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Effect of Ginseng Extract on Lipid and Sugar Metabolism. II. Nutritional States in Rats

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Investigations were carried out to determine whether the nutritional status of an animal changes the effect of ginseng extract. Incorporation of ^{14}C-acetate into total lipid and the glycogen content in the liver were determined 4 hr after the intraperitoneal administration of ginseng extract (fraction 4) to rats. The results indicated a striking stimulation of metabolism in rats fed a fat-free diet.

Previously, we found that ginseng extract showed a stimulating effect on lipid and sugar metabolism, and it was suggested that this effect might indicate a metabolic correlation between liver and adipose tissue.[2] We also found that starvation cancelled lipogenesis in the liver, and a significant stimulation of lipogenesis was observed on refeeding.[3] This prompted us to examine whether the nutritional status of the animals fluctuated the effect of ginseng extract in the liver, and its result is reported in this paper.

Materials and Methods

Animals and Diet——Male Wistar strain rats (120—130 g) were employed throughout the present experiment. The animals were housed in air-conditioned animal quarters kept as closely as possible at 25° and 60% relative humidity, with lighting regulated to give 12 hr of uniform illumination and 12 hr of darkness each day. Rats were fed either laboratory pellet chow or synthetic diet. A laboratory pellet chow was purchased from CLEA Japan Inc., Tokyo, and contained about 3.5% of fat. The composition of the synthetic diet is shown in Table I. Diet and water were available freely for animals throughout the experiments.

Table I. Composition of the Synthetic Diet

	High-fat diet (g)	Fat-free diet (g)
Casein	30	30
Dextrin	33	53
Sucrose	10	10
Salt mixture[a]	4	4
Vitamin mixture[b]	1	1
Cellulose powder	2	2
Choline chloride	0.1	0.1
Oil mixture[c]	20	—

a) Salt mixture obtained from Tanabe Pharmaceutical Co., Ltd., contains 29.29% $CaCO_3$, 0.43% $CaHPO_4 \cdot 2H_2O$, 34.31% KH_2PO_4, 25.06% NaCl, 9.98% $MgSO_4 \cdot 7H_2O$, 0.623% $Fe(C_6H_5O_7) \cdot 6H_2O$, 0.156% $CuSO_4 \cdot 5H_2O$, 0.121% $MnSO_4 \cdot H_2O$, 0.02% $ZnCl_2$, 0.0005% KI, 0.0025% $(NH_4)_6 Mo_7O_{24} \cdot 4H_2O$.
b) Vitamin mixture obtained from Tanabe Pharmaceutical Co., Ltd., contains 0.059% thiamine-HCl, 0.059% riboflavin, 0.294% nicotinic acid, 0.235% calcium pantothenate, 0.029% pyridoxine-HCl, 0.006% menadione, 0.001% biotin, 0.002% folic acid, 0.0002% vitamin B_{12}, 1.176% inositol, 0.588% ascorbic acid, 97.551% lactose.
c) Oil mixture obtained from Tanabe Pharmaceutical Co., Ltd., contains 80% soybean oil, and 20% cod liver oil.

1) Location: *3190 Gofuku, Toyama, 930, Japan.*
2) T. Yokozawa, H. Seno, and H. Oura, *Chem. Pharm. Bull.* (Tokyo), **23**, 3095 (1975).
3) T. Yokozawa and H. Oura, unpublished data.

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 root of *Panax ginseng* C.A. Meyer produced in Kumsan, Korea, as previously described.[4] 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.[5] This fraction also contained 91.7% saponin as detected by the vanillin–H_2SO_4 reaction.[6]

Extraction of Total Lipid and Determination of Radioactivity——Extraction of total lipid was described in the preceding paper.[2] 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).

Liver Glycogen——A portion of the liver (200—300 mg) was digested with 3 ml of 1 N 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.[7] Liver glycogen was determined by the anthrone–H_2SO_4 method, with glucose used as the standard.[8]

Table II. Effect of Fraction 4 on the Incorporation of ^{14}C-Acetate into Total Lipid of Rat Liver in Different Nutritional States

	Lab chow			18 hr fasted		
	Control	Fraction 4	%[a]	Control	Fraction 4	%[a]
Total lipid (mg/g liver)	56.8±0.8	61.2±3.6	108	62.0±1.2	60.4±1.2	97
Lipid synthesis indices						
(cpm/mg lipid)	47.5±3.3	81.3±2.1	171	29.9±5.2	26.5±1.3	89
(cpm/g liver)	2696±168	4948±152	184	1836±304	1600±92	87
(cpm/whole liver)	17450±1482	30216±2399	173	9073±1253	7951±753	88

	High-fat diet			Fat-free diet		
	Control	Fraction 4	%[a]	Control	Fraction 4	%[a]
Total lipid (mg/g liver)	75.6±3.2	72.8±2.4	96	66.4±0.8	64.4±0.8	97
Lipid synthesis indices						
(cpm/mg lipid)	33.4±4.7	36.0±2.6	108	113.7±6.8	262.0±24.9	230
(cpm/g liver)	2533±403	2695±187	106	7548±426	16873±1610	224
(cpm/whole liver)	17657±3783	19228±1715	109	43654±3907	106332±13835	244

Rats kept on above diets were given an intraperitoneal administration of fraction 4 (10 mg). 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 5 to 6 rats were used in each experiment. The data presented are mean ± S.E.
a) percent of control

Results

Effect of Fraction 4 on Lipid Metabolism of Rat Liver in Different Nutritional States

The effect of feeding on lipid synthesis is shown in Table II. The accelerating effect of ginseng extract on hepatic lipogenesis was confirmed in rats fed a laboratory pellet chow, and fasting drastically reduced that in the liver of a normal rat. This indicates that a nutritional state in the body may play an important role in regulating the lipogenic capacity of the ginseng extract. Table II lists lipid synthesis indices calculated on the basis of the incorporation

4) H. Oura, S. Hiai, S. Nakashima, and K. Tsukada, *Chem. Pharm. Bull.* (Tokyo), **19**, 453 (1971).
5) A. Tsugita and S. Akabori, *J. Biochem.* (Tokyo), **46**, 695 (1959).
6) S. Hiai, H. Oura, Y. Odaka, and T. Nakajima, *Planta Medica*, **28**, 363 (1975).
7) J.H. Roe and R.E. Dailey, *Anal. Biochem.*, **15**, 245 (1966).
8) N.V. Carroll, R.W. Longley, and J.H. Roe, *J. Biol. Chem.*, **220**, 583 (1956).

per mg of liver lipid, per g of wet liver, and per g of total wet liver. From this table it is apparent that there are no considerable difference in the effect of ginseng extract calculated from these three indices of lipid synthesis.

It is generally known that lipogenic capacity of the liver is related to the ingested fat.[9] Therefore, an attempt was made to check the influence of dietary fat on hepatic lipogenesis accelerated by the ginseng extract. Comparative data on hepatic lipogenesis of rats fed a high-fat and fat -free diet (see Table I) are shown in Table II. In the first experiment, rats were fed a synthetic diet containing 20% oil mixture for 5 days and, at the end of that time, they were injected intraperitoneally with the ginseng extract. Exactly 4 hr after the administration they were killed, and their liver was rapidly excised and assayed. Under this dietary condition, the ginseng extract showed a slight increase in the incorporation of ^{14}C-acetate into total lipid, but no significant difference existed between the control and fraction 4-treated group. Likewise, no difference in the lipid content was apparent in the two groups of animals.

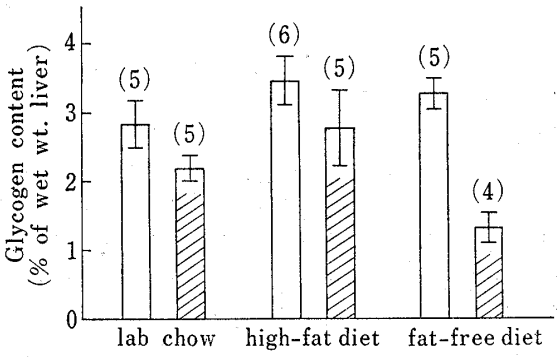

Fig. 1. Effect of Fraction 4 on the Glycogen Content of Rat Liver in Different Nutritional States

Rats were fed either laboratory pellet chow or synthetic diet for 5 days. At the end of the feeding period, each rat was given an intraperitoneal injection of fraction 4 (10 mg). Control animals were treated with an equal volume of saline. Rats were killed by decapitation 4 hr after the administration, and their liver was rapidly excised and assayed. Glycogen content is expressed as % of wet weight liver. Vertical bars show standard error. Numbers in parentheses are number of animals used.
□ control group; ▨ fraction 4-treated group

The experiments described so far were carried out with the liver of rats fed a high-fat diet. In the next experiment, the amount of oil mixture was decreased from 20 to 0%, by varying the level of dextrin in the diet from 33 to 53%. As shown in Table II, administration of the ginseng extract produced a significant increase over the control level, but did not alter the relative difference on the effect of ginseng extract calculated from these three indices of lipid synthesis. In addition, no change in the lipid content was observed in the control and fraction 4-treated group.

It may be considered that the stimulating action by ginseng extract is a matter of a response to the fat content of the diet, and excess fat feeding depressed the total utilization of ^{14}C-acetate by the liver. In addition, it is necessary to consider the possibility that the lower ^{14}C recovery reflects a greater dilution of the injected ^{14}C in the liver of the high fat-fed animals.

Effect of Fraction 4 on the Glycogen Content of Rat Liver in Different Nutritional States

It is also known that the glycogen content of rat liver is altered over a wide range by varying the antecedent diet.[10] Therefore, it seemed of interest to investigate whether or not difference in dietary fat could modify the effect of ginseng extract on liver glycogen content. The results of this experiment are presented in Fig. 1. The observed effect of ginseng extract in the normally fed rats agrees with a previous data.[2] Fig. 1 shows, in addition, that a decrease of liver glycogen content by ginseng extract in rats fed the fat-free diet was more marked than that in the high-fat diet groups. These data, in conjunction with the preceding experiments on the incorporation of acetate[1-^{14}C] into total lipid, lends support to the view that glycogen utilization is linked in some manner to the extent of lipogenesis.

9) J.C. Bartley and S. Abraham, *Biochim. Biophys. Acta*, **280**, 258 (1972).
10) T. Kimura and K. Ashida, *Agr. Biol. Chem.* (Tokyo), **32**, 1440 (1968).

Discussion

It was shown in our previous report that the administration of ginseng extract increased the incorporation rate of labeled precursor into total lipid in the rat liver. In addition, we have demonstrated a decrease in liver glycogen content.[2] In this paper, the factor influencing the effect of ginseng extract on the liver has been investigated.

A general procedure consisted of modifying the nutritional state of rats and then measuring the capacity of their excised liver. As shown in Table II, the lipogenic capacity of ginseng extract was observed in the experiments carried out with the liver of non-fasted rats. However, fasting almost completely cancelled capacity of the liver. This phenomenon is observed as early as 18 hr after food is withheld. It became clear that the elimination of food resulted in a loss of the ability of the liver to convert acetate carbon to total lipid. Further, it seemed of interest to see whether or not capacity of the liver for lipogenesis modified by ginseng extract is also regulated by dietary fat. For this, amount of fat in the diet used for these groups was raised at the expense of carbohydrate. Because of the importance of dietary carbohydrate in the maintenance of hepatic lipogenesis,[11] however, diets used in this study were rich in carbohydrate and, contained exactly 10% sucrose. Under the conditions employed in the experiments shown in Table II, a striking observation was the high level of lipogenesis in rats fed a fat-free diet. These findings led us to conclude that ginseng extract shifts the pattern of metabolism. This conclusion is fully borne out by the result obtained in the study shown in Fig. 1. Thus, this increased lipogenesis by the ginseng extract undoubtedly reflects the glycolytic pathway. The data in Table II further indicate that the effect of ginseng extract on hepatic lipogenesis was completely abolished by high-fat feeding. As a corollary, the failure in lipogenesis might be considered to result from the reduction in glycolytic activity. Indeed, the feeding of this diet did not influence the glycogen content of the liver by the administration of ginseng extract. In general, capacity of the liver for lipogenesis is regulated by hormones, particularly by insulin,[12] and a diet rich in fat has been shown to lower the insulin content of the pancreas.[13] In view of the role of this hormone in carbohydrate utilization we were led to consider the possibility that the depressed lipogenesis by the ginseng extract produced by fat feeding may have resulted from a decrease of available insulin.

Thus, these present results emphasize a remarkable similarity in hepatic lipogenesis between the high-fat fed and fasted rats and, furthermore, differentiate sharply between animals in these conditions and the fat-free fed diet.

In the experiment described above, rats were allowed free access to food at any time. However, rats are nocturnal and eat mainly at night. Consequently, food intake during the night increases. From these, it is considered that food intake, especially the fasting-and-eating cycle is significant in controlling the effect of ginseng extract. However, the stimulating action of ginseng extract on liver glycogen content was observed regardless of the cycle of food intake.[3]

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11) I. Lyon, M.S. Masri, and I.L. Chaikoff, *J. Biol. Chem.*, **196**, 25 (1952).
12) M.J. Osborn, I.L. Chaikoff, and J.M. Felts, *J. Biol. Chem.*, **193**, 549 (1951).
13) R.E. Haist, *Physiol. Rev.*, **24**, 409 (1944).