[Chem. Pharm. Bull. 23(5)1009—1016(1975)]

UDC 547.92'455.04 : 615.322.011.5 : 542.98

Effect of Ginseng Saponins on Cholesterol Metabolism. I. The Level and the Synthesis of Serum and Liver Cholesterol in Rats treated with Ginsenosides

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(Received September 13, 1974)

The *in vivo* effect on cholesterol metabolism of ginsenoside-Rb_1, -Rc, -Rd, -Re, and -Rg_1 purified from Ginseng (the root of *Panax ginseng* C.A. Meyer) was investigated. Four hours after the intraperitoneal injection of 5 mg of each pure ginsenoside into rats, the concentrations of serum and liver cholesterol and the incorporation of ^{14}C-acetate into serum and liver cholesterol were determined. The results indicated the enhancement of cholesterol biosynthesis by administered saponins, particularly by ginsenoside-Rb_1, a predominant component of Ginseng saponins.

Biochemical effect of an extract of Ginseng has been widely studied, and it has been revealed that the extract stimulates various metabolic reactions, as reviewed in recent papers.[2-4] Since most of the active preparations from the extract contained saponins as main constituents, it should be investigated whether the stimulating effect is definitely due to the saponins or to other minor components. This has become possible by the success in isolating and purifying various saponins contained in Ginseng.[5-7] It is also interesting to study the difference in biochemical effect of saponins having different chemical structures.

The present paper reports a research concerning with the effect of five purified saponins of Ginseng on the level and the biosynthesis of rat serum and liver cholesterol. A partially purified preparation of the extract of Ginseng was found by Yamamoto[8] to reduce the concentration of cholesterol and triglycerides in blood of hyperlipemic rats, and to accelerate the rate of disappearance of ^{14}C-labeled cholesterol from circulation. Administering the same preparation, Oura, *et al.*[4,9] observed a stimulation in the incorporation of ^{14}C-acetate into the total lipid fraction of liver and an accumulation of fat in adipose tissues. The preparations used in these studies were rich in saponins. In addition, Yamamoto[10] reported a similar effect of Saiko-saponin, a saponin of *Bupleurum falcatum L.*

Experimental

Saponins——Ginsenoside-Rb_1, -Rc, -Rd, -Re, and -Rg_1[11] were isolated and purified from Ginseng.[5-7] All the preparation used in the present study was found to be pure by chemical and physicochemical analyses.

General Procedures——Unless otherwise indicated, 5 mg of each purified saponin was injected intraperitoneally into rats weighing 100—120 g. Rb_1, Rc, and Rg_1 were dissolved in saline, and Rd and Re in 20% ethanol. The solvents were found not to disturb the present experiments. Total and free cholesterol

1) Location: *1-5-8, Hatanodai, Shinagawa-ku, Tokyo.*
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11) These are abbreviated as Rb_1, Rc, Rd, Re, and Rg_1.

in serum and in liver were assayed 4 hr after the injection of saponins. In labeling experiments, 10 μCi of sodium acetate-1-^{14}C (per 100 g of body weight) was injected intraperitoneally into normal and saponin-treated rats at a definite time prior to sacrifice. The saponin-treated rats were killed 4 hr after the injection of saponins. Incorporation of ^{14}C-acetate into serum cholesterol was measured for the period of 30 min, and that into liver cholesterol was determined for the period of 90 min.

Assay of Cholesterol in Serum——Total and free cholesterol in serum was measured by a modification[12] of Zak's method.[13]

Assay of Liver Cholesterol——Liver cholesterol was determined by a modification of the method reported by Ichida.[14] One gram of liver was first homogenized with about 5 ml of ethanol and was diluted further with about 30 ml of ethanol. The mixture was treated at 50—60° in a water bath for 30 min, and was filtered through defatted filter paper (No. 7, Toyo Roshi). The residue was extracted again with about 15 ml of ethanol–ether (3: 1) at 50—60° for 30 min. The combined filtrates were adjusted the volume to 50 ml. The concentrations of total and free cholesterol in this extract were assayed by the same method as employed for the serum cholesterol.

Measurement of ^{14}C Incorporated into Serum Cholesterol——^{14}C-Labeled acetate was injected 30 min prior to sacrifice, *i.e.*, 3.5 hr after the injection of saponins in the case of saponin-treated rats. Serum cholesterol was isolated as follows. The procedure was originally described by Entenman[15] and later modified by De Matteis[16] for isolating liver cholesterol. To 1 ml of serum were added 5 ml of 21% KOH and the mixture was kept in boiling water for 1 hr. Ethanol was added at a final concentration of 50%, and the mixture was heated to boil. After cooling extraction was carried out twice with use of each 10 ml of petroleum ether (for 5—10 min). The petroleum ether layer was evaporated to dryness under a reduced pressure. The residues were dissolved in 21 ml of ethanol and filtered through a defatted cotton-wool plug into 18 ml of acetone. Five milliliters of the extracts were mixed with 1 ml of 1% digitonin solution in 50% ethanol, and the mixture was kept standing overnight in an incubator (37°). The precipitated digitonide of cholesterol was separated by centrifugation, washed once with a small volume of ethanol–acetone (1: 1), and dried. For counting the radioactivity, the materials were dissolved in 9 ml of scintillation fluid and assayed with a scintillation spectrometer (Beckman Ls-100 C). For quantitative determination of cholesterol, the materials were dissolved in 6 ml of glacial acetic acid and assayed by a modification of *Z*ak's method. The data were expressed as cpm per mg of cholesterol.

Measurement of ^{14}C Incorporated into Liver Cholesterol——^{14}C-Acetate was injected 90 min before sacrifice, *i.e.*, 2.5 hr after the injection of saponins in the case of saponin-treated rats. Liver cholesterol was isolated as similarly as in the case of serum cholesterol. One gram of liver was digested and saponified with KOH and extracted with 50% ethanol at 80—100° for 1—4 hr until clear solution was obtained, followed by extraction with 20 ml of petroleum ether (twice). Cholesterol was separated as digitonide, as described above, and the amount and the radioactivity were measured using appropriate aliquots.

Chemicals——Sodium acetate-1-^{14}C (specific radioactivity: 48—49 mCi per m mole) was purchased from Daiichi Pure Chemicals Co., Tokyo. Digitonin was obtained from E. Merck, Darmstadt.

Result

Amount of Cholesterol in Serum

The amount of cholesterol in serum of the rats treated with various saponins was determined, and the results are presented in Fig. 1 (total cholesterol) and Fig. 2 (free cholesterol). Rb_1 caused a slight elevation both in the total and the free cholesterol concentrations. Rc showed a similar effect as Rb_1 for the total cholesterol, but decreased the amount of free cholesterol. Rg_1 reduced only the free cholesterol level, whereas Rd and Re reduced both free and total cholesterol.

The ratios of free/total cholesterol were calculated, and shown in Fig. 3. Except for the case of Rb_1, saponin-treated rats exhibited lower ratios of free/total cholesterol than those of normal rats.

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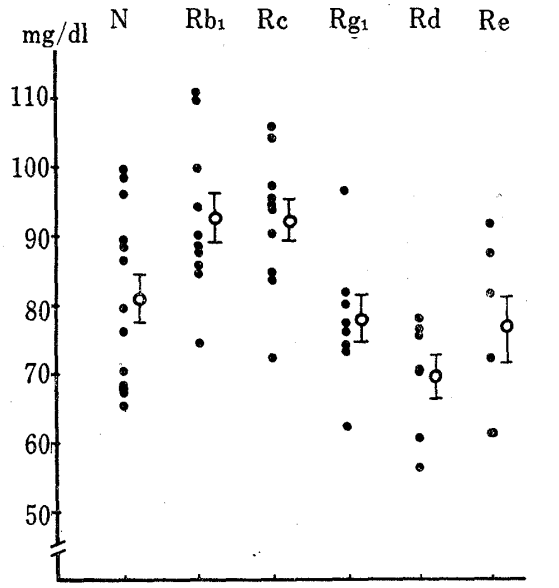
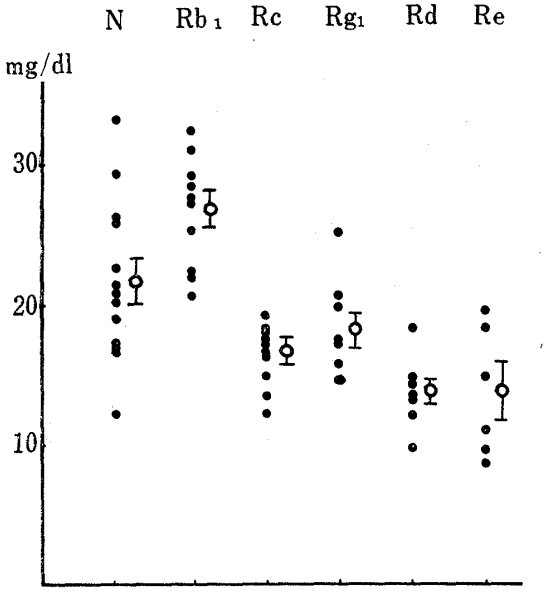


	N	Rb_1	Rc	Rg_1	Rd	Re
mean	81.0	92.5	92.4	78.0	69.6	76.0
±S.E.	3.54	•3.64	•3.16	3.45	•3.11	5.28

Fig. 1. Amount of Total Cholesterol in Serum

Cholesterol was assayed 4 hr after the injection of 5 mg of each ginsenoside. Data are expressed as mg of cholesterol in 100 ml of serum.
N; normal rat serum
Figures are the mean ± standard error.
○: $p<0.01$, ●: $p<0.05$.
The data without these marks are not statistically significant.



	N	Rb_1	Rc	Rg_1	Rd	Re
mean	21.8	26.7	16.4	18.0	13.6	13.6
±S.E.	1.62	•1.22	°0.74	1.29	°0.99	°1.86

Fig. 2. Amount of Free Cholesterol in Serum

See the legend to Fig. 1

Amount of Cholesterol in Liver

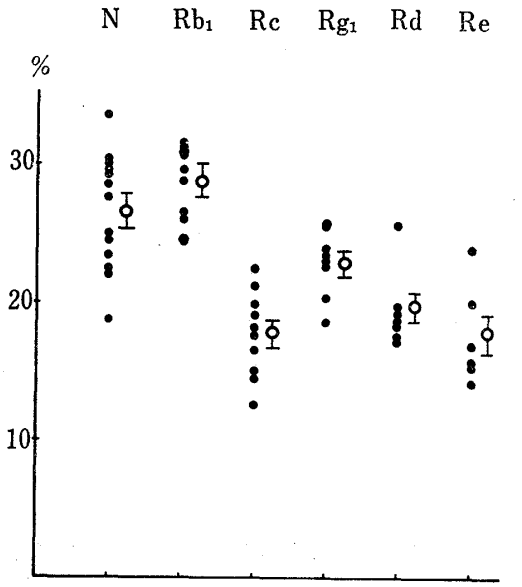
Fig. 4 and 5 show the amounts of total and free cholesterol in the liver of rats injected with various saponins. Administration of Rc resulted in a considerable reduction of both types of cholesterol. Rb_1 and Rg_1 affected slightly the level of total cholesterol. Re caused a remarkable decrease in free cholesterol (50%) associated with an increase in esterified cholesterol, and on the contrary, Rd elevated the concentration of free cholesterol with concomitant decrease of the esterified one.

In Fig. 6 the ratios of free/total cholesterol in liver are shown. The ratio was found to be extremely low in Re-treated rats, and high to some extent in Rd-treated rats.

Incorporation of ^{14}C-Acetate into Serum Cholesterol

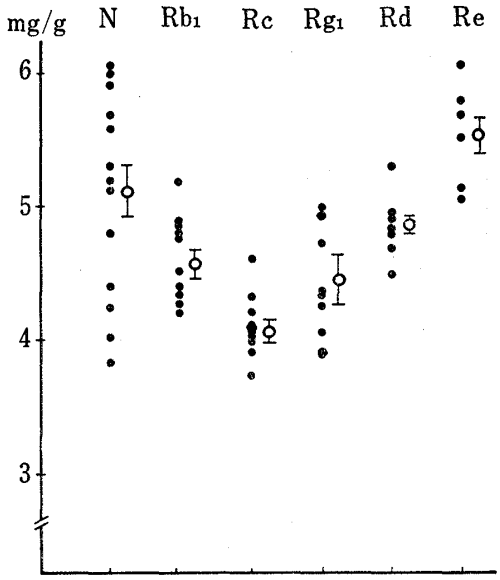
Time course of the incorporation of ^{14}C-acetate into serum cholesterol was investigated with the normal and the Rb_1-treated rats. The results are illustrated in Fig. 7. An apparent peak in the specific radioactivity was observed 30 min after the injection of radioactive acetate in Rb_1-treated rats. Based on this evidence the labeling of serum cholesterol by injected ^{14}C-acetate was compared at 30 min after the injection between the normal and the saponin-treated rats.

Fig. 8 demonstrates the effect of various saponins on the labeling of serum cholesterol by injected ^{14}C-acetate. Serum cholesterol in Rb_1-treated rats was labeled almost 10 times as highly as that in normal rats, and in Rc-treated rats 5 times as much ^{14}C-radioactivities were incorporated compared with the control animals. The effect of other three saponins were found comparatively less than those of Rb_1 and Rc.



	N	Rb1	Rc	Rg1	Rd	Re
mean	26.6	28.9	17.9	22.9	19.5	17.6
±S.E.	1.16	1.15	○0.97	●0.87	○1.06	○1.49

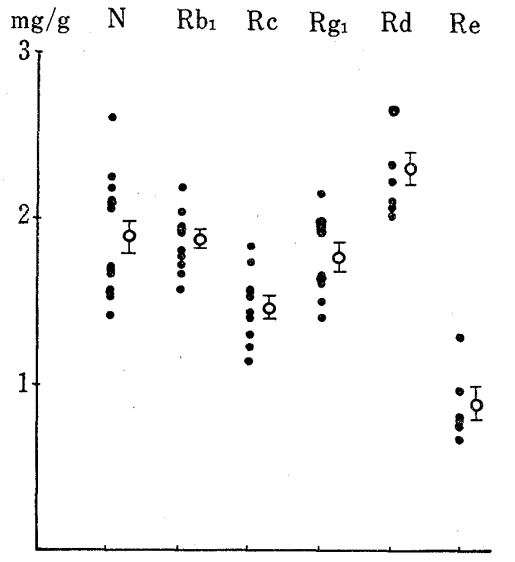
Fig. 3. Ratio of Free/Total Cholesterol in Serum



	N	Rb1	Rc	Rg1	Rd	Re
mean	5.12	4.58	4.15	4.42	4.86	5.54
±S.E.	0.21	●0.10	○0.08	●0.17	0.10	0.16

Fig. 4. Amount of Total Cholesterol in Liver

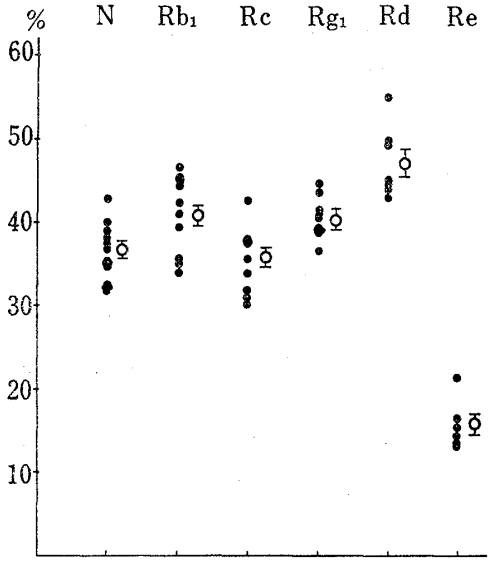
Cholesterol was assayed 4 hr after the injection of 5 mg of each ginsenoside. Data are expressed as mg of cholesterol per g of liver.
N: normal rat liver
Figures are the mean ± standard error.
○: $p < 0.01$, ●: $p < 0.05$.
The data without these marks are not statistically significant.



	N	Rb1	Rc	Rg1	Rd	Re
mean	1.86	1.86	1.47	1.79	2.29	0.88
±S.E.	0.11	0.05	○0.07	0.09	○0.10	○0.09

Fig. 5. Amount of Free Cholesterol in Liver

See the legend to Fig. 4.



	N	Rb1	Rc	Rg1	Rd	Re
mean	36.2	40.8	35.6	40.4	47.1	15.8
±S.E.	1.01	○1.47	1.24	○0.85	○1.66	○1.22

Fig. 6. Ratio of Free/Total Cholesterol in Liver

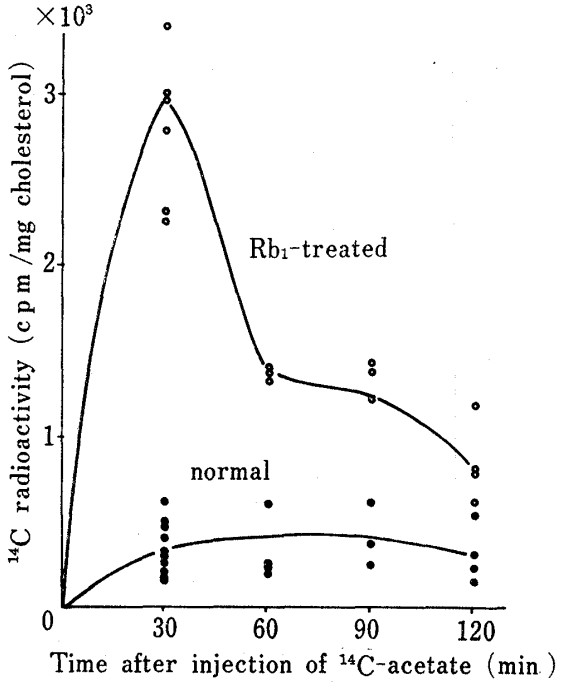


Fig. 7. Kinetics of Incorporation of ^{14}C-Acetate into Serum Cholesterol in Normal and Rb_1-treated Rats

Rb_1-treated rats were killed 4 hr after receiving the ginsenoside, and were injected with ^{14}C-acetate 30, 60, 90 and 120 min prior to sacrifice.
○: Rb_1-treated rats
●: normal rats

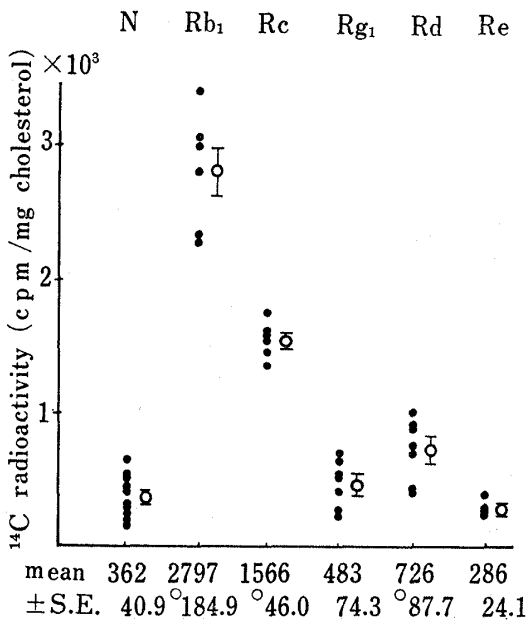


Fig. 8. Incorporation of ^{14}C-Acetate into Serum Cholesterol

Saponin-treated rats were killed 4 hr after receiving the saponin, and were injected with ^{14}C-acetate 30 min before sacrifice. The data are expressed as cpm per mg of cholesterol.
N: normal rats receiving ^{14}C-acetate 30 min before sacrifice.
Figures are the mean ± standard error.
○: $p < 0.01$

Incorporation of ^{14}C-Acetate into Liver Cholesterol

Kinetics of the incorporation of radioactive acetate into liver cholesterol are shown in Fig. 9. The curve for the specific radioactivity of liver cholesterol in Rb_1-treated rats revealed a peak at 90 min after the injection of ^{14}C-acetate, as is the case in normal rats. However, the values in the former were significantly higher than the corresponding ones in the latter. From this finding, it was decided that the effect of saponins on the labeling of liver cholesterol by injected radioactive acetate was investigated 90 min after the isotope injection.

In Fig. 10 are summarized the specific radioactivities of liver cholesterol in saponin-treated rats. As seen in the labeling of serum cholesterol, Rb_1 enhanced the labeling of liver cholesterol most extensively. The other four ginsenosides also stimulated the incorporation to some extent.

Dose Response for Ginsenoside-Rb_1 in Incorporation of ^{14}C-Acetate into Liver Cholesterol

Varying amounts of Rb_1 were administered to rats and their effects on the incorporation of ^{14}C-acetate into liver cholesterol were investigated. The results are presented in Fig. 11, where the data are expressed as percentage of the incorporation in the rats receiving 5 mg of Rb_1.

Discussion

The present results clearly demonstrate that the saponins contained in Ginseng generally stimulate the biosynthesis of cholesterol when administered to rats. The data are summarized in Table I, being expressed as percentage of the control ones. Among five ginsenosides so

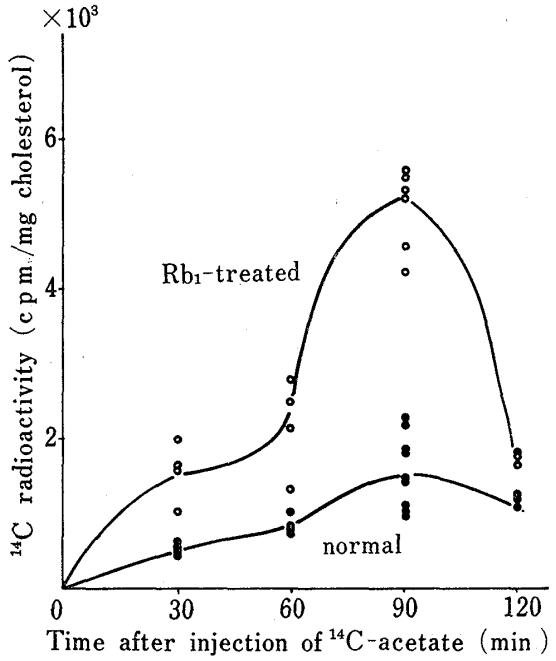


Fig. 9. Kinetics of Incorporation of ^{14}C-Acetate into Liver Cholesterol in Normal and Rb_1-treated Rats

See the legend to Fig. 7.
○: Rb_1-treated rats
●: normal rats

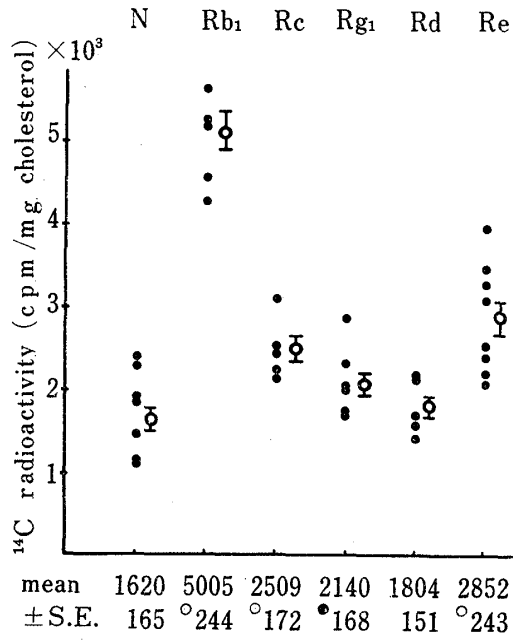


Fig. 10. Incorporation of ^{14}C-Acetate into Liver Cholesterol

Saponin-treated rats were killed 4 hr after receiving the saponins, and were injected with ^{14}C-acetate 90 min before sacrifice. Data are expressed as cpm per mg of cholesterol.
N: normal rats receiving ^{14}C-acetate 90 min before sacrifice.
Figures are the mean± standard error.
○: $p < 0.01$, ●: $p < 0.05$

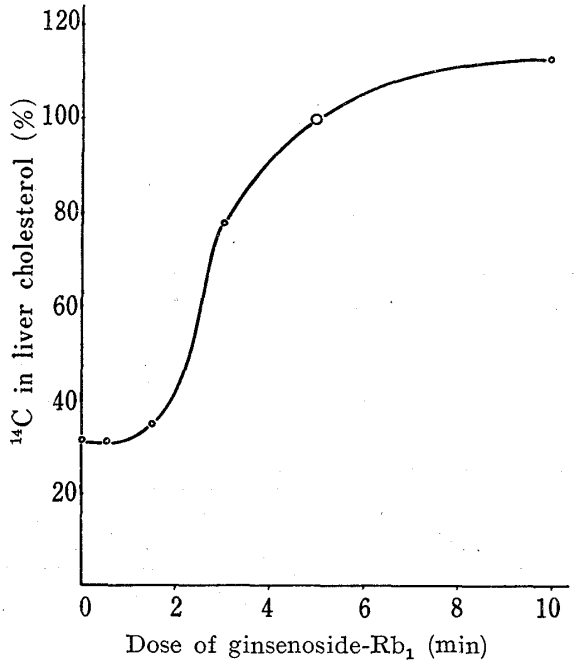


Fig. 11. Incorporation of ^{14}C-Acetate into Liver Cholesterol in Rats Receiving Various Doses of Ginsenoside-Rb_1

Ginsenoside-Rb_1 and ^{14}C-acetate were injected 4 hr and 90 min prior to sacrifice, respectively. The incorporation with rats receiving 5 mg of Rb_1 was taken as the standard (100%).

far examined, ginsenoside-Rb_1 was most strikingly active. This is a predominant saponin in quantity in Ginseng, thus it can be concluded that the activity of Ginseng for stimulating the cholesterol metabolism is mainly accounted for by ginsenoside-Rb_1.

As seen in Chart 1, the difference in the structure between ginsenoside-Rb_1 and -Rc is only in a sugar component at the end of each molecule, *i.e.*, glucose in ginsenoside-Rb_1 and arabinose in ginsenoside-Rc. Replacement of arabinose by glucose doubles the incorporation of ^{14}C-acetate into cholesterol. The sugar component at this site in the molecule of ginsenoside seems to be significant for exerting the enhancement of cholesterol synthesis, because ginsenoside Rd lacking a sugar molecule in this position showed a less activity.

It may be considered that ginsenosides containing panaxatriol (Rg_1 and Re) appeared to be less effective than those containing panaxadiol (Rb_1, Rc and Rd). However, ginsenoside-Re containing rhamnose showed

TABLE I. Effect of Various Ginsenosides on Cholesterol Metabolism

Ginsenoside			Rb_1	Rc	Rg_1	Rd	Re
Serum cholesterol	amount	total	114	114	96	86	94
		free	123	75	83	62	62
		F/T[a)]	109	67	86	73	66
	biosynthesis		773	433	133	201	79
Liver cholesterol	amount	total	90	81	86	95	108
		free	100	79	96	123	47
		F/T[a)]	113	98	112	130	44
	biosynthesis		309	155	132	111	176

The results in Fig. 1—10 were summarized in this table. The values are expressed as percent of the control.
a) free/total cholesterol × 100

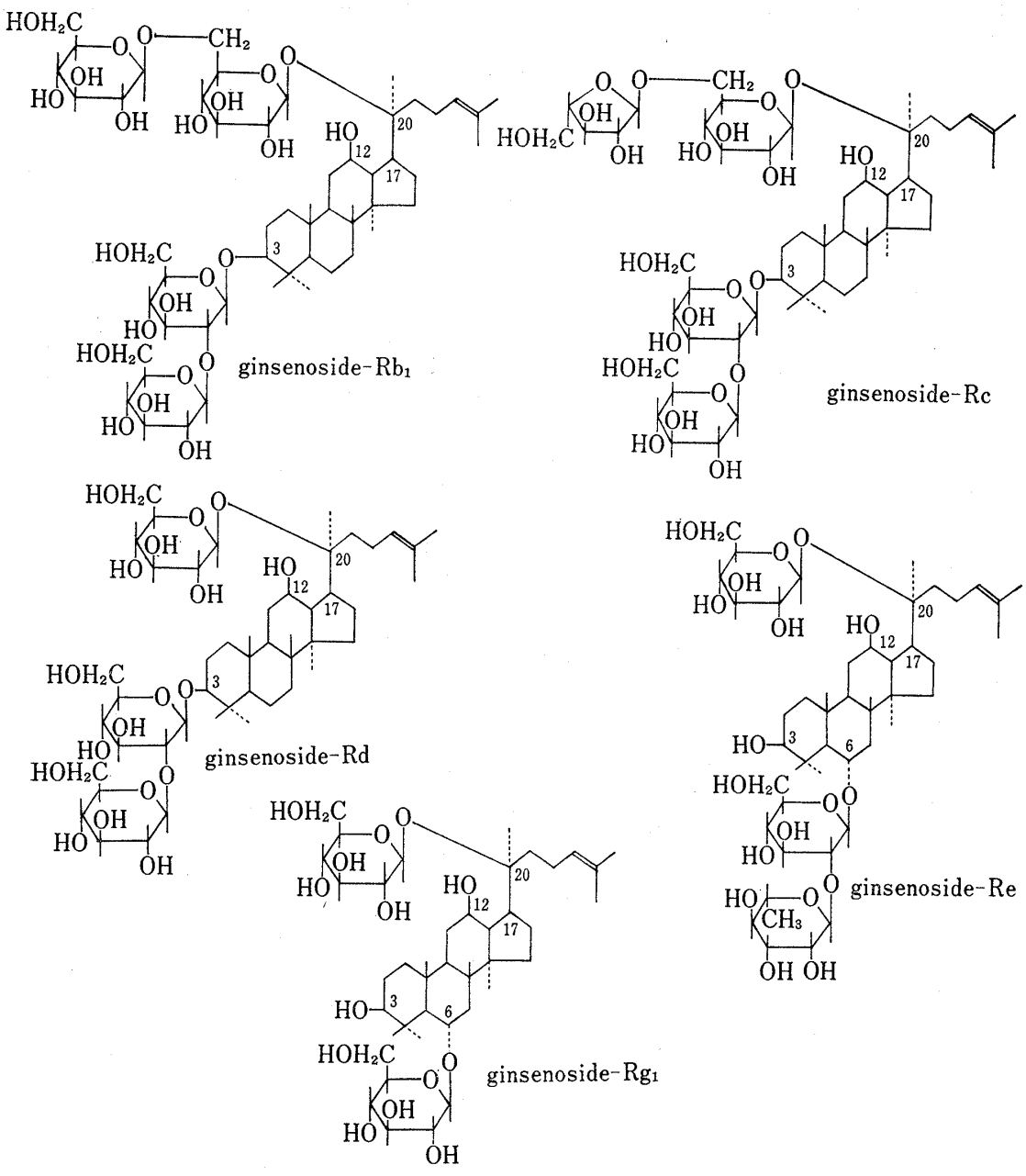


Chart 1. Structure of Ginsenosides Used in the Present Study

rather unique features in affecting the free cholesterol level in liver and the labeling of serum cholesterol by injected ^{14}C-acetate.

Compared to the significant increase in biosynthesis of cholesterol, particularly by ginsenoside-Rb_1, the amounts of cholesterol in serum and in liver were affected to a lesser extent. This fact will evidently suggest that the saponins stimulate not only the synthesis but also the catabolism of cholesterol, including the conversion into other steroid compounds. Yamamoto[8] observed an increased rate in the excretion of injected ^{14}C-cholesterol into bile and feces by administrating the partially purified preparation of Ginseng extracts. In this connection, glycyrrhizin in licorice was reported to behave similarly.[8]

Following the experiments reported in the present paper, the authors have been studying *in vitro* cholesterol synthesis using liver slices from the ginsenoside-treated rats. The results so far obtained indicate an enhanced synthesis by those slices compared with slices from normal rats. A report dealing with such study will follow this paper shortly.

Acknowledgement The authors' thanks are due to Miss Nobuko Sugiyama for her technical assistance in assaying serum and liver cholesterol.