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The Action of Panax Ginseng on the Glucose Oxidation of the Rat Liver *in vitro**,**

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Panax Ginseng was investigated for its effect on carbohydrate and protein metabolism. Some investigators have discussed the hypoglycemic action and change of liver glycogen, while others have found the change of nitrogen excretion in urine and the decrease of serum protein level.(1)~(6)

Recently Petkov(7) has shown that Ginseng regulates carbohydrate metabolism in experimentally induced hyperglycemia, potentiates the action of insulin, and, in higher doses, has itself a hypoglycemic effect.

Therefore, in this study, the author have attempted to observe the action of Ginseng on the glucose oxidation of rat liver in vitro, and tried to find the passage of glucose of oxidation using glucose-1-C^{14} and glucose-6-C^{14}

In another way, it has been also tried to find that the in vitro action of Ginseng on the glucose oxidation in a thyroidectomized rat liver as compared with that in the normal rats.

Experimental Procedures

1. Treatment of Rats

Male rats of Sprague-Dawley and local strain, weighing 250 to 300 gm were used throughout the experiments.

Thyroidectomy was performed by subcutaneous injections of 0.1, 0.5 mc I^{131} respectively, (8)~(10) and the rats had been fed for 60 days before they were sacrificed and then nothing was fed to them for 12 hours before the experiment except in the first group of I^{131} thyroidectomized rat in the experiment.

2. Incubation Procedures:

The rats were sacrificed by decapitation and their liver was quickly excised. Each liver was placed in a Petri dish containing cold Krebs-Ringer phosphate or Krebs-Ringer bicarbonate buffer, and sliced free hand with razor blade. Then slices were gently blotted, weighed and transferred to incubation flask, 300-500 mg per flask.

Each flask contained Krebs-Ringer buffer, 0.5 μc of glucose-1-C^{14} or glucose-6-C^{14} with 1mg per ml. of carrier glucose. Besides, certain amount of Ginseng extract was added and the total volume of incubation media was adjusted to 4.0 ml.

3. Analytical Procedures

The collection and determination of carbon dioxide ($C^{14}O_2$) followed the method of Lyon et al. (11)-(13)

Incubation flask of 25 ml. with center well and capped rubber stopper was put in water bath adjusted to 37 degree C, and shaked for one to two hours with wrist-action shaker. The gas phase was pure oxygen in phosphate buffer and 95% oxygen and 5% carbon dioxide in bicarbonate buffer.

Incubation was terminated by injecting 10N sulfuric acid, and carbon dioxide ($C^{14}O_2$) was collected in 20% KOH introduced into the central wells.

4. Measurements of Radioactivity

All radioactivities of carbon dioxide ($C^{14}O_2$) were determined as barium carbonate, collected as disks on filter paper mounts. The counting of disks was

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performed in the end window G. M. tube for a sufficient length of time to insure a probable error of less than five percent. Counts were corrected for thickness and normalized to 100 mg of fresh liver slices.

5. Preparation of Reagents

1% Ginseng solution was prepared from ethanol extracted Panax Ginseng. This solution was put in 4 deg. C refrigerator for several days, filtered, discarded the insoluble precipitates and kept in 4 deg. C refrigerator for the experiment.

Glucose-1-C^{14}, glucose-6-C^{14}, glucose-(U)-C^{14} and I^{131} were obtained from The Radiochemical Centre, England.

Results

1. Effect of Ginseng Extract on Glucose Oxidation in the Normal Rat Liver

Table 1. Panax Ginseng stimulation of carbon dioxide production by liver slices.

Each flask contained 4ml. of phosphate buffer, 1 mg per ml. of carrier glucose and 0.5 μc of glucose-1-C^{14}

Results are the average of closely agreeing duplicate.

Concentration of Panax Ginseng	0.01mg (%)	0.05mg (%)	0.25mg (%)	0.50mg (%)	1.0mg (%)
	8.5	10.1	28.9	35.7	—
	18.7	19.1	13.7	20.1	—
	6.9	—	—	8.1	—
	—	—	26.8	35.3	—
	—	—	—	32.3	87.0
	—	—	—	34.0	35.9
Average	11.2	14.6	21.8	27.8	36.9

Table 2. Panax Ginseng stimulation of carbon dioxide production by liver slices.

Each flask contained 4 ml. of bicarbonate buffer, 1 mg per ml. of carrier glucose and 0.5 μc of glucose-1-C^{14} and glucose-6-C^{14}

Results are the average of closely agreeing duplicate.

No. of rat	Glucose	0.1 mg/ml of Panax Ginseng	1.0mg/ml of Panax Ginseng
1	G-1-C14	18.5	103
	G-6-C14	6.4	6.4
2	G-1-C14	22.9	120.7
	G-6-C14	25.0	110.0
3	G-1-C14	2.4	80.3
	G-6-C14	4.7	65.7
4	G-1-C14	12.1	83.4
	G-6-C14	—	66.7
5	G-1-C14	37.9	78.0
6	C-1-G14	—	96.7
	G-6-C14	8.2	23.3
7	G-1-G14	—	99.0
	C-6-C14	—	36.3
8	G-1-G14	—	120.4
	C-6-C14	—	21.0
Average	G-1-G14	18.8	98.3
	C-6-C14	11.1	47.1

The action of Ginseng was observed in comparison with the effect of this agent on liver slices. The results of various amount of Ginseng extract added to rat liver slices are shown in Table 1 and 2. The data, indicated in the tables, were calculated in the percentage of control activity.

In the first experiment (Table 1), the production of $C^{14}O_2$ was determined for 60 minutes incubation periods and glucose-1-C^{14} was used as substrate.

There was a definite increase of $C^{14}O_2$ production in the concentration of 0.01 mg Ginseng extract and the effect of this agent was much more pronounced in higher concentrations. It could be observed that the effect of Ginseng extract was roughly proportional with its concentration as the usual manner of drug action.

Furthermore, the stimulation of $C^{14}O_2$ production by Ginseng extract was compared with different kind of radioactive glucose which was used as substrate.

In Table 2, these data in 0.1 and 1.0 mg per ml. concentration of Ginseng extract are shown. The effects were more prominent in this experiment than the previous one, and also the difference of oxidation effect caused by the radioactive carbon position in glucose was observed.

In the concentration of 0.1 mg Ginseng extract, the effect of glucose-1-C^{14} was 18.8 percent while the effect of glucose-6-C^{14} was 11.1 percent as compared with controls. This differences were more pronounced in the concentration of 1.0 ml. Ginseng extract, i.e., the effect in glucose-1-C^{14} was 98.3 percent and that in glucose-6-C^{14} was 47.2 percent. It is assumable that this result would be related with the pathway of glucose oxidation, since the glucose-6-C^{14} does not produce carbon dioxide($C^{14}O_2$) in the oxidation through hexose monophosphate shunt.

2. Effect of Thyroidectomy by I^{131} on the Glucose Oxidation and the Action of Ginseng Extract on the Glucose Oxidation of Thyroidectomized Rat Liver

Table 3. Oxidation of glucose-1-C^{14} into carbon dioxide in the normal and I^{131} thyroidectomized rat liver slices.

Each flask contained 4 ml. of buffer, 1 mg per ml. of carrier glucose and 0.5 μc of glucose-1-C^{14}.

Results are the average of closely agreeing duplicate.

Experiment	Control rats	0.1mc of I^{131} treated rats	0.1mc of I^{131} treated rats
	cpm/100mg tissue	cpm/1000mg tissue	cpm/100mg tissue
1	833	477	309
	269	440	515
	377	352	483
	355	486	212
	328	—	—
Average	271.9	255.9	195(0
2	146	210	184
	265	312	244
	324	194	199
	364	208	169
	286	307	155
	246	291	189
	—	256	227
	—	266	193
Average	271.9	225.9	195.0

Table 4. Comparative effects of Panax Ginseng on glucose-1-C^{14} oxidation into carbon dioxide($C^{14}O_2$) in the normal and I^{131} thyroidectomized rat liver slices.

Each flask contained 4 ml. of buffer, 1 mg per ml, of carrier glucose and 0.5 μc of glucose-1-C^{14}.

Results are the average of closely agreeing duplicate.

Concentration of Panax Ginseng	0.1mg (%)	0.5mg (%)
Control group	5.7	60.3
	—	14.4
	7.5	60.0
	16.8	91.2
	13.8	29.1
	39.0	82.7
	—	51.3
	—	40.2
	—	65.1
	147.8	90.3
Average	38.4	64.2
Thyroidectomized group with 0.1mc of radioiodine	39.9	55.2
	—	19.2
	9.9	27.0
	10.2	55.2
	5.1	69.0
	—	51.6
	—	50.1
	19.8	79.8
	19.8	120.8
	—	52.8
	—	72.9
Average	17.7	59.4
Thyroidectomized group with 0.5mc of radioiodine	17.1	31.5
	17.1	20.1
	2.7	22.2
	52.9	—
	4.8	42.9
	16.8	29.4
	—	30.0
	—	47.4
	—	85.5
	—	39.0
	—	20.4
Average	16.8	36.4

As illustrated in the experimental methods, rats were treated with large amount of raioiodine I^{131} and the hypothyroidism status were obtained after the lapse of two months.

The experimental data are illustrated in Table 3, in which the rates of glucose oxidation were decreased by the thyroidectomy though the individual variances were remarkable. It would be difficult to evaluate these differences statistically; however, the tendency of diminished carbon dioxide($C^{14}O_2$) production by the thyroidectomy was clear. The similar results could be seen in the action of Ginseng in these animals. As shown in Table 4, carbon dioxide($C^{14}O_2$) production of the Ginseng extract was diminished in the thyroidectomized animals. And animals treated with 0.5mc of I^{131} have shown more remarkable diminishing effect than animals treated with 0.1mc of I^{131}.

Any significant differences could not be determined in statistically also in this experiment, but the tendency could be observed in the result and it indicates that the difference was more prominent than that in usual glucose oxidation of liver slices without Ginseng extract.

Discussion

The finding that Ginseng stimulates the glucose oxidation in liver slices in vitro is a further evidence of the role of Ginseng in carbohydrate metabolism. Effects of Ginseng on the carbohydrate metabolism have been demonstrated by the several methods. Especially the action of Ginseng on blood sugar was the most interesting problem, and has been investigated by many workers.

Petkov[7] and Yamada[6] have reported the hypoglycemic action of Ginseng in high doses, but the action was prominent in hyperglycemia induced by

adrenaline and alloxane. Petkov has shown that Ginseng potentiate the action of insulin and Yamada reported that Ginseng does not have curative effect on alloxane diabetes in regard to contents of blood residual nitrogen and urinary ketone body. This would indicate that the action of Ginseng on the carbohydrate metabolism has different nature from that of insulin. Also it does not have remarkable hypoglycemic action as compared with insulin.

In another way, the action of Ginseng on the body weight, body temperature, and basal metabolic rate were discussed. Hahn[14] observed that Ginseng has the tendency of decreasing body weight continuously, and Yamada[6] observed initial decrease of body weight in the rabbits treated with Ginseng for 30 days. With this phenomenon, it would be possible to assume that Ginseng act on carbohydrate or protein metabolism either way. Also Yoshida[16] reported that Ginseng increases total nitrogen amount in urine while Yamada[6] observed that Ginseng decreses the amount of serum protein and albumin globulin ratio in the initial phase of treatment.

On the otherway, Hahn et al.[14] observed the tendency of Ginseng to increse the basal metabolic rate and Oh et al.[15] reported that Ginseng antagonizes against the decreased body temperature by pentobarbital, reserpine and chlorpromazine. In addition to this, increased oxygen consumption of in vitro slices by Ginseng were observed by Joun[17] recently.

The results reported here suggest that the part of pharmacological effect of Ginseng on the metabolism would be related to a direct stimulation of glucose oxidation.

As illustrated by many investigators, glucose decomposes through several ways, Emden Myerhoff pathway and tricarboxylic cycle, hexose monophosphate shunt and glucuronic acid pathway. In the experiment reported here, different effect of carbon dioxide($C^{14}1O_2$) production due to Ginseng was observed when glucose-1-C^{14} or glucose-6-C^{14} are used as the substrate glucose.

Glock et al.[18] have shown that the formation of carbon dioxide($C^{14}O_2$) from glucose -6-C^{14} is significantly reduced whereas that from glucose-1-C^{14} is only slightly reduced in the liver of alloxane diabetic rats, while the treatment of insulin on these animals has partially reversed the change mentioned above by increasing carbon dioxide($C^{14}O_2$) production from both glucose-1-C^{14} and glucose-6-C^{14}. Also they reported that insulin increased carbon dioxide($C^{14}O_2$) production more remarkably from glucose-1-($C^{14}O_2$) than that from glucose-6-C^{14}. And the production of carbon dioxide ($C^{14}O_2$) from glucose-6-C^{14} has been significantly increased in the rat liver in which animals were treated with thyroxine.

It is possible to compare these results with the results of the experiment which was performed with Ginseng extract and reported here. Ginseng extract stimulates both glucose-1-C^{14} and glucose-6-C^{14} significantly in the appropriate concentration but the carbon dioxide($C^{14}O_2$) production from glucose-1-C^{14} was much more pronounced than that from glucose -6-C^{14}. This phenomenon is similar to the action of insulin on the diabetic animals liver and the action of some anterior pituitary hormones on the mammary gland.[12] [13]~[20]

In the animals of thyroidectomy by the radioiodine I^{131}, the glucose oxidation is rather decreased as compared with that of the normal animals. It would be expected by the phenomenon that the thyroxine treatment increased the production of carbon dioxide ($C^{14}O_2$), but these data do not show remarkable difference as that from hyperthyroidism. However, the effect of Ginseng on the glucose oxidation of those rat liver is more significant than that of normal ones.

Those differences are observed more remarkably in the rats liver to which 0.5 mc of I^{131} are administered than the group treated with 0.1 mc of I^{131}. Since effect of thyroxine on the liver has different nature in comparison with Ginseng, it has a prominent effect on the Ginseng action.

By the mechanism which thyroidectomized animals have lesser effect to the action of Ginseng, it would be possible to explain that the action of Ginseng on the glucose oxidation need certain amount of thyroid hormone for the complete effect.

Summary

1. Various concentrations of Ginseng extract increases the oxidation of glucose-1-C^{14}, -and glucose-6-C^{14}

in rat liver slices. Such effects are more prominent in Krebs-Ringer bicarbonate buffer than in Krebs-Ringer phosphate buffer,

2. Carbon dioxide production from glucose-1-C^{14} is much more pronounced than that from glucose -6-C^{14}.
3. Production of carbon dioxide is decreased in the hypothyroid status which induced treating animals with large amount of radioiodine(I^{131}).
4. The effect of Ginseng on the glucose oxidation is more decreased in the liver slices obtained from animals of hypothyroid status than that from the normal animals.

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국 문 초 록

흰쥐 간의 포도당 산화작용에 대한 인삼의 작용

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강 수 상

인삼이 탄수화물 및 단백대사에 어떠한 영향을 미친다는 것은 여러가지로 검토되었는데 그 가운데서도 혈당치를 감소시킨다던지 insulin과 관계가 있다는 일과 결부시켜서 저자는 invitro에서 흰쥐의 간(肝) 절편에 대한 인삼의 효과를 관찰 하였다.

즉 정상 혹은 대량의 방사성옥소(I^{131})를 투여하여 갑상선 기능을 저하시킨 흰쥐의 간절편을 완충액에 넣어서 반응시켰으며 방사성 포도당인 glucose-1-C^{14} 및glucose-6-C^{14}에서 생성된 $C^{14}O_2$를 $BaC^{14}O_3$로 하여 방사능을 측정하였다 이때 인삼의 효과는 인삼의 alcohol 추출액을 완충액에 첨가 하여 비교 관찰 하였다.

실험에서 얻은 성적은 다음과 같다.

1) 각종 농도의 인삼 추출액은 in vitro에서 흰쥐 간(肝)의 포도당 산화작용을 촉진시켰으며 이와 같은 작용은 인산완충액에서 보다 중탄산 완충액에서 더 현저 하였다.
2) 인삼 추출액에 의한 $C^{14}O_2$ 산생물은glucose -6-C^{14}에서 보다. glucore-1-C^{14}에서 더 현저하게 나타났다.
3) 대량의 방사성옥도 (I^{131})의 투여로 동물을 저갑상선 기능 상태로 할때는 $C^{14}O_2$의 생산이 감소 되었다.
4) 저 갑상선 기능상태에서는 정상상태에서 보다 인삼에 대한 간(肝)조직 $C^{14}O_2$ 산생물의 효과가 감소 되었다.

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