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Effect of *Ginseng* Extract on Lipid and Sugar Metabolism. I. Metabolic Correlation between Liver and Adipose Tissue

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A single intraperitoneal administration of the extract (fraction 4) from the roots of *Panax ginseng* C.A. MEYER increased the incorporation of labeled precursor into total lipid of liver and epididymal adipose tissue in rats. A maximum increase in both tissues was found 4 hr after the treatment, but the response curve to *ginseng* extract was not the same. In addition, accumulation of lipid was observed in adipose tissue, showing a maximum increase 6 to 8 hr after the treatment with fraction 4. In contrast, the concentration of serum triglycerides was slightly decreased after treatment with fraction 4. Further, reduction of blood sugar level and liver glycogen content by *ginseng* extract administration was observed.

Based on these results, the metabolic interrelation induced by *ginseng* extract was discussed.

It is widely accepted that in mammals, liver and adipose tissue play an important role as a site of nutritional and hormonal regulation of both lipid and sugar metabolism.[2-4)] We have already revealed the reduction of liver glycogen and reducing sugar in rats by administration of *ginseng* extract *in vivo*.[5)] Yamamoto[6)] also demonstrated that oral administration of *ginseng* extract increased lipid synthesis in rat liver. From such findings, we became interested in obtaining further information on the effect of *ginseng* extract on lipid and sugar metabolism.

In the present work, an attempt was made to clarify the metabolic correlation between liver and adipose tissue, with special reference to lipid and sugar metabolism when *ginseng* extract was administered *in vivo*.

Materials and Methods

Animals and Diet——Male Wistar strain rats, weighing 120—130 g, were used throughout. A group of 5 to 6 rats was usually housed in a stainless steel cage. Animals were maintained on a laboratory pellet chow obtained from CLEA Japan Inc., Tokyo, for 5 to 7 days before the experiments. Rats had free access to food and water. The room temperature was kept at 25±1°, and relative humidity was maintained at 60—80%.

Chemicals——Sodium acetate [1-^{14}C] (46.1 mCi/mM) was a product of Daiichi Pure Chemicals Co., Ltd., Tokyo. All other chemicals used in this study were of analytical or reagent grade and were used without further purification.

Extraction and Partial Purification of Ginseng Radix——Fraction 4 was prepared from the extract of roots of *Panax ginseng* C.A. MEYER produced in Kumsan, Korea, as previously described.[7)] Roots of *ginseng* were powdered and extracted with 0.05M Tris-HCl buffer (pH 7.6) under stirring for 48 hr in a cold room. The filtrate was brought to 70% saturation of $(NH_4)_2SO_4$. The precipitate formed was collected by centrifugation, dissolved in distilled water, dialyzed against deionized water until salt-free, and then the residual solution was lyophilized (fraction 3). Fraction 3 was extracted with 99% MeOH by refluxing on a water

1) Location: *3190 Gofuku, Toyama, 930, Japan.*
2) G. Weber, G. Banerjee, and J. Ashmore, *Biochem. Biophys. Res. Commun.*, 3, 182 (1962).
3) B. Jeanrenaud, *Metabolism*, **10**, 535 (1961).
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bath, and MeOH solution was concentrated. To this residual solution was added 15 volumes of cold ether. The white or slightly yellowish precipitate (fraction 4) was collected and dried overnight *in vacuo*. Preliminary chemical examination showed that fraction 4 gave a positive Liebermann-Burchard reaction, and contained 70—75% of hexose (glucose equivalent) which was determined by the Tsugita-Akabori method.[8] This fraction also contained 91.7% saponin as detected by vanillin-H_2SO_4 reaction.[9]

Extraction of Total Lipid and Determination of Radioactivity——Liver and epididymal adipose tissue were quickly removed after decapitation of the rat, cooled on ice, and weighed. The adipose tissue was placed immediately in 20 ml of $CHCl_3$–MeOH mixture (2: 1, v/v). The cooled liver was homogenized with 3 volumes of ice-cold 0.9% NaCl solution in a Potter-Elvehjem type glass homogenizer with a Teflon pestle. The homogenate was filtered through 4 layers of gauze, and 1 ml of the homogenate was mixed with 20 ml of $CHCl_3$–MeOH (2: 1, v/v). Total lipid was extracted from both tissues by shaking. The respective residual tissues were then removed, and the $CHCl_3$–MeOH solution partitioned and washed by the method of Folch, *et al.*[10] The organic solution was evaporated and the residue was dried over P_2O_5 overnight. The concentration of total lipid was determined by gravimetry and its radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer, Model 3003 after the dried residue was suspended in 10 ml of the scintillator (0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis[2-(5-phenyloxazolyl)]benzene in toluene).

Cholesterol in Blood Serum——Cholesterol was determined by a modification of the method of Zak-Henly.[11]

Triglycerides in Blood Serum——Triglycerides was determined by the method of Fletcher,[12] using a kit supplied by Wako Chemicals Co., Ltd., Osaka.

Blood Sugar——Blood sugar was determined by the method of Momose, *et al.*[13]

Liver Glycogen——A portion of the liver (200—300 mg) was digested with 3 ml of 1N NaOH in a boiling water for 40 min and glycogen was precipitated by the addition of 2 ml of EtOH and purified according to the method of Roe and Dailey.[14] Liver glycogen was determined by the anthrone–H_2SO_4 method, with glucose used as the standard.[15]

Results

Effect of Fraction 4 on Lipid Metabolism of Rat Liver

First, an attempt was made to determine the dose-response relation of graded doses of *ginseng* extract. The radioactivity of total lipid was determined 4 hr after treatment with fraction 4. As shown in Table I, it was found that the incorporation depended directly on the amount of fraction 4 administered to a rat.

TABLE I. Dose-Response Relationship of Fraction 4 on Lipid Synthesis in Rat Liver

Material	Dose (mg)	^{14}C-acetate (cpm/mg lipid)	%[a]
Control (saline)	—	52.2± 3.6	100
Fraction 4	2.5	94.4± 0.7	181
Fraction 4	5	98.2± 1.5	188
Fraction 4	10	105.9± 6.0	203
Fraction 4	20	120.4±10.3	231

Graded dose of fraction 4 was administered intraperitoneally to rats. Control animals were treated with an equal volume of saline. At 3.5 hr after treatment, ^{14}C-acetate (2.5 μCi/rat) was injected intraperitoneally and the animals were killed 30 min later by decapitation. Groups of 4 to 5 rats were used in each experiment. The data are presented as mean±S.E.
a) percent of control

7) H. Oura, S. Hiai, S. Nakashima, and K. Tsukada, *Chem. Pharm. Bull.* (Tokyo), **19**, 453 (1971).
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14) J.H. Roe and R.E. Dailey, *Anal. Biochem.*, **15**, 245 (1966).
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To obtain further evidence on the effect of fraction 4 on total lipid synthesis, the time course of the incorporation of ^{14}C-acetate into total lipid was followed. As shown in Table II, a significant increase was seen 4 hr after the treatment, but this increase seemed transitory, since this response showed only a 29% increase over the control animals 6 hr after the administration of fraction 4 and returned almost to the control level 8 hr after the treatment. On the contrary, lipid content in the liver showed no appreciable change (Fig. 1). Thus, further studies are needed to clarify the reason for an increase of the radioactivity in the liver.

Table II. Effect of Fraction 4 on Lipid Synthesis of Rat Liver

Time after fraction 4 treatment (hr)	^{14}C-acetate (cpm/mg lipid)	%[a]
Control (saline)	38.0±1.2	100
2	44.5±1.3	117
4	71.5±0.8	188
6	48.9±6.9	129
8	42.0±1.0	111
16	42.9±5.1	113

At the indicated time after intraperitoneal administration of fraction 4 (5 mg), ^{14}C-acetate (2.5 μCi/rat) was injected intraperitoneally and the animals were killed 30 min later by decapitation. Control animals were killed 4 hr after saline-treatment. Three rats in each group were used. The data are presented as mean±S.E.
a) percent of control

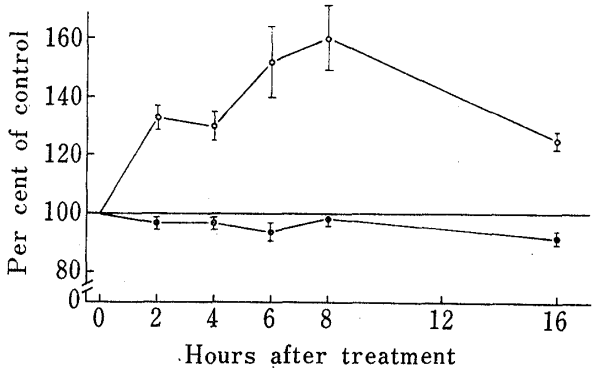

Fig. 1. Effect of Fraction 4 on the Lipid Content of Rat Liver and Epididymal Adipose Tissue

Experimental conditions are as given in Table II and III. Data are expressed as percentage of control group. —○—: epididymal adipose tissue; —●—: liver

Effect of Fraction 4 on Lipid Metabolism of Rat Epididymal Adipose Tissue

From the previous experiments, it was considered that fraction 4 plays an important role in the lipid metabolism of other tissues. To examine this, the effect of pretreatment on the incorporation of ^{14}C-acetate into total lipid was followed in epididymal adipose tissue. As

Table III. Effect of Fraction 4 on Lipid Synthesis of Rat Epididymal Adipose Tissue

Time after fraction 4 treatment (hr)	^{14}C-acetate (cpm/mg lipid)	%[a]
Control (saline)	11.4± 9.7	100
2	57.8±45.7	507
4	65.7±17.4	576
6	37.2±22.7	326
8	24.4±10.7	214
16	5.1± 3.4	45

At the indicated time after intraperitoneal administration of fraction 4 (10 mg), ^{14}C-acetate (2.5 μCi/rat) was injected intraperitoneally and the animals were killed 30 min later by decapitation. Control animals were killed 4 hr after saline-treatment. Groups of 3 to 4 rats were used in each experiments. The data are presented as mean±S.E.

a) percent of control

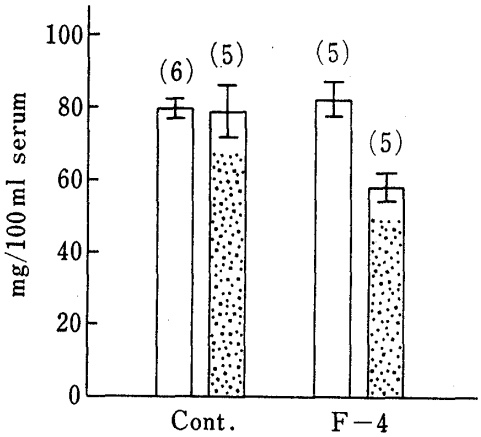

Fig. 2. Effect of Fraction 4 on Serum Lipids Level of Rats

Fraction 4 (10 mg) was administered intraperitoneally to rats. Control animals were treated with an equal volume of saline. At 4 hr after treatment, rats were killed by decapitation. Blood was collected in a conical centrifuge tube, allowed to clot at room temperature for 2—3 hr, and then centrifuged. The supernatant was obtained and cholesterol and triglycerides were determined. Vertical bars show standard errors. Numbers in parentheses are number of animals used.
□: cholesterol ▨: triglycerides

shown in Table III, a striking change was observed 2 hr after intraperitoneal administration of 10 mg of fraction 4. It reached the maximum increase 4 hr after the treatment, and the rate of synthesis returned gradually to the control level. However, a depression was observed 16 hr after treatment. This may suggest that *ginseng* extract exhibits a negative feed-back for lipogenesis.

Further, the lipid content extracted from epididymal adipose tissue was determined. The result was expressed as the lipid content in a pair of adipose tissue. As shown in Fig. 1, the maximum lipid content was observed 8 hr after fraction 4 treatment. Two and 4 hr after the treatment, there were 33 and 30% increase in the lipid content, respectively, and 16 hr after administration of fraction 4, there was 25% increase over the control animals.

Accordingly, the maximum increase in lipid content occurs later than a striking increase of the radioactivity.

Effect of Fraction 4 on the Concentration of Serum Cholesterol and Triglycerides

The result of above experiments poses the question of the functional significance of blood lipids. Therefore, we examined the effect of *ginseng* extract on the concentration of serum cholesterol and triglycerides. The results are shown in Fig. 2. Four hours after the intraperitoneal administration of 10 mg of fraction 4, *ginseng* extract did not affect serum cholesterol level, whereas it caused 26% decrease of serum triglycerides; the mean values in the control and fraction 4-treated group were 78.7 and 57.9 mg/100 ml, respectively.

Effect of Fraction 4 on Sugar Metabolism

In the preceding experiments, we elucidated the effect of *ginseng* extract on lipid metabolism. It has been shown that the extent of lipogenesis is closely linked to carbohydrate utilization. Therefore, we examined the effect of *ginseng* extract on sugar metabolism, and the effect of pretreatment with *ginseng* extract on blood sugar level was first assayed. As shown in Table IV, a slight decrease in blood sugar level was observed 2 hr after the intraperitoneal administration of *ginseng* extract, this tendency continued until 8 hr after treatment, and returned almost to the control level 14 hr after the treatment. A significant decrease in blood sugar level was observed in adrenalectomized rats when *ginseng* extract was administered.[5]

Determination of liver glycogen content indicated, as shown in Table V, a dramatic decrease 8 hr after the treatment and thereafter glycogen content returned to the control level.

Thus, reduction of carbohydrate was observed by the administration of *ginseng* extract. But, blood sugar level was only a slight change compared with that of glycogen content.

Table IV. Effect of Fraction 4 on Blood Sugar Level in Rats

Time after fraction 4 treatment (hr)	Blood sugar (mg%)		%[a]
	Control	Experiment	
0	137±3	—	
2	135±6	124±5	92
4	123±7	107±3	87
6	124±7	105±2	85
8	125±7	112±3	90
11	125±8	120±3	96
14	128±7	127±5	99

Rats were administered intraperitoneally 10 mg of fraction 4. Control animals were treated with an equal volume of saline. At the indicated time after treatment, rats were killed by decapitation. Blood was collected in a conical centrifuge tube, allowed to clot at room temperature for 2—3 hr, and then centrifuged. The supernatant was obtained and blood sugar was determined by the method of Momose, *et al.*[13] Five rats in each group were used. The data are presented as mean±S.E.

a) percent of control

TABLE V. Effect of Fraction 4 on Liver Glycogen Content in Rats

Time after fraction 4 treatment (hr)	Glycogen content (% of wet wt. liver)		%[a]
	Control	Experiment	
0	3.34±0.28	—	
2	2.66±0.05	2.51±0.04	94
4	2.38±0.11	2.18±0.19	92
6	3.25±0.25	2.32±0.21	71
8	2.62±0.22	1.45±0.23	55
12	2.31±0.12	1.83±0.11	79
16	3.23±0.47	3.70±0.19	115

Rats were administered intraperitoneally 10 mg of fraction 4. Control animals were treated with an equal volume of saline. At the indicated time after treatment, rats were killed by decapitation. Five rats in each group were used. The data are presented as mean±S.E.
a) percent of control

Discussion

It was found that *ginseng* extract had an effect on lipid and sugar metabolism when administered to rats *in vivo*. The results presented in Tables I and II indicate that *ginseng* extract accelerates the hepatic lipogenesis in normal fed rats, as judged by the incorporation of ^{14}C-acetate into total lipid. Sensitivity of hepatic lipogenesis is shown by determining the dose-response relation of graded doses of the *ginseng* extract. Our observations also suggest that the lipogenic capacity of epididymal adipose tissue is more sensitive to the extract than that of the liver in the maximum level. In addition, accumulation of lipid in adipose tissue was observed. From these results, it seems quite clear that the *ginseng* extract has a stimulating action on the two tissues, and these experiments have given some insight into the regulation of the lipogenic pathways.

On the other hand, lipogenesis by these tissues is markedly influenced by the nutritional status of the animals. Fasting of the animals results in the reduction of lipogenesis.[16] Upon refeeding, lipogenesis returns to the normal level.[17] Therefore, the effect of *ginseng* extract was determined from the viewpoint of the nutritional status of animals. In these experiments, starvation destroyed lipogenesis in the liver and adipose tissue, and upon refeeding, a significant stimulation of lipogenesis was observed.[18] These experimental results confirmed that the nutritional state of an animal is an important factor determining the effect of *ginseng* extract.

In addition, we have revealed the effect of *ginseng* extract on sugar metabolism. A slight decrease in blood sugar level was observed (Table IV). While there was a dramatic decrease in liver glycogen content (Table V). In recent years many investigations indicate that a number of physiological phenomena exhibit a rhythmic fluctuation. Watanabe, *et al.*[19] showed the presence of a circadian variation in rat liver glycogen content under controlled lighting conditions and feeding schedules. However, the effect of *ginseng* extract on liver glycogen content appeared legardless of circadian variation.

The experimental results described above suggested that *ginseng* extract turns the metabolic flow in the direction of lipogenesis by the conversion of sugar.

On the basis of the present evidence, we have summarized the sequential stimulation of biological action on lipid and sugar metabolism as a result of a single dose injection of *ginseng* extract in a rat. As shown in Fig. 3, the first phenomenon observed was a striking stimulation

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18) T. Yokozawa and H. Oura, unpublished data.
19) M. Watanabe, V.R. Potter, and H.C. Pitot, *J. Nutr.*, **95**, 207 (1968).

in the incorporation of radioactivity into the adipose tissue beginning 2 hr after the administration of *ginseng* extract. Almost simultaneously a slight decrease of blood sugar level was observed. These indicate that blood sugar plays a possible role in facilitating the re-esterification of free fatty acids in the adipose tissue. At this time, a change in hepatic lipogenesis was observed a little and later a temporary increase was observed only 4 hr after the treatment. In succession, a significant decrease in liver glycogen content was observed 6—8 hr after the administration of *ginseng* extract, while accumulation of lipid in adipose tissue was observed about the same time. From these observations, it may be concluded that *ginseng* extract brings about the accumulation of lipid in adipose tissue as a result of its stimulating action on lipid and sugar metabolism. The stimulating action by *ginseng* extract returned almost to the control level 16 hr after the treatment, with the exception of the lipid content in adipose tissue.

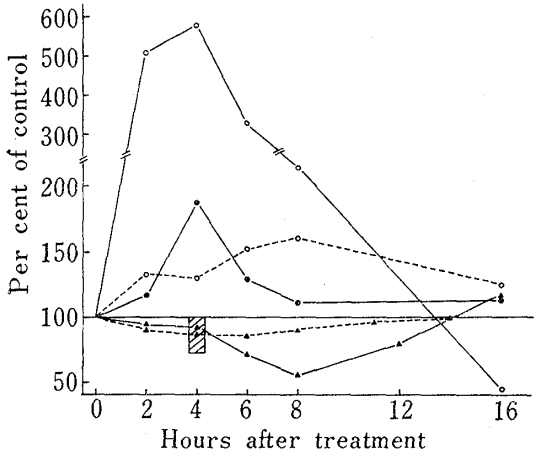

Fig. 3. Summary of Data on the Sequential Stimulation by *ginseng* extract (Fraction 4)

—○—: cpm/mg lipid, epididymal adipose tissue; —●—: cpm/mg lipid, liver; ---○---: lipid mg/adipose tissue; ▨: triglycerides mg/100 ml serum; ---▲---: blood sugar mg/100 ml serum; —▲—: glycogen content % of wet wt. liver.

Further, a decrease in the concentration of serum triglycerides was observed 4 hr after treatment with *ginseng* extract. It is generally accepted that lipoprotein lipase controls the hydrolysis of triglycerides of chylomicrons and lipoprotein to free fatty acids and glycerol before their utilization by tissues.[20~22] This observation raises the question of the functional significance of lipoprotein lipase.

Thus, we have clarified the metabolic correlation between liver and adipose tissue, with special reference to lipid and sugar metabolism. Further studies are in progress on the relationship between hormone and enzyme regulation.

Acknowledgement We express our gratitude to Mr. Y. Odaka for technical assistance.

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