BIOCHEMICAL ACTION OF *PANAX GINSENG* PRINCIPLE

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Our topic is concerned mainly with the biochemical action of ginseng and its mechanism, and with determining which component of ginseng is biochemically active. In 1965, we started biochemical research in Japanese and Asian herbal medicines at our Reserach Institute for WAKAN-YAKU. Our desire was to give quantitative and scientific supports to the traditional remedies. Soon we encountered that mysterious panacea, that is, the root of *Panax ginseng* C. A. Meyer, after a preliminary screening of some tonic crude drugs, in which nuclear RNA synthesis of rat liver was measured as an index of biochemical activity. The term "tonic" is very vague. So we have given a tentative and simplified definition to it. That is, the tonic effect is a stimulatory effect on RNA and protein biosyntheis from the biochemical point of view.

Stimulation of RNA and Protein Biosynthesis

Aqueous extracts of ginseng and other crude drugs were injected intraperitoneally to rats, and radioactive orotic acid was pulsed for 20 min. Four hours after ginseng administration, the incorporation of labeled orotic acid i nto liver nuclear RNA increased about 50% to 60% compared with saline-treated controls (Table 1). After this we carried out experiments in dose-response and multiple-dose response and determination of the pool size of pyridine nucleotide. Thus we thought we found that *Panax ginseng* root can clearly stimulate the synthesis of rat liver nuclear RNA, and ginseng contained a certain active principle. Then the synthesized RNA was characterized as a mixture of messenger and ribosomal RNA by sedimentation profiles and base analysis. And then we found that *in vitro* synthesis of protein increased by microsomes and polysomes fractions from treated rat liver and that *in vivo* synthesis of serum protein also was increased by the ginseng treatment.

Figure 1. summaries our results on RNA and protein synthesis. First of all, after ginseng treatment, the activity of liver nuclear RNA polymerase did increase at one hour and the maximal increase occured at two hours, then gradually recovered to

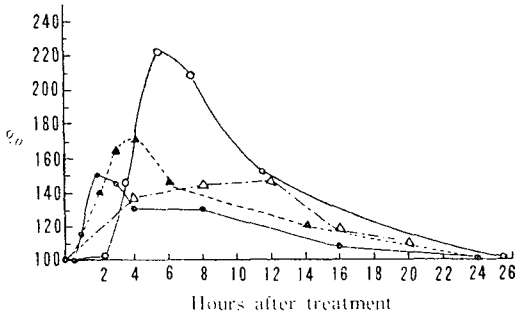


Fig. 1. Summary of Data on the Sequential Stimulations by *ginseng* Extract (Fraction 3 or 4)
●, liver nuclear RNA polymerase activity; ▲, nuclear RNA synthesis; ○, polysomal RNA synthesis; △, serum protein synthesis.

Table 1. Effects of Several Tonic Crude Drugs on the Incorporation of Labeled Orotic Acid into Rat Liver Nuclear RNA

Expt. No.	Material	No. of rats	Labeled orotic acid (cpm/mg RNA)	%
1	control (saline)	6	58250 ± 1150	100
	radix Rehmanniae (China)[a]	3	49200	85
	fractus Lycii (China)[b]	3	65000	111
	rhizoma Cnidii (Hokkaido, Japan)[c]	3	57600	99
	radix Ginseng (Kumsan, Korea)[d]	3	87900	151
2	control (saline)	6	22200 ± 400	100
	radix Ginseng (Nagano, Japan)[e]	3	35800	161
	rhizoma Panacis Japonici (Tohoku, Japan)[f]	3	25600	115
	radix Glycyrrhizae (Tohoku, Japan)[g]	3	22700	102
	semen Cuscutae (China)[h]	3	28300	128
	radix Bupleuri (China)[i]	3	27200	123
3	control (saline)	12	40800 ± 2600	100
	radix Scrophulariae (China)[j]	6	46500 ± 3800	114
	cortex Lycii Radicis (China)[k]	3	42600	105
	rhizoma Atractylodis (China)[l]	3	35000	86
	tuber Ophiopogonis (Osaka, Japan)[m]	3	46800	115
	radix Asparagi (China)[n]	3	38200	94
	herba Cistanches (China)[o]	3	35800	88
4	control (saline)	12	47750 ± 4500	100
	fructus Schizandrae (China)[p]	6	53100 ± 3400	111
	radix Dioscoreae (China)[q]	6	52600 ± 1700	110
	rhizoma Alimatis (China)[r]	3	50300	105

Each crude drug extract was injected 0.5 ml per rat intraperitoneally, corresponding to 0.15 g of dried crude drug. Expt. 1, ^{14}C-orotic acid 2.5 μCi; Expt. 2, ^{3}H-orotic acid 4.0 μCi; Expt. 3 and 4, ^{3}H-orotic and 7.0 μCi were injected per rat respectively. The data are expressed as percent of the values of untreated control rats which are taken as 100%. The data are presented as mean ± S.E.

Scientific and Japanese name of each crude drug: *a*) *Rehmannia glutinosa* (Gaertn) Libosch forma *hueichingensis* (Chao et Schih) Hsiao; Kaikei-jio. *b*) *Lycium chinense* Miller; Kukoshi. c) *Cnidium officinale* Makino; Senkyu. *d*) *Panax ginseng* C.A. Meyer; Ninjin. *e*) *Panax ginseng* C.A. Mayer; Ninjin. *f*) *Panacis japonici* C.A. Meyer; Chikusetsuninjin. *g*) *Glycyrrhiza uralensis* Fisch et D.C.; Tohokukanzo. *h*) *Cuscuta chinensis* Lamark; Toshishi. *i*) *Bupleurum falcatum* L.; Saiko. *j*) *Scrophularia ningpoensis* Hemsl; Genjin. *k*) *Lycium chinense* Miller; Jikoppi. *l*) *Atractylodis lancea* DC. var. *chinensis* Kitamura Sojyutsu. *m*) *Ophiopogon japonicus* Ker-Gawler; Bakumondo. *n*) *Asparagus cochinchinenensis* Merill. Tenmondo. o) *Cistanche salsa* Benth. et Hook fil; Nikujyuyo. *p*) *Schizgandra Chinensis* Billon. Gomishi; *q*) *Dioscorea batatas* Decaisne; Sanyaku. *r*) *Alisma plantago-aquatica* L. subsp. *orientale* Samuelsson; Takusha.

Table 2. Effect of Fraction 3 on Ribosomal RNA Contents in Rat Liver

		Expt. 1			Expt. 2		
		Total ribosome	Membrane bound ribosome	Free ribosome	Total ribosome	Membrane bound ribosome	Free ribosome
Cont.	mg/g liver	3.16	2.15	1.01	3.20	2.25	0.95
	ratio	100	68	32	100	70	30
F-3	mg/g liver	4.10	3.25	0.86	3.90	2.92	0.97
	ratio	100	78	21	100	75	25
Percent of cont		130	151	85	122	130	102

Fraction 3 (1.0g/kg body weight/day) was administered to rat orally for two weeks.

normal or control level. Second is the increase in the rate of nuclear RNA synthesis, its maximum occurred at 4 hours. The third was the rate of synthesis of cytoplasmic polysome RNA, and the peak was at 5.5 hours. Then the last one was the synthesis of serum protein, the maximum occurred around 8 hours after the administration of ginseng extracts.

We carried out long-term administration experiments. Rats were fed on a diet containing ginseng extracts for 4 weeks. Figure 2A is an electronmicroscopic picture of normal hepatocytes. Here we see hepatocytes from an experimental animal (Fig. 2B). From these pictures, we can see that the amount of rough endoplasmic reticulum increased, as did the rate of RNA synthesis in the isotopic experiments.

Further we confirmed the results by a colorimetric determination of the amount of total polysomes and of the membrane-bound ribosomes fractions separated from the treated rat liver. Table 2 is the result of two weeks of oral administration of ginseng extract. For convenience, we named the active principle in ginseng roots "prostisol", which implies the protein biosynthesis stimulating factor.

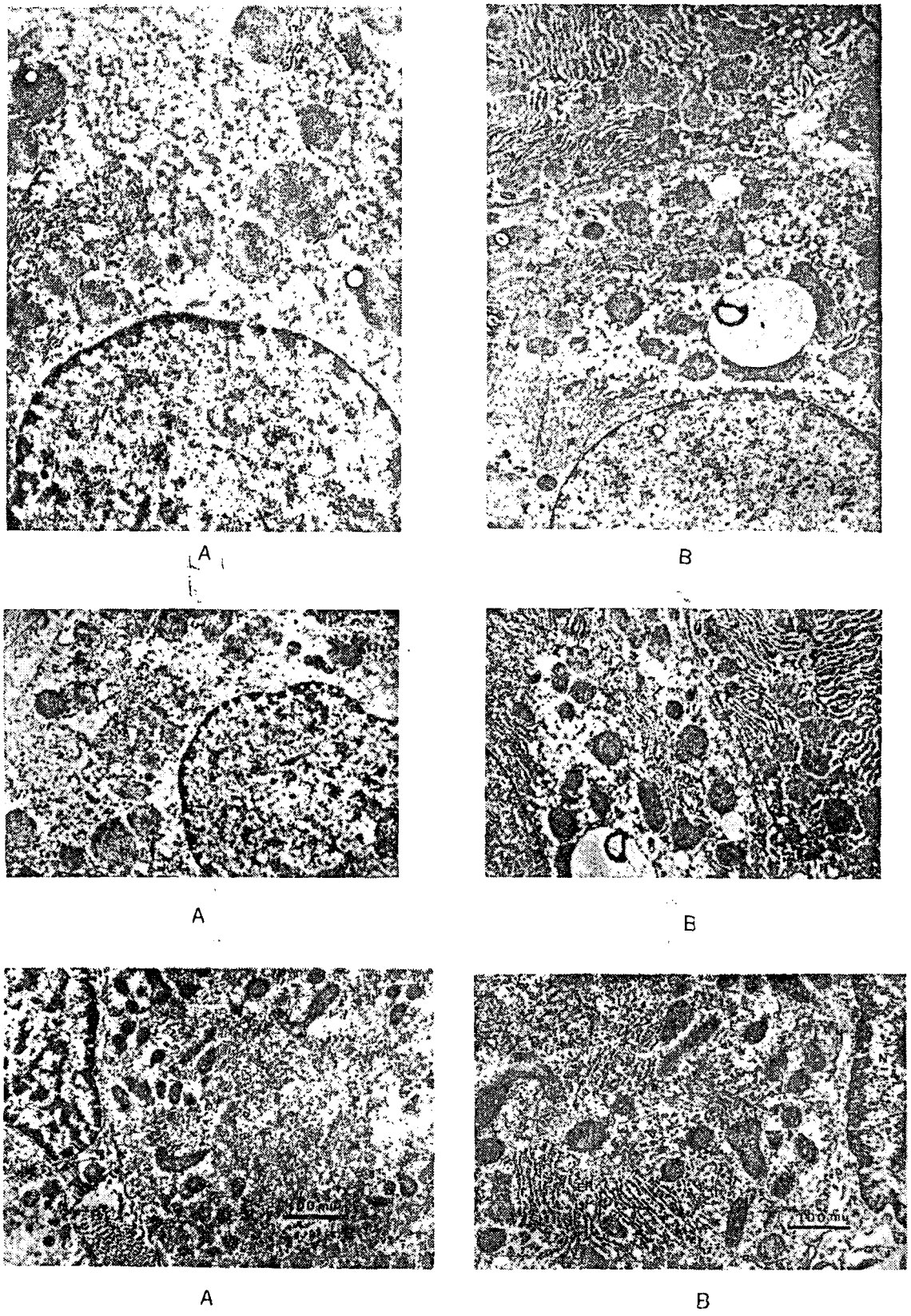
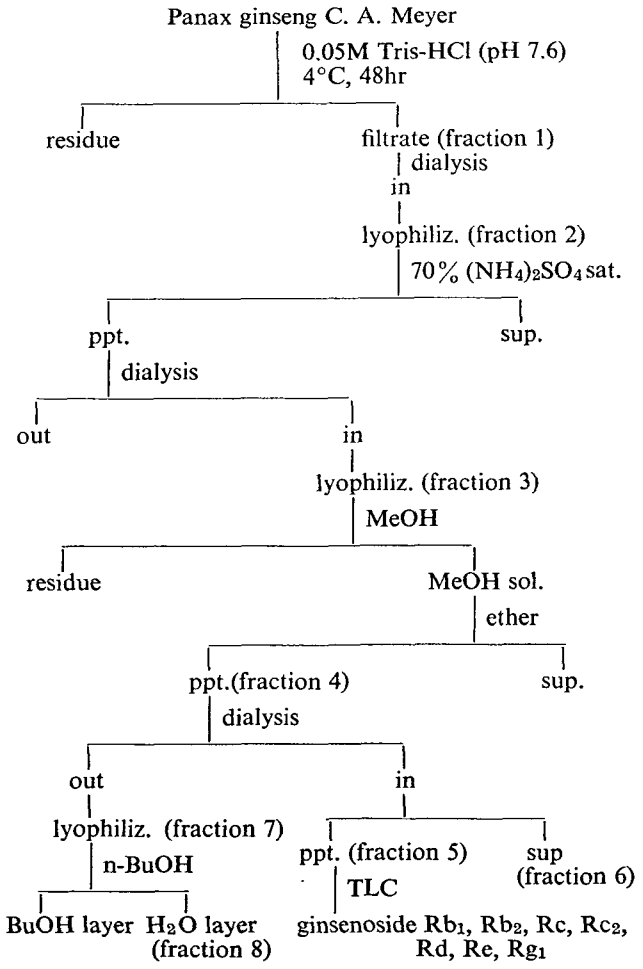


Fig. 2. Effect of fraction 3 on endoplasmic reticulum of rat hepatocyte. A: control, B: experimental.

Scheme 1. Purification and Isolation of Ginseng Saponin



Active Principle in Ginseng

Our second problem was to determine which component of ginseng is biochemically active. We prepared several fractions from ginseng roots as shown in Scheme 1. Fraction 1, an aqueous extract was obtained by stirring powdered ginseng for 48 hours with 0.05 M tris buffer solution, pH 7.6, at 4 . Fraction 2 was the inside solution of fraction 1 which was dialized against distilled water. Fraction 3 was precipitate from fraction 2 which was brought to 70% saturation with ammonium sulfate. Fraction 4 was obtained as a precipitate by the addition of ethylether to a methylalcohol extract of fraction 3. When fraction 4 was dialized, the white precipitate found in the dializing tube was fraction 5, its inside solution was fraction 6, and its outside solution was fraction 7. Fraction 8 or the non-saponin fraction was obtained from the water phase of butylalcohol extract of fraction 7.

Table 3. Effect of Ginseng Fractions on the Incorporation of ^{3}H-leucine into Mice Sera.

Fraction	No. of mice	^{3}H-Leucine (cpm/mg protein)	%	Yield (%)
Control (saline)	79	820 ± 13	100	—
Fraction 2	5	873 ± 17	106	20.0
Fraction 3	9	1371 ± 48	167	3.0
Fraction 4	9	1395 ± 73	170	1.2
Fraction 5	10	1607 ± 28	196	0.5
Fraction 6	9	1430 ± 70	174	0.5
Fraction 7	27	1293 ± 31	158	0.2
Fraction 8*	5	839 ± 108	102	0.06

At 5 hr after each sample (5 mg/mouse) administration, each mouse received intraperitoneally 5 μCi of ^{3}H-leucine (46 Ci/m*M*). One hr later, blood sample were taken by heart puncture. The data are expressed as per cent of the values of untreated control mice which are taken as 100%. The data are presented as mean ± S.E. Yield are expressed as per cent of the weight values obtained from ginseng powder. Control were assayed at 6 hr after saline-treatment.
*2 mg/mouse

Then we assayed these fractions by measuring the labeled leucine incorporation into the serum protein of a rather small animal, the ddYS strain of mouse. It was determined that "fraction 5" or the saponin fraction was the most active (Table 3). We also determined the amino acid pool size or free amino acid of the mouse livers. Both leucine and the total amino acid contents of the liver were increased rather than decreased by the ginseng treatment. Thus we were convinced that the increase in the rate of protein synthesis is not superficial.

Then we isolated seven saponins from fraction 5, that is, ginsenoside Rb_1,Rb_2,Rc,Rc_2,Rd,Re, and Rg_1, These were identified by Professor Shoji of Showa University, Japan. Then we determined their activities. The most active one was ginsenoside Rd(Table 4). Only ginsenoside Rb_1 seemed to be inactive. Thus we concluded finally that the active principle of ginseng is saponin when the activity was measured as protein biosynthesis.

We developed the colorimetric determination method to apply to ginseng saponin and its sapogenin in a crude extract of ginseng. Purified saponin and sapogenin gave a reddish purple color with vani-

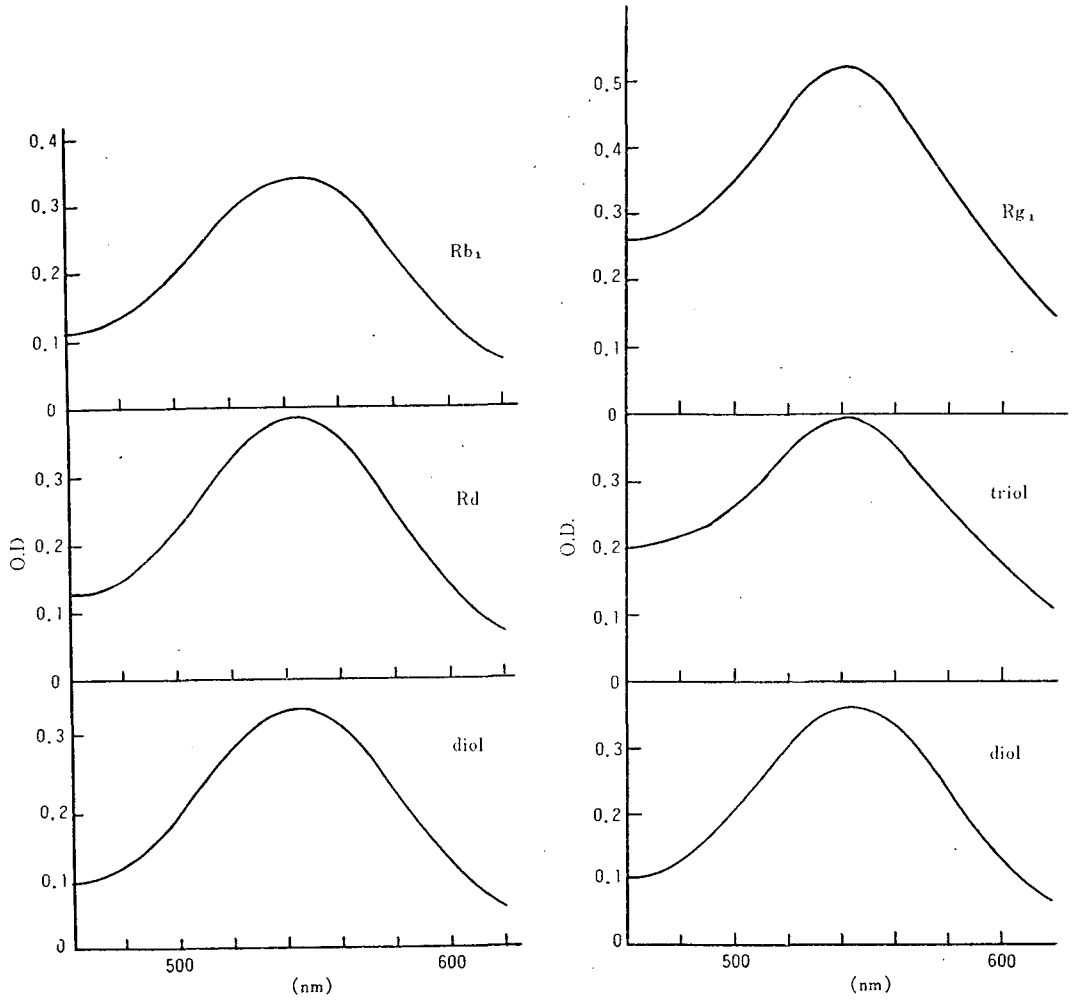


Fig. 3. Absorption spectra of ginseng saponin or sapogenin in vanillin-sulfuric acid reagent.

Table 4. Effects of Ginseng Saponins on the Incorporation of (^{3}H)-Leucine into Mice Sera

Material	No. of mice	(^{3}H)-Leucine (cpm/mg protein)	%
Control (saline)	20	1082 ± 38	100
Fraction 5	5	1307 ± 73	121
Spot 1 (Ginsenoside-Rb$_1$)	9	1142 ± 42	106
Spot 2 (Ginsenoside-Rb$_2$)	10	1320 ± 58	122
Spot 3 (Ginsenoside-Rc)	8	1310 ± 36	121
Spot 4 (Ginsenoside-Rc$_2$)	8	1278 ± 51	118
Spot 5 (Ginsenoside-Rd)	9	1593 ± 85	147
Spot 6 (Ginsenoside-Re)	10	1430 ± 80	132
Spot 7 (Ginsenoside-Rg$_1$)	10	1294 ± 79	120

At 5 hr after each sample (2 mg/mouse) administration, each mouse received intraperitoneally 5 μCi of (^{3}H)-leucine (52 Ci/mM). One hr later, blood sample were taben by heart puncture. Control mice were assayed at 6 hr after saline-treatment.

llin-sulfuric acid reagents under heating at 60° for 10 minutes. The absorption maximum was 544 nm for ginsenoside Rb$_1$ and Rd and panaxadiol, 542 nm for minor component, ginsenoside Rg$_1$ and panaxatriol (Fig. 3). The absorption of one μmole of Rb$_1$ and Rd in 6 ml of reaction mixture was 4.1, and one of Rg$_1$ was 4.5. Thus we may be able to grade the ginseng root by determining the amount of saponin in the crude saponin fraction extracted from the root.

Stimulation of Sugar and Lipid Metabolism

The third topic is the effect of ginseng saponin on the metabolism of sugar and lipid. At first we encountered the fact that ginseng extract markedly reduced the blood glucose level of adrenoectomized rats (Fig. 4). Then we could detect a similar but rather weak effect in normally fed rats (Fig. 5) and in fasted rats infused with glucose solution. Thereafter we found that liver glycogen content was reduc-

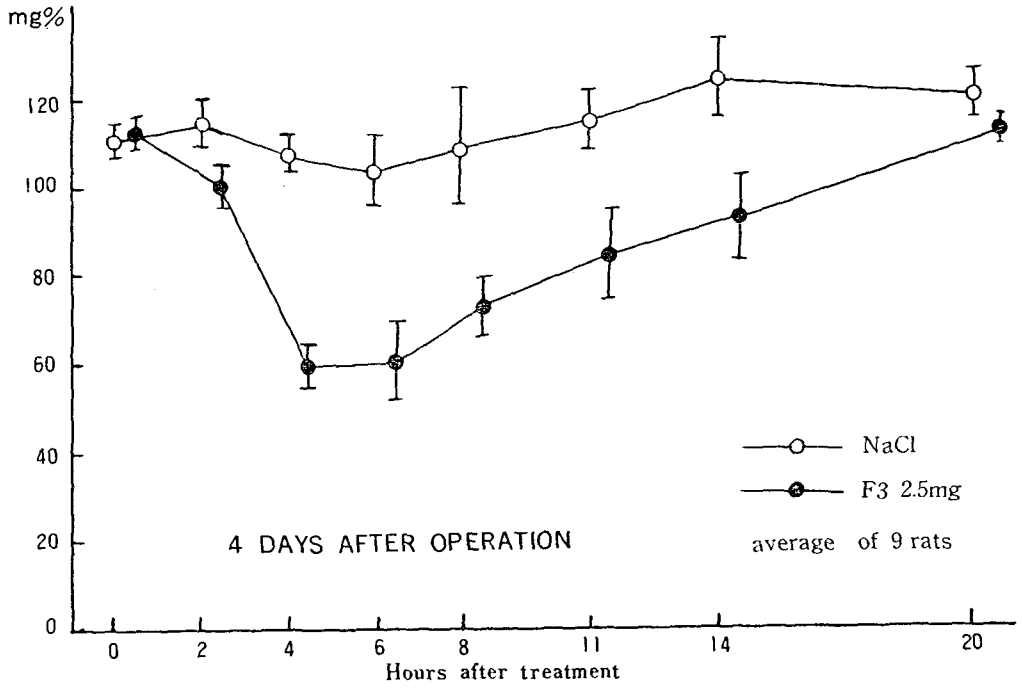


Fig. 4. Effect of fraction 3 on blood glucose level of adrenoecomized rats.

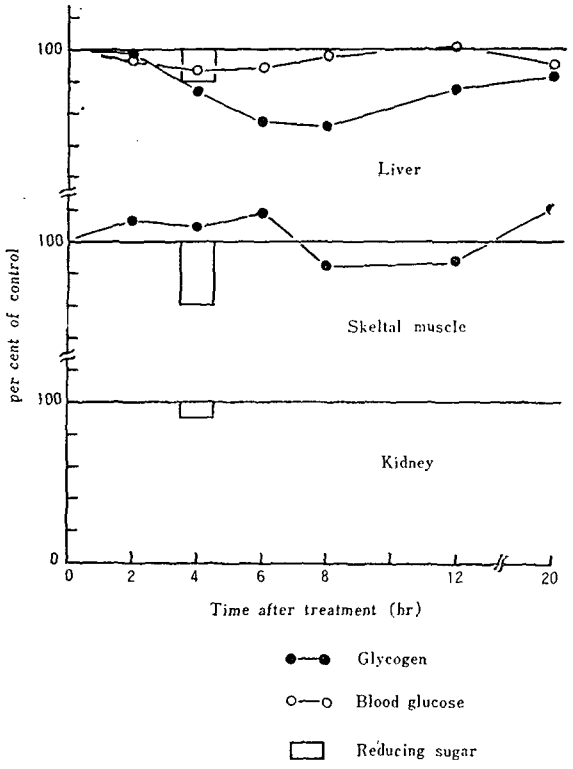


Fig. 5. Effect of fraction 3 on glycogen, blood glucose and reducing sugar. Five mg of fraction 3 was administered to rat.

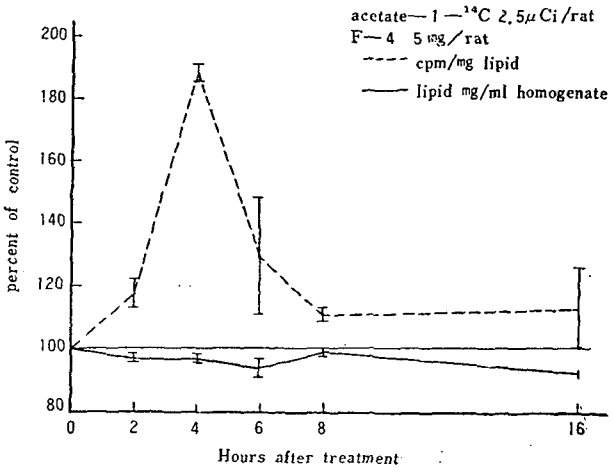


Fig. 6. Effect of fraction 4 on lipid synthesis of rat liver.

ed markedly in the normally fed rats, and that the reducing sugar level in liver, kidney, and muscle was also decreased by the ginseng treatment (Fig. 5). Thus ginseng extract was somehow causing a decrease in total carbohydrate level in the animal. Anyway, in the next step, C^{14}-acetate incorporation into liver lipid was determined. The rate of lipid synthesis was increased transiently by ginseng treatment. The peak occurred 4 hours after the ginseng treatment

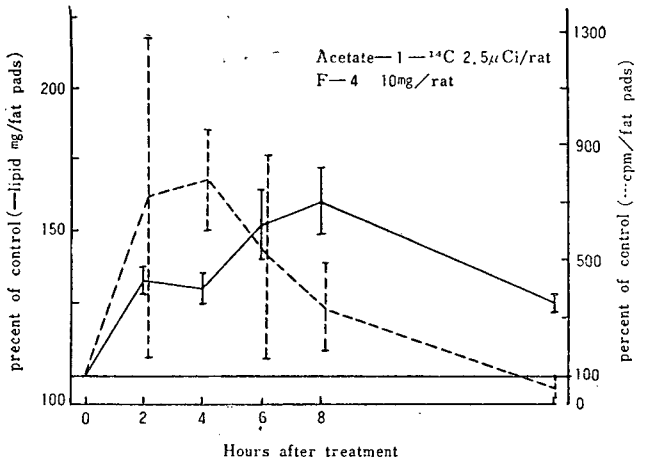


Fig. 7. Effect of fraction 4 on lipid synthesis of rat epididymal fat pads.

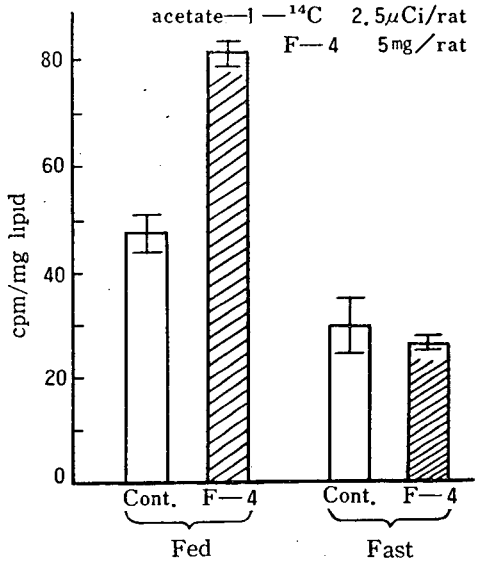


Fig. 8. Effect of fraction 4 on liver lipid synthesis of normally fed rat and fasted rat for 18 hours.

(Fig. 6). However, changes in the total amount of lipid in rat liver could not be detected.

So we assayed the rate of lipid synthesis and the total amount of lipid in an adipose tissue. Figure 7 will show that both the rate of synthesis and the amount of lipid in epididymal fat pads were increased by ginseng treatment. Thus ginseng principle or ginseng saponin caused an increase in lipid synthesis and concomitantly reduced blood glucose and liver glycogen. From the view point of the metabolic pathway and the inter-organal relations, we may say that ginseng saponin caused a transformation from carbohydrate to lipid, and its transference from liver to the adipose tissue.

Regulational Features of Ginseng Action

Our fourth topic is the mode of action of ginseng saponin as a kind of metabolic regulator. At first we tested the effect of saponin on lipid synthesis in rats fasted for 18 hours. Figure 8 is the result. Increase in C^{14}-acetate incorporation into liver lipid did not occur in the fasted rats. I think this is one of the special features of ginseng action, different from the usual metabolic effectors.

Figure 9 shows the results of dietary conditions. Four groups of rats were fed on normal laboratory chow, fat-free or high carbohydrate, high fat and high protein diets. We assayed the effect of ginseng on lipid synthesis in the rat liver of the groups. With high carbohydrate, high protein and normal

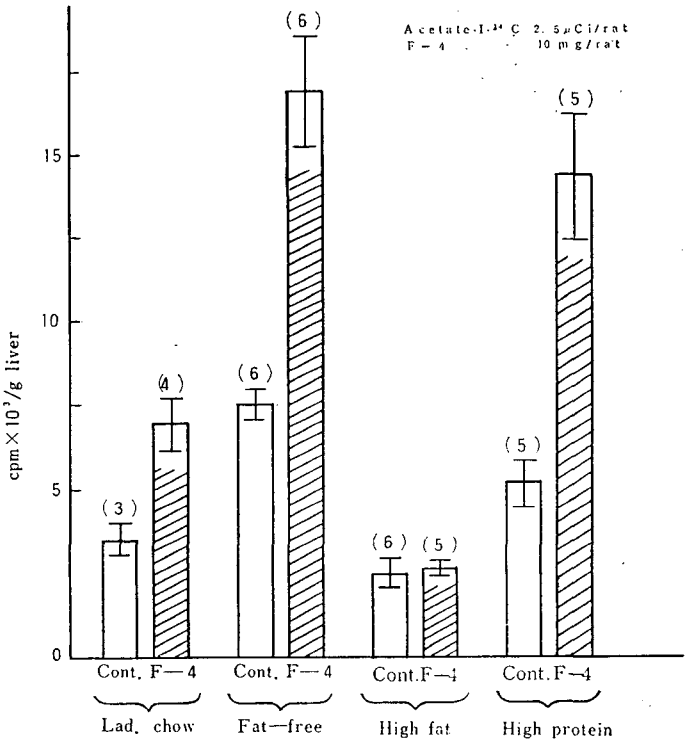


Fig. 9. Effect of fraction 4 on liver lipid synthesis of rat fed on various dietary conditions.

groups, we could reconfirm the lipid increasing effect of ginseng. However, with the high lipid group, ginseng did not show the increasing effect on lipid synthesis. This is the second special feature of gin-

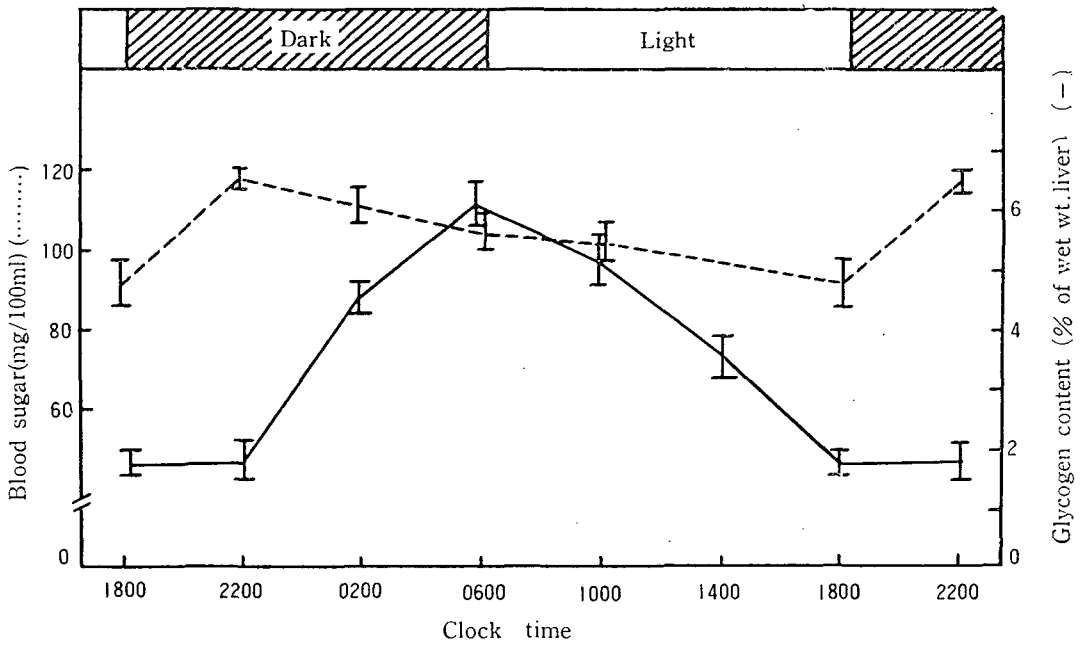


Fig. 10. Circadian rhythm of liver glycogen content and blood glucose level.

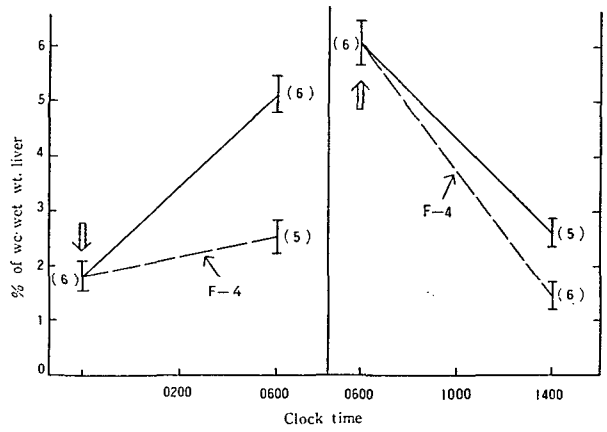


Fig. 11. Effect of fraction 4 on liver glycogen content. Ten mg of fraction 4 was administered to rat at 10 P. M.or 6 A.M.

seng action.

Recently it was reported in detail that many metabolic activities of living organisms change rhythmically. Figure 10 shows our data on the circadian rhythm or 24 hour rhythm of blood glucose and liver glycogen level of normally fed rats. The dark phase is from 6 P. M. to 6 A. M., and the light phase from 6 A. M. to 6 P. M. Liver glycogen content has its lowest or basal level between 6 P. M. and 10 P. M., and it increases almost linearly from 10 P. M. to 6 A. M. From 6 A. M. to 6 P. M. it decreases linearly.

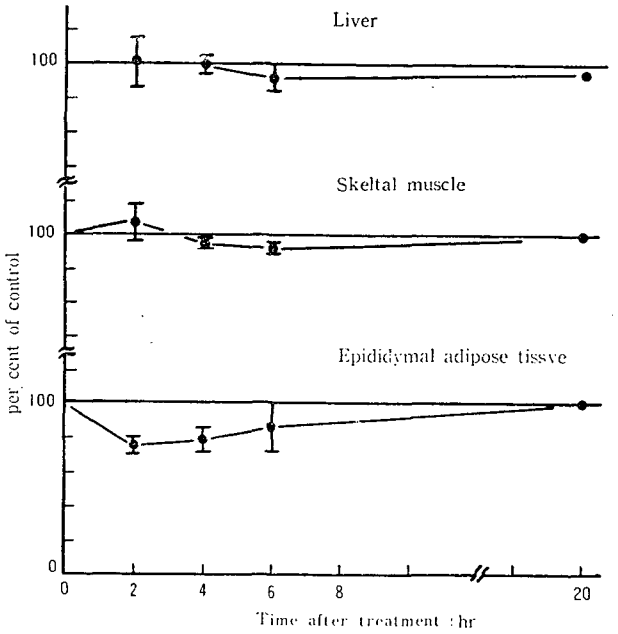


Fig. 12. Effect of fraction 5 on cAMP content of rat tissues. Ten mg of fraction 5 was administered to rat intraperitoneally. Cyclic AMP was determined by the Gilman's binding-protein method.

When ginseng was adminstered at the early stage of the increasing phase or dark phase, the final glycogen level markedly decreased but some increment of glycogen was still observed (Fig. 11). When administered at the early stage of the decreasing phase or

light phase, the effect of ginseng was also clear.

It is a well known fact that many hormones, for example, insulin, corticosterone, epinephrine, glucagon and growth hormone are involved in the metabolism of carbohydrate and lipid. These hormones, except corticosterone, produce their specific physiological responses in the cells of the target organs by aids of endogenous cyclic AMP as the second messenger. So we assayed the cAMP level in liver, muscle, adipose tissue and adrenal gland upon the administration of ginseng saponin. In liver and muscle tissue, we could not detect any significant changes in the cAMP level (Fig. 12). However, in adipose tissue cyclic AMP level clearly reduced. The decreasing effect and the phase of the decrease in cyclic AMP seem reasonable given the generally accepted facts of lipid metabolism.

In addition to these, cyclic AMP content of the

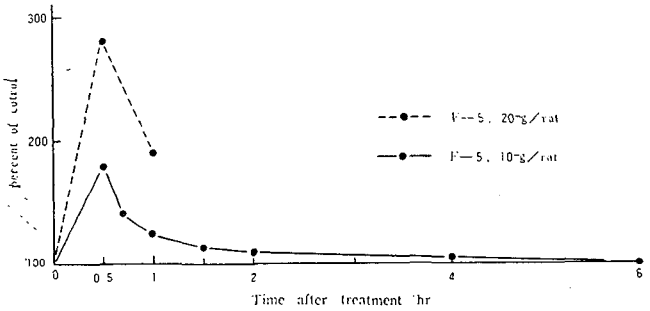


Fig. 13. Increase in adrenal cAMP content by fraction 5 administration. Rats were sacrified in the period, 9–11 A.M.

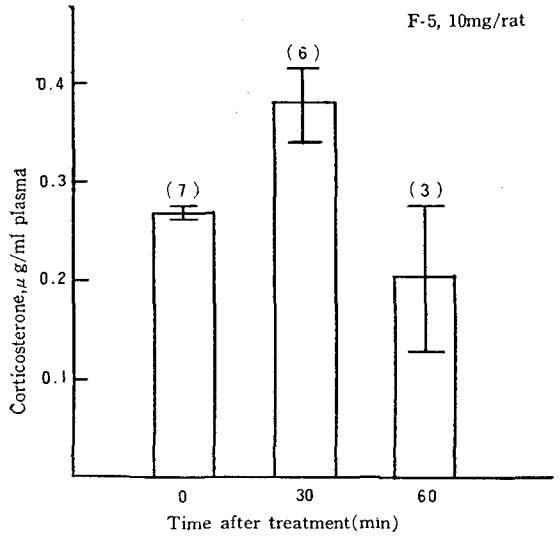


Fig. 14. Effect of fraction 5 on plasma corticosterone level.

adrenal gland was also assayed. A transient increase of cyclic AMP was found (Fig. 13). The peak came about 30 minutes after ginseng treatment. This response was the earliest one among the many biochemical changes already noted. Then we observed that serum corticosterone also was increased transiently by treatment with ginseng saponin (Fig. 14).

As the next step, we made an experiment with hypophysectomized rats to test the possibility of increase in secretion of ACTH, adrenocorticotropic hormone. Table 5 will clearly show that ginseng saponin had no effect on the cyclic AMP level in the adrenal gland, although ACTH had an effect with the hypophysectomized rats.

Table 5. Effect of fraction 5 on adrenal cAMP content in normal and hypophysectomized rats.

cAMP content, pmole/mg wet wt

	No. of Rat	Saline	F-5 (30 min)	F-5 (60 min)	ACTH (30 min)
Normal rat	6	4.46 ±0.26 (100)	17.1 ±3.50 (384)	4.20 (97)	—
Hypox. rat	4	3.45 ±0.21 (100)	3.71 ±0.38 (108)	3.38 ±0.11 (98)	102.1 ±20.3 (2960)

Fraction 5: 10 mg/rat, ACTH: 200mU/rat.

Therefore, it seems to me that ginseng saponin administration caused ACTH secretion to increase by some rapid process, and then the endogenous ACTH increased the cAMP level in the adrenal gland, and the cAMP increased the secretion and/or synthesis of corticosterone. We do not know how deeply these findings are concerned with the many metabolic effects already mentioned. However, I would like to say that we found that the administration of ginseng saponin to rat resulted in metabolic changes and an increase in hormone level.

Summary and Concluding Remark

Now we may summarize our results. The first. Ginseng can stimulate the synthesis of rat liver RNA and of serum protein in rat and mouse. Ginseng

can also stimulate the synthesis of bone marrow DNA and the mitoses of bone marrow cells, as found by Yamamoto *et al.* and by us. The second. Ginseng can stimulate carbohydrate metabolism in the liver and can increase the lipid content of adipose tissue. The third. The biochemically active principle of ginseng is saponin. The fourth. The action of ginseng has some special features in its mode of action. Or we may say that ginseng saponin is a kind of metabolic regulator or hormone-like substance.

I think that the action of ginseng roots, or the traditional remedy has now obtained some biochemical supports. And some of the tonic effects of ginseng may be explained by our biochemical results. Thus we may safely say that the mysterious panacea which was found and used by ancient people, is very useful even in modern medicine. And I hope that the great progress in medical and clinical studies of ginseng will be continued.

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