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Effect of Ginseng Saponins on Cholesterol Metabolism. II.[1] Effect of Ginsenosides on Cholesterol Synthesis by Liver Slice

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Following the previous study, biosynthesis of cholesterol was measured by the incorporation of ^{14}C-acetate in liver slices from rats treated with ginsenoside $-Rb_1$, $-Rc$, $-Rd$, $-Re$, and $-Rg_1$. Five mg of each ginsenoside was given to rats by intraperitoneal injection, and after 2.5 hr liver slices were incubated with ^{14}C-acetate to determine its incorporation into cholesterol. The enhancement of cholesterol synthesis was observed in most of the slices from ginsenoside-treated rats, confirming the conclusion in the previous study on *in vivo* incorporation of ^{14}C-acetate into liver and serum cholesterol. On the other hand, the ginsenoside did not exhibit any effect on the cholesterol synthesis by normal liver slices when added to the incubation mixture.

The previous paper[1] reported the *in vivo* effect on cholesterol metabolism of five ginsenosides purified from Ginseng (the root of *Panax ginseng* C.A. Meyer). By intraperitoneal injection of 5 mg of each pure ginsenoside into rats, the incorporation of ^{14}C-acetate into serum and liver cholesterol was mostly stimulated with little concomitant increase in the level of choresterol in serum and liver.

The present paper is concerned with the incorporation of ^{14}C-acetate into cholesterol in liver slices prepared from rats treated with the five ginsenoside as previously described.

Experimental

Saponins——Ginsenoside-Rb_1, -Rc, -Rd, -Re, and -Rg_1[3] were isolated and purified from Ginseng,[4–6] and proved to be pure by chemical and physicochemical analyses.

Animals——Male rats weighing 100—150 g were used. Five mg of each purified ginsenoside was injected intraperitoneally into rats as described previously.[1] Rats were killed 2.5 hr after the injection of saponins.

Cholesterol Synthesis in Liver Slices——Liver slices (approximately 500 mg) were incubated at 37° for 1.5 hr with 2 μCi of sodium acetate-1-^{14}C in 5 ml of 0.1 M potassium phosphate buffer, pH 7.4, containing 30 mM nicotinamide and 4 mM magnesium chloride. A glass rod was placed in each flask and the flasks were shaken. At the end of the incubation period, the contents of each flask were saponified at about 70° with 5 ml of 15% potassium hydroxide in 90% ethanol for 30 min. After cooling, extraction was carried out twice with 20 ml of petroleum ether, and the petroleum ether layer was evaporated to dryness under a reduced pressure. The residues were dissolved in 7 ml of ethanol and filtered through a defatted cotton-wool plug into 6 ml of acetone. One ml of 1% digitonin was added to 5 ml of the extracts, and the mixture was placed in an incubator (37°) overnight. The precipitated digitonide of cholesterol was separated by the same method described previously.[1] The precipitates were washed three times to remove contaminating ^{14}C-labeled compounds. Then the materials were dried, and used both for counting the radioactivity and for determining the quantity. The methods for both assays were as described in the previous paper,[1] except that the materials were dissolved in 12 ml of scintillation fluid for counting.

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3) These are abbreviated as Rb_1, Rc, Rd, Re, and Rg_1.
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Chemicals——Sodium 1-^{14}C-acetate (specific radioactivity: 48—49 mCi per m mole) was purchased from Daiichi Pure Chemicals Co., Tokyo. Digitonin was obtained from E. Merck, Darmstadt. Glacial acetic acid and sulfuric acid for cholesterol assay were obtained from Chugai Pharmaceutical Co., Tokyo.

Results

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

Time course for the incorporation of ^{14}C-acetate into cholesterol was investigated using normal liver slices. The results are illustrated in Fig. 1. The specific radioactivity of cholesterol increased rapidly with time of incubation, until reaching a plateau after 90 min.

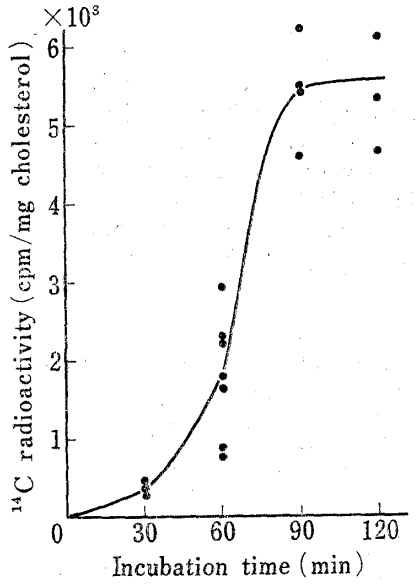

Fig. 1. Kinetics of Incorporation of ^{14}C-Acetate into Cholesterol in Liver Slices

Cholesterol Synthesis in Liver Slice from Rats Treated with Ginsenosides

The *in vitro* incorporation of ^{14}C-acetate into cholesterol was studied with liver slices from rats receiving five kinds of ginsenoside. The specific radioactivities were measured after 90 min incubation, based on the results shown in Fig. 1. As seen in Table I, Rb_1 exhibited the most significant stimulation on cholesterol synthesis, and Rg_1 and Re also showed an enhancing effect. However, the slice from rats treated with Rc or Rd incorporated radioactive acetate into cholesterol at the same rate as the control ones.

In Vitro Effect of Ginsenoside-Rb_1 on Cholesterol Synthesis

In order to study whether the ginsenosides affect the cholesterol synthesis directly or indirectly, various amounts of Rb_1 were added to the incubation mixture consisting of liver slices from normal rats and ^{14}C-acetate

Table I. Synthesis of Cholesterol by Liver Slices

	Normal	Rb_1	Rc	Rg_1	Rd	Re
Specific radioactivity (c.p.m./mg cholesterol)	5740	21811	6314	8244	7128	8424
	4828	23659	4346	7886	6888	7216
	6480	25272	5184	7776	5311	6138
Average	5683	23581	5281	7969	6442	7259
%	100	415	93	140	113	128

Table II. *In Vitro* Effect of Ginsenoside-Rb_1 on Cholesterol Synthesis in Liver Slice

Added Rb_1(mg)	0	0.01	0.1	1.0
Specific radioactivity (c.p.m./mg cholesterol)	4456	6106	5592	4868
	4056	5856	4488	5840
	5392	4664	5126	4906
	4900	4204	4864	4610
	5120	4410	4812	4720
Average c.p.m.	4784	5048	4976	4988
%	100	106	104	104

Indicated amounts of Rb_1 were added to the incubation mixture (5 ml) containing 500 mg of liver slices and 2 μCi of sodium acetate-1-^{14}C. Incorporation of ^{14}C into cholesterol was measured after 90 min.

as a precursor, and the radioactivities incorporated into cholesterol were determined. As demonstrated in Table II, 0.01—1.0 mg of Rb_1 did not show any stimulating effect on cholesterol synthesis.

Discussion

The stimulating effect of ginsenosides on the synthesis of cholesterol, which was demonstrated in the previous *in vivo* study,[1] was confirmed by the present *in vitro* experiments using slices obtained from saponin-treated rats. Ginsenoside-Rb_1, a predominant constituent in Ginseng saponins, is most active among five saponins employed in these investigations. The results presented in this paper are well consistent with those of *in vivo* study, except for ginsenoside-Rc. The treatment with Rc increased the *in vivo* incorporation of ^{14}C-acetate into liver cholesterol by 55% over the control, but did not stimulate the *in vitro* incorporation in slice. This discrepancy can not be explained at present.

Cholesterol metabolism is considered to be strongly affected by the age and the condition of feeding. The authors observed that the biosynthesis of cholesterol in young rats was more intensive than that in aged rats. Therefore, the attention should be paid to the factor of age in further studies. Many steps are involved in the synthetic pathway of cholesterol from acetate, and the authors interest will concern with the step most strikingly influenced by the treatment of Ginseng saponins. The most probable one may be the reaction from HMG–CoA to mevalonate, which has been considered to be rate-limiting for biosynthesis of cholesterol.[7]

From the evidence that Rb_1, the most effective ginsenoside so far examined, showed no stimulation on cholesterol synthesis by liver slice when added *in vitro*, it seems to be concluded that most of the ginsenosides would affect the synthesis of cholesterol not directly but through certain intermediate agents, which might include some of the hormones. At the authors' laboratory, it was also found that most of these ginsenosides increased the synthesis of proteins[8] and ribonucleic acids[9] *in vivo*, but no effect was detected when supplemented *in vitro*. Therefore, in order to clarify the mechanism for the effect of Ginseng saponins, which appears to be general in character, research should be directed to the investigation for a possible common mediator(s).

As will be reported elsewhere,[10] the synthesis of lipoproteins was most significantly stimulated among the serum proteins in the ginsenoside-treated rats. This would suggest a relationship between the synthesis of lipids and that of apo-lipoproteins transferring them, and provide the authors with a new interesting project.

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