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Effect of Radix Ginseng Extract on Cytoplasmic Polysome in Rat Liver[1]

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It was shown that a single intraperitoneal injection of extract (fraction 4) from roots of *Panax ginseng* C.A. Meyer, increased the incorporation rate of labeled precursor into cytoplasmic polysomal ribonucleic acid (RNA) of rat liver. The content of heavy polysomes (over hexamer) in postmitochondrial supernatant from liver treated with fraction 4 was observed to increase at 6 and 10 hr after treatment. Sedimentation analysis of ribosomal RNA and messenger RNA derived from polysomes showed that the newly induced RNA by fraction 4 is evenly distributed over all RNA. Particularly, 29S ribosomal and 9—10S messenger RNAs were stimulated significantly. In an amino acid incorporation system *in vitro*, stimulatory activities of microsomes and polysomes from treated rat liver are more active by 85 and 67% than those from normal liver, respectively.

In previous paper, we reported that extracts from roots of *Panax ginseng* C.A. Meyer, had an action to increase the rate of the synthesis of liver nuclear and cytoplasmic polysomal ribonucleic acid (RNA) in rat.[3] The synthesized nuclear RNA was identified with ribosomal and desoxyribonucleic acid (DNA)-like messenger RNA.[4] It was also shown that the administration of *ginseng* extract increased the activity of RNA polymerase of nuclei of rat liver.[5] Furthermore, intraperitoneal administration of *ginseng* extract was shown to cause an increase in the rate of synthesis of serum proteins such as albumin and γ-globulin.[6]

Recently some hormone have been shown to stimulate protein synthesis in target organs as a consequence of prior increase of RNA synthesis.[7,8] It was found with testosterone acting on the seminal vesicle,[9] estrogen on the uterus,[10,11] and thyroid hormone on the liver[12] that the hormonal stimulation of cytoplasmic protein synthesis was accompanied by the appearance of new ribosome, although acceleration of nuclear RNA synthesis occurred much earlier. Therefore, it has been considered that many homones stimulate the synthesis of ribosomal and messenger RNA, and increase the content of heavy polysomes in cytoplasm, and the cytoplasmic protein synthesis is regulated by newly formed ribosomes and messenger RNA after stimulation of nuclear RNA synthesis.[13–15]

1) This work was presented at the 86th Annual Meeting of the Pharmaceutical Soceity of Japan, Sendai, October 1966.
2) Location: *3190 Gofuku, Toyama, 930, Japan.*
3) H. Oura, S. Hiai, S. Nakashima, and K. Tsukada, *Chem. Pharm. Bull.* (Tokyo), **19**, 453 (1971).
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8) J.R. Tata, "Progress in Nucleic Acid Research and Molecular Biology," Vol. 5, Academic Press, Inc., New York, 1966, p. 191.
9) W.D. Wicks and F.T. Kenney, *Science*, **144**, 1346 (1964).
10) R.J. Moore and T.H. Hamilton, *Proc. Natl. Acad. Sci. U.S.*, **52**, 439 (1964).
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13) A. Korner, *Biochem. J.*, **92**, 449 (1964).
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As described previously,[3] the administration of *ginseng* extract increased the incorporation rate of labeled precursor into polysomal RNA. The present studies were carried out to define further the effect of *ginseng* extract (fraction 4) on microsomes, polysomes and cytoplasmic protein synthesis in rat liver.

Material and Method

Animals and Chemicals——Male wistar rats weighing about 100 g were used for all the experiments. Rats were obtained locally and fasted for 16—18 hr prior to the experiments. 5-^{3}H-Orotic acid (5 mCi/3.49 mg) and U-^{14}C-L-leucine (0.1 mCi/0.0558 mg) were obtained from the New England Nuclear Corp., U.S.A. ^{32}P-Orthophosphate (54.5 Ci/mg P) was the product of the Radiochemical Centre, England.

Extraction and Partial Purification of Radix Ginseng——Fraction 4 was prepared from the extract of roots of *Panax ginseng* C.A. MEYER produced in Kumsan, Korea, as previously described.[3] 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 ammonium sulfate. The precipitate was dissolved in distilled water, dialyzed against running cold water to be salt-free, and then the inside solution was lyophilized (fraction 3). Fraction 3 was extracted with 99% MeOH on a water bath under refluxing, 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 dried overnight *in vacuo*. Preliminary chemical examination of fraction 4 gave positive Liebermann-Burchard reaction, and it contained 70—75% of hexose by the Tsugita and Akabori's method.[16] Mp 180—190°.

Polysomal RNA Labeling——Fraction 4 (0.5 ml of saline solution) was given to rats intraperitoneally. Control rats were treated with an equal volume of saline. At 4 hr after treatment, except for the designated time experiment, ^{3}H-orotic acid (20 μCi/rat) was injected intraperitoneally and the animals were killed 1.5 hr later by decapitation. In the labeling of ^{32}P-orthophosphate, the rats were given fraction 4, saline solution of ^{32}P-orthophosphate was administered intraperitoneally 3.5 hr later, and the animals were killed 2.5 hr later by exsanguination after cutting the abdominal aorta under ether anesthesia.

Preparation of Cell-Free Fractions——Three rats were used in one group. Liver was removed quickly, weighed, minced with scissors, and homogenized with Littlefield's Medium A[17] (0.25M sucrose, 0.025M KCl, 0.005M $MgCl_2$, and 0.05M Tris-HCl buffer, pH 7.6), 2.5 ml/g tissue, in a Potter–Elvehjem device with 8 strokes of a motor-driven Teflon pestle in a cold room. The supernatant fluid obtained after removing unbroken cells, debris, nuclei, and mitochondria by centrifugation at 20000 $\times g$ for 10 min was clarified by the addition of sodium deoxycholate at a final concentration of 1.3%. This deoxycholate-treated 20000 $\times g$ supernatant was used (i) to analyze the heavy polysome content by sucrose density gradient centrifugation and (ii) to separate the highly purified polysomes by the method of Wettstein, *et al.*,[18] with two layers of 2.0M and 0.5M sucrose solutions containing Medium A salt buffer.

Microsome fraction was prepared by centrifugation of the 20000 $\times g$ supernatant without the addition of deoxycholate at 105000 $\times g$ for 1 hr at 2—4°.

Polysome Content of Sucrose Density Gradient Analysis——The deoxycholate treated postmitochondrial supernatant was analyzed by sucrose density gradient centrifugation by layering it over 22.5 ml of 0.3—0.8M linear sucrose density gradient with a cushion of 5 ml of 2.0M sucrose solution containing Medium A at the bottom of the cellulose tube. After centrifugation for 2.5 hr at 25000 rpm and 2—4°, the bottom of the tube was punctured with a hypodermic needle, and absorbance was analyzed continuously in a flow-through cuvette (at 254 mμ by Uvicord) attached to a strip chart recorder (New York Photo Volt Co.) as described in a previous paper.[4]

Distribution and Incorporation Pattern of Purified Polysomes——Highly purified polysomes obtained from postmitochondrial supernatant were suspended in Medium A salt buffer, and then layered over 27.5 ml of 0.3—1.0M convex sucrose density gradient containing Medium A salt buffer. After centrifugation at 25000 rpm for 2.5 hr and 2—4°, absorbance was analyzed continuously by Uvicord and fractions of 1.25 ml were collected by a fraction collector. The radioactivity of each fraction was determined by the procedure of Staehelin, *et al.*[19] in a Packard Tri-Carb liquid scintillation spectrometer, model 3003.

Zone Centrifugation of Polysomal RNA——The RNA components were liberated by adding SDS (U.S.P. grade from Difco Co.) to the polysomes suspended in distilled water to give a final concentration of 0.5%. After a short (20 sec) heating of the mixture to 37°, this mixture was layered on top of a pre-cooled 0.5—1.0M linear sucrose gradient, and centrifuged for 40 hr at 24000 rpm at 2—4°. The gradient contained 0.005M

16) A. Tsugita and S. Akabori, *J. Biochem.* (Tokyo), **46**, 695 (1959).
17) J.W. Littlefield and E.B. Keller, *J. Biol. Chem.*, **224**, 13 (1957).
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19) T. Staehelin, F.O. Wettstein, H. Oura, and H. Noll, *Nature*, **201**, 264 (1964).

Tris-HCl buffer, pH 7.2. Fractions of 1.25 ml collected from gradients were assayed for radioactive RNA and sedimentation coefficients were determined essentially by the procedure of Staehelin, *et al.*[19]

Incorporation of Amino Acid into Protein *in Vitro*——Assay mixture contained, in 0.5 ml; 20 μmoles Tris-HCl buffer, pH 7.8; 1.5 μmoles $MgCl_2$; 1.0 μmole mercaptoethanol; 0.5 μmole ATP; 0.2 μmole GTP; 5 μmoles phosphoenolpyruvate; 10 μg pyruvic kinase; 0.1 μCi U-^{14}C-L-leucine (5 μCi per μmole); 0.13 μmole of each of 19 amino acids (leucine omitted); 3 mg soluble protein prepared from normal rat liver 105000 $\times g$ supernatant (without the addition of sodium deoxycholate) and microsome or polysome fractions. This mixture was incubated for 10 min at 37°, the reaction was stopped by the addition of 5% trichloroacetic acid, and the radioactivity of protein was determined by the procedure of Staehelin, *et al.*[19]

Protein Determination——The protein of microsome and polysome were assayed by Lowry's method.[20]

Result

Time Course of the Effect of Fraction 4 on the Polysomal RNA Synthesis

In previous experiments,[3] we reported that the partial purification of extracts from Radix Ginseng was carried out by the index of the increase in the incorporation rates of labeled precursors into nuclear RNA and cytoplasmic polysomal RNA. It was found that the incorporation rate was directly dependent on the amount of fraction 4 administered to a rat, and the administration of 0.5 mg of fraction 4 increased the incorporation rate by 48% in polysomal RNA at 5.5 hr after treatment over the control level. Also the administration of 5 mg of fraction 4 showed 130% increased rate in polysomal RNA and 134% in nuclear RNA over the control animals. To obtain further evidence on the effect of fraction 4 in polysomal RNA synthesis, the effect of pretreatment on the incorporation of ^{3}H-orotic acid into polysomal RNA was assayed at 1,2,4,6,10, and 24 hr. Groups of normal and fraction 4-treated rats were injected intraperitoneally with ^{3}H-orotic acid at designated time, the animals were

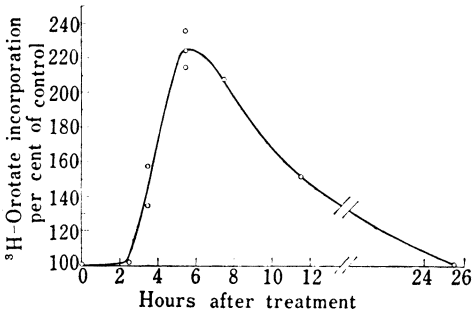

Fig. 1. Time Course of Effect of Fraction 4 Treatment on the Incorporation of ^{3}H-Orotic Acid into Cytoplasmic Polysomal RNA

Fraction 4 (5 mg) was administered intraperitoneally to rats at the designated time, ^{3}H-orotic acid (25 μCi/rat) was injected intraperitoneally to animals, and the animals were killed 1.5 hr later by decapitation. The purified polysomes were prepared, and the specific radioactivity (cpm/absorbance unit at 260 mμ) was assayed. The control values are 262 ± 18 cpm/absorbance unit polysomal RNA. For each period of time tested for fraction 4 action, separate control experiments were performed. The control values given here at zero hr for specific polysomal RNA activity represent the mean for 5 control groups (*i.e.*, five for each-time perod of fraction 4 action tested). The data are expressed as percentage of control value. Three rat livers were pooled for each experimental or control group.

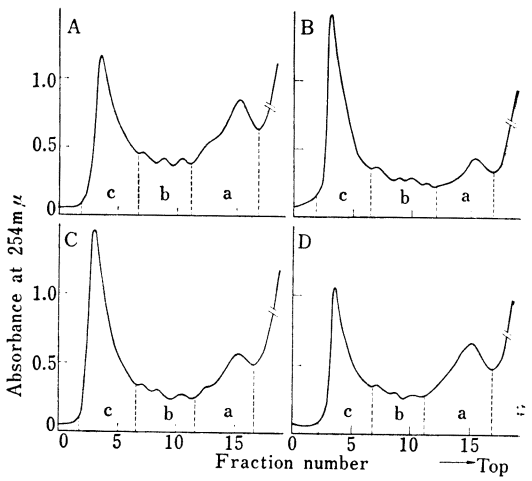

Fig. 2. Sucrose Density Gradient Patterns Illustrating the Increase of Liver Polysomes after Injection of Fraction 4 to Rats

A) control (saline) input: 0.3 ml postmitochondria supernatant corresponds to 0.5 g of wet weight liver tissue; centrifugation time: 2.5 hr at 25000 rpm
B) intraperitoneal injection of 5 mg of fraction 4 and sacrificed 6 hr after injection; input and centrifugation as in (A)
C) intraperitoneal injection of 5 mg of fraction 4 and sacrificed 10 hr after injection; input and centrifugation as in (A)
D) intraperitoneal injection of 5 mg of fraction 4 and sacrificed 16 hr after injection; input and centrifugation as in (A

20) O.H. Lowry, N.J. Rosenbrough, A.L. Farr and R.L. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

killed 1.5 hr later, and the polysomes were isolated. The specific radioactivity of polysomal RNA was determined by absorbance unit at 260 mμ. Fig 1 shows the effect of fraction 4 pretreatment on the incorporation of ^{3}H-orotic acid into polysomal RNA. From these data, it was evident that the greatest specific radioactivity of polysomal RNA was at 5.5 hr after treatment, and increased 124% over the control level by fraction 4 administration. At 3.5 and 11.5 hr after treatment, there were 39—59 and 53% respective increase in the specific radioactivity. Also, this response returned almost to the control level 25.5 hr after treatment.

These experimental results showed almost the same response pattern with the maximum radioactivity to nuclear RNA at 4 hr after treatment, as described in a previous paper.[4] These observations support the assumption that the nuclear RNA induced by *ginseng* extract treatment is transferred rapidly to the cytoplasm without accumulation in the nucleus. Also, this pattern is consistent with the conclusion drawn from kinetic analysis in various mammalian systems that cytoplasmic RNA is formed in the nucleus.[21]

Effect of Fraction 4 on the Polysome Content in Postmitochondrial Supernatant

Administration of fraction 4 has been observed to cause an increase of heavy polysomes and a decrease of monomer ribosomes in postmitochondrial supernatant of rat liver, as shown in Fig. 2. Since these effects might be an expression of changes in the cellular protein synthesis, the effect of fraction 4 administration on the heavy polysomes was studied periodically. The polysome profile from postmitochondrial supernatant was examined by sucrose density gradient centrifugation as described in Methods at 6,10, and 16 hr after treatment. Content of polysomes (hexamer or more) was found to be 35% of the total absorbance applied on the gradient in the normal liver. They increased to 49% at 6 hr after fraction 4 treatment (Table I). On the other hand, at the region of monomer and dimer, they showed dramatic decrease from 42% to 29% as measured by ultraviolet (UV) absorbance at 254 mμ. The same tendency continued until 10 hr after treatment on the polysome content. At 16 hr after treatment, however, these patterns returned almost to the control profile at region of monomer, dimer, and heavy polysomes.

Table I. Population of Polysomal Size of Rat Liver Treated with Fraction 4

Time after Fraction 4 treatment (hr)	Aggregate number of ribosomes		
	(a) 1—2 (%)	(b) 3—5 (%)	(c) 6 over (%)
0 (control)	42	23	35
6	29	22	49
10	31	18	51
16	44	18	38

Fraction 4 (5 mg/rat) was administered intraperitoneally at designated time to experimental rats. The deoxycholate-treated postmitochondrial supernatant was analyzed by sucrose density gradient. The figures express percentage of the values of each absorbance peak to each total absorbance which are taken as 100% in Fig. 2.

On the other hand, the contents of trimer-pentamer (aggregate number of ribosomes, 3—5) at 6,10, and 16 hr after treatment were not different from that in control liver (Table I). This effect suggests that the fraction 4 administration has a constitutive action on the polysomes.

Effect of Fraction 4 on the Incorporation of ^{3}H-Orotic Acid and Population of Polysomes

Since the increase of polysome content was observed in cytoplasmic postmitochondrial supernatant of liver by fraction 4 treatment, the incorporation rate of ^{3}H-orotic acid into

21) D.M. Prescott, "Progress in Nucleic Acid Research and Molecular Biology," Vol. 3, Academic Press, Inc., New York, 1964, p. 33.

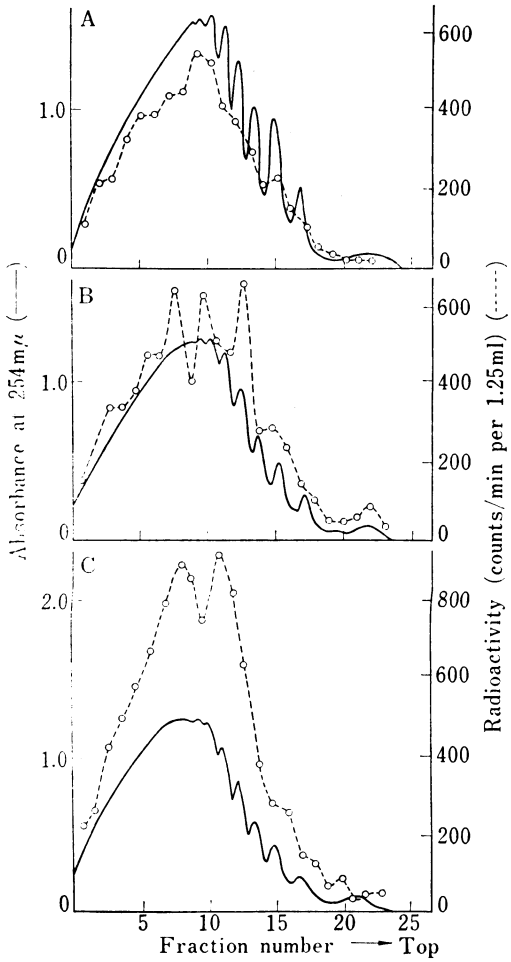

Fig. 3. Sucrose Density Gradient Patterns of Purified Polysomes Prepared at 1.5 hr after Injection of ^{3}H-orotin Acid into the Fraction 4-treated Rats at Designated Time

A) control (saline) Input, 57 absorbance unit at 260 mμ of polysome; a 0.3—1.0 M convex sucrose gradient centrifugation at 25000 rpm for 2.5 hr
B) Fraction 4 (5 mg/rat) was administered intraperitoneally. At 2 hr after treatment, ^{3}H-orotic acid (25 μCi/rat) was injected intraperitoneally, and 1.5 hr later the animals were killed by decapitation. Input and centrifugation as in (A).
C) At 4 hr after fraction 4 (5 mg/rat)-treatment, ^{3}H-orotic acid (25 μCi/rat) was injected intraperitoneally and 1.5 hr later the animals were killed by decapitation. Input and centrifugation as in (A).

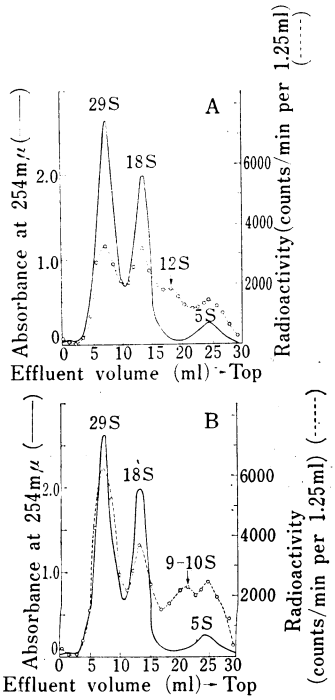

Fig. 4. Sucrose Density Gradient Analysis of Polysomal RNA from Control and Fraction 4-treated Rats

A) control (saline) At 6 hr after intraperitoneal administration saline, ^{32}P-phosphate 3.0 mCi was injected per rat intraperitoneally at 2.5 hr prior to killing the animals. centrifugation at 24000 rpm for 40 hr.
B) At 6 hr after fraction 4 (5 mg/rat) treatment, ^{32}P-phosphate (3.0 mCi/rat) was injected intraperitoneally at 2.5 hr prior to killing the animals. centrifugation as in (A)

polysomal RNA and population of polysomes were analyzed at 3.5 and 5.5 hr after treatment. Fig. 3 shows that fraction 4 obviously enhanced the incorporation rate by 52 and 92%, respectively, in the total counts of sucrose gradient, over the control rate. It was also found that the absorbance peaks of monomer ribosome, dimer, trimer, and tetramer polysomes markedly decreased by fraction 4 treatment in contrast to the region of heavy (over hexamer) polysomes at 5.5 hr, by sucrose density analysis as shown in Fig. 3C.

Characterization of Polysomal RNA Induced by Fraction 4 Treatment

An attempt was made to characterize polysomal RNA induced by fraction 4 administration. At 6 hr after pretreatment, ^{32}P-phosphate was injected intraperitoneally to animals,

2.5 hr prior to killing. Sedimentation analysis of ribosomal RNA and messenger RNA derived from SDS-treated polysomes was carried out by the sucrose density gradient as shown in Fig. 4.

Fig. 4 is the sedimentation pattern of RNA derived from a polysome population of mixed aggregate sizes. The sedimentation diagram shows four radioactivity peaks, three of which coincide in position with the absorbance peaks of 29S, 18S ribosomal RNAs and mixture of 5S ribosomal RNA and 4S transfer RNA. The messenger RNA peak which contributes no significant UV absorption, appears to be heterogeneous, as indicated by the wide sedimentation range corresponding to 11S to 14S, and it indicates some overlapping of the radioactivity peaks corresponding to messenger RNA and 18S ribosomal RNA.[19]

On the other hand, the sedimentation behavior of RNAs induced by fraction 4 (Fig. 4B) indicates that by this time the newly induced RNAs are evenly distributed over the zone spectrum of all RNAs. Particularly, 29S ribosomal RNA showed significant increase of the incorporation. In addition, we found that the radioactivity peak corresponding to messenger RNA shifted to 9S to 10S region from 11S to 14S of control liver.

Stimulatory Activity of Microsomes and Polysomes by Fraction 4 Treatment in an Amino Acid Incorporating System *in Vitro*

It has been found that microsome and polysome fractions obtained from the liver of fraction 4-treated rats are more active in the incorporation of amino acid than those obtained from normal liver. The results shown in Table II indicate that the microsomes and polysomes from treated liver are more active by 85% and 67%, respectively.

TABLE II. Stimulation of ^{14}C-Leucine Incorporation into Protein by Microsomes and Polysomes from Control and Fraction 4-treated Rats *in Vitro*

Preparation	Treatment	^{14}C-Leucine (cpm/mg protein)	%
Microsomes	control (saline)	994	100
	fraction 4	1840	185
Polysomes	control (saline)	1210	100
	fraction 4	2019	167

At 6 hr after administration of fraction 4 (5 mg/rat), the animals were killed by decapitation. In assay mixture, microsomes and polysomes were used respective 0.4 mg and 0.05 mg as protein by Lowry's method.

Discussion

Growth hormone,[13,22] testosterone,[9,23] estrogen,[24] thyroid hormone,[12,25] and cortisone,[11,26] soon after their administration, produce a 2 to 4 fold increase in the specific activity of rapidly labeled nuclear RNA and a stimulation of RNA polymerase in the animals whose organs of secretion were removed by operation. It was also found that many, but not all, hormones stimulate the synthesis of ribosomal RNA and messenger RNA, and increase the content of heavy polysomes in cytoplasm, and the cytoplasmic protein-synthesizing capacity is stimulated by newly formed ribosomes and messenger RNA after stimulation of nuclear RNA synthesis.[12–15,27]

22) G.P. Talwar, S.L. Gupta, and F. Gros, *Biochem. J.*, **91**, 565 (1964)
23) D.L. Greenman, W.D. Wicks, and F.T. Kenney, *J. Biol. Chem.*, **240**, 4420 (1965).
24) J. Gorski and N.J. Nelson, *Arch. Biochem. Biophys.*, **110**, 284 (1965).
25) C. Kidson and K.S. Kirby, *Nature*, **203**, 599 (1965).
26) F.T. Kenney and F.J. Kull, *Proc. Natl. Acad. Sci. U.S.*, **50**, 493 (1963).
27) D.L. Greenman and F.T. Kenney, *Arch. Biochem. Biophys.*, **107**, 1 (1964).

In previous paper,[3,4] we reported that the accelerated synthesis of rapidly labeled nuclear RNA occurs early by *in vivo* administration of *ginseng* extract to rat. Also, we observed the increase of DNA dependent nuclear RNA-polymerase activity at 2 hr after treatment.[5]

The present work, as described here showed that the effect of fraction 4 to increase the protein synthesis in the cytoplasm was well detected by the assay of polysomes and microsomes. The effect of fraction 4 on the incorporation rate of labeled precursor into polysomal RNA showed a maximum increase at 5.5 hr after treatment, and returned almost to the control level at 25.5 hr after administration. In addition, both the heavy polysome content in the mitochondrion-free supernatant and the incorporation rate of labeled precursor into purified polysomal RNA were accelerated by fraction 4 at 5.5—6.0 hr after treatment. The sedimentation profile of labeled polysomal RNA from treated liver (Fig. 4B) showed the increase of 29S ribosomal RNA and 9S to 10S messenger RNA in specific radioactivity. These experimental results suggest that the increase in synthesis of ribosomal RNA and messenger RNA after fraction 4 administration produces an increase in the population and amount of polysomes. Also, the stimulation of cytoplasmic protein synthesis was identified with microsomes and polysomes *in vitro*.

We have summarized the sequential effects of a single dose injection of fraction 4 into rat on the stimulation of biological activity in the liver nucleus and cytoplasm. The first phenomenon observed recorded a stimulation in the Mg^{2+}-activated RNA-polymerase activity beginning at 1 hr after *ginseng* extract administration *in vivo*, and reaching a value of 50% increase at 2 hr.[5] The second phenomenon observed was an increase in the specific radioactivity of rapidly labeled nuclear RNA which has a maximum rate at 4 hr after treatment.[4] A substantial rise in the polysomal RNA synthesis of cytoplasm took place at 5.5—6.0 hr after the fraction 4 was given, thus elevating the hepatic polysome content and the protein synthetic activity. Finally, the synthesis of serum protein was gradually stimulated up to 10 hr after treatment.[6] However, all the effects induced by *ginseng* extract returned almost to the control level about 24 hr after administration.

Similar phenomena in mammals were shown by the administration of thyroid hormone to the thyroidectomized rat by Tata and Widnell,[12] except for a longperiod response. Fraction 4 did not increase the activities of tryptophan pyrrolase and tyrosine-α-ketoglutarate transaminase.[6] In adrenalectomized rats, we also observed that fraction 4 enhanced the incorporation rate of labeled precursor into liver nuclear and cytoplasmic polysomal RNAs after treatment.[28] These experimental results eliminate the possibility that the action of fraction 4 is due to stressing the animals to increase the secretion of steroid hormones.

On the basis of these experimental results, we suggest that the action of fraction 4 is the metabolic stimulation or hormone-like action on the RNA and protein biosynthesis. Therefore, it might be expected that the *ginseng* extract acts at the level of transcription on DNA of liver chromatin by direct or indirect action. Further characterization and biological action of fraction 4 are now in progress.

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28) H. Oura and H. Seno, at the 88 th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1968.