The radioactivity of methanol-water phase (unreacted glucose and other water soluble intermediates) of *E. coli* extract, grown in the presence of 10^{-3}% of saponin was lowest suggesting that moderate mounts of saponin(10^{-3}%) stimulated both lipid and protein synthesis of this organism. This result using glucose-1-^{14}C was in good agreement with the above experiment observing the effect of ginseng saponin on growth, lipid and protein synthesis of *E. coli* (ADO1) and glucose oxidation of *E. coli* (w 3110).

Distribution of radioactivities of methanol-water phase, chloroform phase and insoluble fraction of the extract of disrupted *E. coli* (ADO1) cells, which were incubated in the mixture or phosphate buffer (pH 7.4) 1.2×10^{-2}M, Na-acetate 5.6×10^{-4}M, ATP 1.1×10^{-4}M, $KHCO_3$ 1.1×10^{-4}M, CoASH 1.1×10^{-4}M, $MgSO_4$ 1.1×10^{-4}M, various concentration of saponin and acetate-1,2-^{14}C 0.0095 Ci (20900 DPM), showed that a very small fraction was incorporated into lipid fraction but quite high degree of incorporation into insoluble fraction (protein and nucleic acid) were observed (Table 22). Incorporation of (methyl-^{3}H)-thymidine to insoluble action (DNA) of *E. coli* cells incubated in basic medium in the presence of saponin was also found highest when the concentration in saponin was 10^{-3} — 10^{-2}% (Table 23).

Table 22. Distribution of radioactivies of methanol-water phase, chloroform phase and insoluble fraction. Disrupted *E. coli* (ADO1) cell preparation (0.5 ml) was incubated in the mixture of 0.55 ml of 0.1M phosphate buffer (pH 7.4), 0.5 ml of 5mM Na-acetate, 0.5 ml of 1mM ATP, 0.5 ml of 1mM $KHCO_3$, 0.5 ml of 0.1 mM CoASH, 0.5 ml of 1mM $MgSO_4$, 0.45 ml of saponin solution, 0.5 ml of Na-acetate-1,2-^{14}C solution (0.019 Ci) at 37°C for 30 min. The radioactivities were expressed in DPM.

Fraction / Conc. of saponin (%)	MeOH-H_2O phase	% recovered	Chloroform phase	% recovered	Insoluble fraction	% recovered
0	16,889	80.8	58	0.28	3,730	17.9
10^{-1}	15,509	74.2	140	0.67	4,471	21.4
10^{-2}	14,729	70.5	133	0.64	5,458	26.1
10^{-3}	14,098	67.5	151	0.73	6,111	29.2
10^{-4}	16,111	77.1	118	0.56	3,833	18.3

These experimental results suggested again that the moderate counts of saponin (10^{-3}%) stimulated the *in vitro* synthesis of lipid protein and nucleic acid of this organism.

Table 23. The effect of ginseng saponin on the incorporation of [methyl-^{3}H]-thymidine to insoluble fraction (DNA) of *E. coli* cell. *E. coli* (ADOl) suspension (0.45 ml) kept at 4° C was allowed to stand at room temperature for 20 minutes and incubated in the mixture of basic medium (3 ml), 0.45 ml of saponin solution of various concentrations (0–1%), and 0.6 ml of [methyl-^{3}H]-thymidine-solution (0.01 μCi/ml) at 37°C for 30 minutes. The reaction was terminated by adding 0.5 ml of 30% TCA and the radioactivity of the precipitated were counted using ALoKa Liquid Scintillation counter (Model 601). The radioactivities were expressed in DPM.

Conc. of saponin (%)	Radioactivity (DPM)	% incorporated
0	669	5.07
10^{-1}	710	5.38
10^{-2}	809	6.13
10^{-3}	791	5.99
10^{-4}	681	5.15

The Effect of Ginseng Saponin on Alcohol Metabolism

Present knowledge on the metabolism of alcohol shows that over consumption of alcohol can cause cirrhosis and death not only because alcoholism promotes malnutrition but also because alcohol and its products disturb liver metabolism and damage the liver cells. And a number of the metabolic effects of alcohol are known to be directly linked to the two first products of its oxidation, hydrogen and acetaldehyde.

It appears that a central role in the toxicity of alcohol may be played by acetaldehyde, which is extremely reactive and affects most tissues in the body (Lieber, 1976).

It has been proposed that ALDH activity might be the main regulator for acetaldehyde oxidation during ethanol metabolism in rat liver (Eriksson, 1973). And acetaldehyde oxidation has little effect on the cytosolic redox state (Lindros *et al.*, 1972) but a relatively large effect on the mitochondrial redox state (Eriksson, 1973; Parilla *et al.*, 1974) suggesting that the oxidation of acetaldehyde during alcohol metabolism mainly occurs in mitochondria. Furthermore, a number of investigators have shown that the existence of a low Km (10mM for acetaldehyde) ALDH in mitochondria and high Km (in the molar range) enzymes in the cytosol, microsomal fraction and mitochondria (Marjanen, 1972; Tottmer *et al.*, 1973; Koivula and Koivulalo, 1975) support the importance of the mitochondrial oxidation of ethanol derived from acetaldehyde.

It was also found in this laboratory that most

Table 24. The effect of ginseng saponin on the oxidation of ethanol by rat hepatic cytosolic preparation *in vitro*

Condition / Fraction	Acetaldehyde fraction			Acetate fraction		
Conc. of saponin in assay mixture (%)	Radioactivity (DPM)	%* of administered isotope incorporation	μ atoms** of ^{14}C-incorporated	Radioactivity (DPM)	%* of administered isotope incorporation	μ atoms** of ^{14}C-incorporated
0	39,855	1.07	0.160	42,150	1.13	0.169
1.4×10^{-4}	31,980	0.87	1.128	60,315	1.62	0.242

The assay volume was 5ml. It contained (final concentrations): glycine buffer (pH 9.0) 0.044M, NAD^+ 4.2×10^{-4}M, nicotinamide 0.028M, ethanol 3×10^{-3}M containing ethanol-1-^{14}C 1.08μCi (3.37×10^6DPM), ginseng saponin 1.4×10^{-4}% and 1ml of rat hepatic cytoplasmic preparation. After 5min. incubation at 25°C, the reaction was terminated by the addition of 1ml of 20% TCA and radioactivity of acetaldehyde and acetate formed were assayed using Tricarb Scintillation spectrometer.

* % of administered isotope incorporation was calculated according to the equation of

$$\frac{\text{(Total DPM per fraction)}}{3.73\times10^6\times100}\times100$$

** No. of microatoms of ^{14}C-incorporation into the corresponding fraction was calculated according to the equation of

$$\text{(Total DPM per fraction)} \div \frac{3.73\times10^6}{3\times5}$$

Table 25. The distribution of radioactivity of hepatic lipids of saponin (1.8mg/mouse) administered mice (C-54, weighed 32g to 34g) intraperitoneally 2 hours prior to the intraperitoneal injection of 33% ethanol containing 0.26μCi of 1-^{14}C-ethanol

	Time of continuous administration of ethanol-1-^{14}C (min)	Radioactivity of total lipid in liver (DPM)	%* of isotope incorporation into hepatic lipid fraction	Radioactivity (DPM) in		
				Triglyceride fraction	Cholesterol fraction	Other lipid fraction
Control	50	8,650	1.51	3,058	380** (461)	4,838
Test	50	11,298	1.98	5,304	478** (525)	5,392
Test	60	14,203	2.48	7,196	720** (554)	6,037

* % of isotope incorporation into hepatic lipid fraction was calculated according to (DPM in total lipid/5.72 $\times$ 10^5) $\times$ 100.

** Figures in bracket showed specific radioactivity.

of the ALDH activities were present in mitochondria. The Km for acetaldehyde of mitochondrial ALDH was 2.74mM while that of cytosolic ALDH was 66mM, supporting again the significance of mitochondrial acetaldehyde oxidation (Joo *et al.*, 1977).

As shown in Table 24, *in vitro* experiment using rat hepatic cytosolic fraction and radioactive ethanol-1-^{14}C showed that isotope incorporation into acetate in test solution was found 0.242 microatoms while that of control reaction was only 0.169 microatoms. And the percent of ^{14}C recovered in acetaldehyde fraction was higher in the control (1.97%) than in the test (9.87%) suggesting that the saponin stimulated ADH activity as well as the removal of acetaldehyde by activating ALDH.

The test mice were administered ginseng saponin (1.8mg in 0.3ml of saline/mouse) intraperitoneally and control mice were injected only 0.3ml of saline instead. Two hours later, 0.3ml of 33% ethanol containing ethanol-1-^{14}C was injected intraperitoneally and the radioactivities of total lipids, triglyceride and cholesterol of the liver were investigated 50 min and 60 min after the ethanol administration. As shown in Table 25, the isotope incorporations into the hepatic lipids of the test animals were 1.98% (50min. pulse) and 2.48% (60min. pulse) while that of control animals was 1.51%. Radioactivities of the individual lipid fractions were also found higher in test group. Specific radioactivities of hepatic cholesterol and fatty acid of mice killed 30min. after the ethanol-1-^{14}C injection showed again that higher activities were found in test group, particularly in fatty acid fraction as shown in Table 26.

It was realized that moderate amounts of

Table 26. Specific radioactivity of hepatic cholesterol and fatty acids of mice(C-54 weighed 31g to 33g) administered ginseng saponin (1.8 mg/mouse) intraperitoneally 2 hours prior to the intraperitoneal injection of 33% ethanol containing 0.2μCi of 1-^{14}C-ethanol (pulse period was 30min.)

	Specific radioactivity *CPM/mg	
	Cholesterol	Fatty acids**
Control	3.5 $\pm$ 0.8	0.8 $\pm$ 0.5
Test	5.0 $\pm$ 1.2	10.3 $\pm$ 2.0

* Mean values of three mice.

** The amounts of fatty acids liberated following saponification were calculated assuming all fatty acids being stearic acid.

ginseng saponin might play a significant role in ethanol oxidation in the body by stimulating ALDH as well as ADH. It is particularly noticeable that the saponin stimulated ALDH, an important enzyme to remove the most harmful acetaldehyde from the tissue. Furthermore *in vivo* experiment using ethanol-1-^{14}C showed that the saponin stimulated not only ethanol oxidation to acetate, but also the biosynthesis of fatty acid and cholesterol in the body. There are many ways in which the liver cell can rid itself of alcohol's excess hydrogen. Several of them involve the formation of lipids. The hydrogen can be shunted directly into the synthesis of fatty acid and cholesterol. Moderate amounts of ginseng saponin may stimulate both ADH and ALDH resulting in accelerating ethanol oxidation and acetaldehyde removal from the tissue. Furthermore the excess hydrogen can be shunted more quickly into lipid biosynthesis.

When young albino rats (about 150g) were given free access to 12% ethanol instead of water and fed with normal diet, their growth rate was lowered but fed together with 2% ginseng extract (which contained about 7% of saponins) the test animals kept their ordinary growth rate. Photomicroscopic study of the liver sections of alcohol fed rats with normal diet showed severe hepatic injuries. However, the remakable protective effect of ginseng extract keeping the liver safe from alcohol intoxication was observed as shown in Figure 10 and Table 27.

Analysis of hepatic protein, sugar and lipid of the above animals under different feeding conditions showed that alcohol feeding lowered the protein composition and caused sugar and lipid deposition considerably but ginseng extract seemed to maintain the normal levels of the above compononts (Table 28).

Similar results were obtained when the rats had free access to 12% alcohol and were fed normal diet plus 0.15% ginseng saponins.

Protective effect of ginseng saponin on the liver function was reported by Han (1977) who demonstrated the decrease of GPT and GOT activities of rabbits which were administered 95% carbon tetrachloride (0.1ml/kg) intraperitoneally

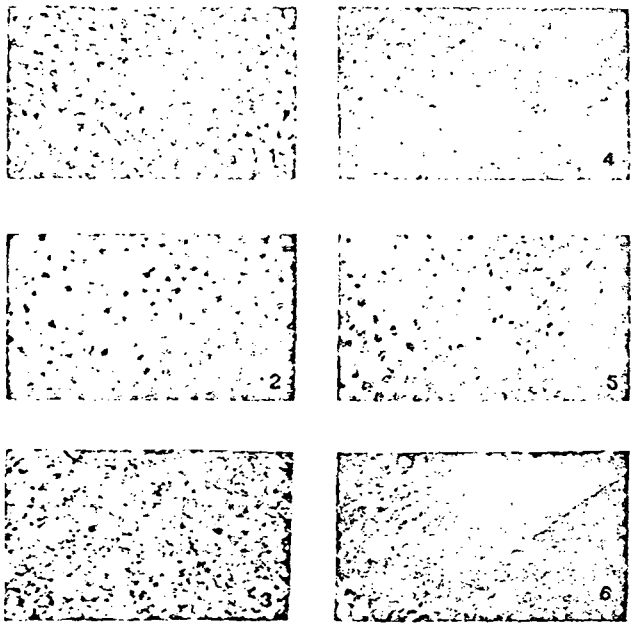

Fig. 10. Photomicrographs of the liver sections of male albino rats which were given free access to 12% ethanol instead of water and fed with normal diet or fed with 2% ginseng extract containing diet for 14 days.
1,4: Hematoxylin eosin stain, X 600.
2,5: PAS hematoxylin stain, X 600.
3,6: Sudan black B stain, X 600.
1,2,3: The liver sections of ethanol fed rats. Severe injuries of the liver was observed (vacuolic degeneration in the hepatocytes, disappearance of nuclei (1), glycogen deposition (2) and fatty degeneration (3)).
4,5,6: The liver sections of 2% ginseng extract plus 12% ethanol fed rats (14 days). Remarkable protective effect of ginseng extract keeping the liver safe from alcohol intoxication was observed.

prior to oral administration of ginseng. He also demonstrated that the saponin accelerated the metabolic process of phenacetin or acetaminophemin within the test animals, suggesting that the saponins prevent unexpected bad influence and untoward effects that could be caused by the accumulation of synthetic drugs within the animal.

From the above considerations, it seems likely that the effect of ginseng saponins might be brought about, at least, partly by their surface activity and physiologically non-toxic and ideal amounts of the saponin might play a significant role in the metabolism of the body.

We know little about how these mechanism work, however, it is expected from the above results that moderate amounts of the saponins may

Table 27. Histochemical observation of the livers of rats-fed with normal diet plus free access to 12% ethanol instead of water, and 12%-ethanol plus normal diet containing ginseng extract for 14 days. The degrees of symptoms were approximated by following signs: — (negative), + (very weak), ++ (weak) and +++ (strong).

Group		Necrosis	Disappearance of nuclei	Appearance of large and giant cells	Irregularity in the nuclear sizes	Occurrence of binuclear cells	Pyknosis	Vacuolic degeneration in the hepatocytes	Glycogen deposition in the hepatocytes	Fatty degeneration	Dilatation of the portal veins	Dilatation of the sinunoids	Congestive hyperemia of the livers
Control	1	+	++	+	+	+	+	++	+	+	−	++	+
	2	−	++	+	+	+	+	+++	+	++	−	+	+
	3	−	++	+	+	−	+	+++	++	+	−	+	−
	4	−	+	+	+	+	+	+	+	+	−	+	+
	5	−	+	+	−	+	−	+	+	−	−	+	−
Test	1	−	−	+	+	−	−	−	−	−	−	+	−
	2	−	−	+	+	−	−	−	+	−	−	+	−
	3	−	+	−	+	+	−	−	−	−	−	+	−
	4	−	+	+	+	+	+	+	+	+	−	+	+
	5	−	+	+	+	+	−	−	+	−	−	+	−

Table 28. The composition of protein, carbohydrate and lipid of alcohol fed male albino rats under various feeding conditions

Feeding conditions	Protein (%)	Carbohydrate (%)	Lipid (%)	Carbohydrate / protein	Lipid / Protein
Normal diet and water only (control)	18.98	2.97	3.47	0.16	0.18
10 days alcohol instead of water plus normal diet	11.62	3.88	5.41	0.33	0.47
20 days' //	8.94	3.64	5.86	0.41	0.66
30 days' //	8.96	4.33	5.62	0.49	0.63
10 days' alcohol instead of water plus 2% ginseng extract containing diet	14.72	3.18	4.96	0.22	0.34
20 days' alcohol instead of water plus normal diet followed by normal diet and water	11.94	3.57	3.83	0.30	0.32
20 days' alcohol instead of water plus normal diet followed by 2% ginseng extract containing diet and water	16.19	2.79	3.06	0.18	0.19

bring about a slight change of an aqueous environment of the enzymes, resulting in a change of enzyme conformation, which would be in favor of the reactions proceeding.

We know that Km values of enzymes tested so far in this laboratory lowered unexceptionally in the presence of moderate amounts of saponins, suggesting again that the saponin acts nonspecifically on various enzyme-catalyzed reactions. We do not know how much the individual glycosides

(diol- and triol-glycosides) behave differently physiologically. Although there are controversial opinions about the actions of individual glycosides, both diol- and triol-saponin have almost similar surface activities.

What mechanism could explain the above mentioned common effect for the enzyme-catalyzed reactions? One of the answers would be, I suggest, that some pharmacological effects of the saponin might be explained with the above-physical property rather than their chemical nature, for which much work has been done.

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