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Stimulating Effect of the Roots of *Panax ginseng* C. A. Meyer on the Incorporation of Labeled Precursors into Rat Liver RNA[1]

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The *in vivo* administration of extract of the roots of *Panax ginseng* C. A. Meyer increased the incorporation rate of labeled precursors into liver nuclear RNA at 4 hr and into cytoplasmic polysomal RNA at 5.5—6.0 hr after a single dose intraperitoneal injection in a rat. The incorporation rate was directly dependent on the amount of a fraction obtained by partial purification. The increased specific radioactivity of the liver RNA is not due to the change in the pool size of endogenous acid-soluble nucleotides.

Many experimental evidences indicate the presence of rapidly synthesized RNA in mammalian tissues.[3] This RNA is synthesized only in the nucleus.[4] Another evidence strongly suggests that this RNA transfers to the cytoplasm, as would be expected of messenger RNA which directs polypeptide chains during translation of the genetic message by protein synthesis upon polysomes.[5]

During studies on several tonic crude drugs, roots of *Panax ginseng* C. A. Meyer showed a significant increase in the incorporation rate of labeled orotic acid into rapidly synthesized liver nuclear RNA after a single dose injection intraperitoneally in a rat.

The present paper describes studies on the partial purification of the stimulating substance and the stimulating effect of ginseng root extract on the incorporation of labeled precursors into rat liver RNA.

Meterial and Method

Materials and Animals——The crude drugs were obtained from the Mikuni Co., Ltd., Osaka. Each crude drug was extracted with 0.05 M Tris–HCl buffer (pH 7.6) under stirring for 48 hr at 4° in a coldroom. Each extract was adjusted to 0.9% saline with 10% NaCl solution.

Male Wistar rats weighing about 100 g were used for all the experiments. Rats were obtained locally, were fasted for 16 to 18 hr prior to the experiments and then given 0.5 ml of saline solution of each sample intraperitoneally. Control rats were treated with an equal volume of saline. At 4 hr after administration, labeled orotic acid was given intraperitoneally 20 min prior to killing the animals by decapitation.

6-^{14}C–Orotic acid (32.6 mCi/mM) and ^{32}P–orthophosphate (54.5 Ci/mg phosphorus) were obtained from the Radiochemical Centre, England. 5-^{3}H–Orotic acid (5 mCi/3.49 mg) was the product of New England Nuclear Corp., U.S.A. Actinomycin D was supplied from the Merck Sharp and Dohme Research Laboratories, U.S.A.

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) *a*) A. Sibatani, S.R. de Kloet, V.G. Allfrey, and A.E. Mirsky, *Proc. Natl. Acad. Sci. U.S.*, **48**, 471 (1962); *b*) G.P. Georgiev and V.L. Mantieva, *Biochim. Biophys. Acta*, **61**, 153 (1962); *c*) C. Kidson, K.S. Kirby,and R.K. Ralph, *J. Mol. Biol.*, **7**, 312 (1963); *d*) S. Penman, K. Scherrer, Y. Becker, and J.E. Darnell, *Proc. Natl. Acad. Sci. U.S.*, **49**, 654 (1963).
4) *a*) H. Hiatt, *J. Mol. Biol.*, **5**, 217 (1962); *b*) H. Harris, H.W. Fisher, A. Rodgers, T. Spencer, and J.W. Wattes, *Proc. Roy. Soc.*, B, **157**, 177 (1963).
5) *a*) S.M. Girard, S. Penman, and J.E. Darnell, *Proc. Natl. Acad. Sci. U.S.*, **51**, 205 (1964); *b*) G. R. Lawford, P. Sadowski, and H. Schachter, *J. Mol. Biol.*, **23**, 81 (1967); *c*) J.R. Warner, P. Knopf, and A. Rich, *Proc. Natl. Acad. Sci. U.S.*, **49**, 122 (1963); *d*) H. Noll, T. Staehelin, and F.O. Wettstein, *Nature*, **198**, 632 (1963).

All other chemicals used in this study were of analytical or reagent grade and were used without further purification.

Extraction of Radix Ginseng——Roots of *Panax ginseng* C. A. Meyer, produced in Kumsan, Korea, were powdered and extracted with 0.05 M Tris–HCl buffer (pH 7.6) under stirring for 48 hr in a cold room. The filtrate was centrifuged and the clear supernatant fluid (fraction 1) was obtained. This fraction was dialyzed against cold water for 48 hr in a cold room, and then the inside solution of the dialysis bag was lyophilized (fraction 2). Fraction 2 was dissolved in water and brought to 70% saturation of ammonium sulfate. The precipitate was dissolved in distilled water, dialyzed against running cold water for 48 hr until salt-free, and then the inside solution was lyophilized (fraction 3). Fraction 3 was obtained in 4% yield from the ginseng powder. Fraction 3 was extracted with 99% MeOH on a water bath under refluxing for 12 hr, 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*. Fraction 4 was obtained in 35% yield from fraction 3.

Separation of Nuclei——Three rats were used in one group. Livers were removed immediately after decapitation and washed with cold 0.25 M sucrose–0.0033 M $CaCl_2$, and pooled. All subsequent steps were carried out at 0—4° unless otherwise noted. These livers were minced with scissors and homogenized with 3 volumes of the same solution in a Potter–Elvehjem device with 10 strokes of a motor-driven Teflon pestle in a cold room. The homogenate was filtered through 4 layers of gauze. The purified nuclei were prepared with 2.2 M sucrose, $105000 \times g$ for 1 hr, by the procedure of Tsukada and Lieberman.[6] The purified nuclear preparation had a ratio of RNA to DNA of about 1:5.

Assay of Specific Radioactivity of Nuclear RNA——The purified nuclei were washed three times with 0.6 N $HClO_4$, three times with 95% EtOH, and three times with ether in a cold room. To the residue 0.5 N KOH was added and the sample was hydrolyzed at 37° for 18 hr. The extract was neutralized with cold $HClO_4$ and centrifuged. The supernatant fluid was used for RNA determination by the Dische and Borenfreund's method.[7] The specific radioactivity (cpm/mg nuclear RNA) was determined by aliquots of this supernatant in the Bray's solution[8] for radioassay in a Packard Tri–Carb Liquid Scintillation Spectrometer, Model 3003.

Assay of Specific Radioactivity of Polysomal RNA——Three rats were used as one group. The purified polysome fraction from rat liver was prepared according to the method of Wettstein, *et al.*[9] The radioactivity of the polysomal RNA was determined by the procedure of Staehelin, *et al.*[10] The specific radioactivity of the polysomal RNA was determined by cpm per optical density unit of polysomal suspension at 260 mμ.

Assay of Specific Radioactivities of Pyrimidine Nucleotides from Liver Acid-Soluble and RNA Fractions——The livers were immediately homogenized in 3 volumes of $HClO_4$ (0.1 M, 0°) and thehomogenates from 3 rats were used as one group. Further $HClO_4$ (5 M) was added to a final concentration of 0.5 M and insoluble material was removed by centrifugation at $10000 \times g$ for 10 min. The acid–insoluble fraction was washed three times with $HClO_4$ (0.5 M), twice with EtOH–ether (3:1), and twice with ether in a cold room. To the residue 0.5 N KOH was added, and the sample was digested at 37° for 18 hr. The extract was neutralized with cold $HClO_4$ and $KClO_4$ was removed by centrifugation. Chromatography of the supernatant fluid was made on a column (0.9 × 5 cm) of Dowex 50 (H^+ form, 200—400 mesh), and UMP was eluted with 0.05 N HCl.[11] Acid-soluble fraction was heated for 30 min at 100°. When cooled, the centrifuged solution was neutralized with KOH (5 M, internal bromothymol blue indicator). Chromatography of the neutralized solution was made on a column (0.9 × 12 cm) of Dowex 1 (Cl^- form, 200—400mesh), and pyrimidine nucleotides were eluted with 0.1 M NaCl in 0.01 M acetate buffer (pH 5.6) by the method of Cohn,[12] and then UMP was separated with Dowex 50 (H^+ form) according to the method of Katz and Comb.[11]

Result

Effect of Several Crude Drugs on the Incorporation of Labeled Orotic Acid into Rat Liver Nuclear RNA

Both groups of normal and experimental rats treated with crude drug extract were injected with labeled orotic acid and the animals were killed 20 min later, the nuclear RNA was

6) K. Tsukada and I. Lieberman, *J. Biol. Chem.*, **239**, 1564 (1964).
7) Z. Dische and E. Borenfreund, *Biochim. Biophys. Acta*, **23**, 639 (1957).
8) G.A. Bray, *Anal. Biochem.*, **1**, 279 (1960).
9) F.O. Wettstein, T. Staehelin, and H. Noll, *Nature*, **197**, 430 (1963).
10) T. Staehelin, F.O. Wettstein, H. Oura, and H. Noll, *Nature*, **201**, 264 (1964).
11) S. Katz and D.G. Comb, *J. Biol. Chem.*, **238**, 3065 (1963).
12) W.E. Cohn, *J. Am. Chem. Soc.*, **72**, 1471 (1950).

Table I. Effects of Several Tonic Crude Drugs on the Incorporation of Labeled Orotic Acid into Rat Liver Nuclear RNA

Expt. No.	Material	No. of rats	Labeled orotic acid (cpm/mg RNA)	%
1	control (saline)	6	58250±1150	100
	radix Rehmanniae (China)[a]	3	49200	85
	fractus Lycii (China)[b]	3	65000	111
	rhizoma Cnidii (Hokkaido, Japan)[c]	3	57600	99
	radix Ginseng (Kumsan, Korea)[d]	3	87900	151
2	control (saline)	6	22200± 400	100
	radix Ginseng (Nagano, Japan)[e]	3	35800	161
	rhizoma Panacis Japonici (Tohoku, Japan)[f]	3	25600	115
	radix Glycyrrhizae (Tohoku, Japan)[g]	3	22700	102
	semen Cuscutae (China)[h]	3	28300	128
	radix Bupleuri (China)[i]	3	27200	123
3	control (saline)	12	40800±2600	100
	radix Scrophulariae (China)[j]	6	46500±3800	114
	cortex Lycii Radicis (China)[k]	3	42600	105
	rhizoma Atractylodis (China)[l]	3	35000	86
	tuber Ophiopogonis (Osaka, Japan)[m]	3	46800	115
	radix Asparagi (China)[n]	3	38200	94
	herba Cistanches (China)[o]	3	35800	88
4	control (saline)	12	47750±4500	100
	fructus Schizandrae (China)[p]	6	53100±3400	111
	radix Dioscoreae (China)[q]	6	52600±1700	110
	rhizoma Alimatis (China)[r]	3	50300	105

Each crude drug extract was injected 0.5 ml per rat intraperitoneally, corresponding to 0.15 g of dried crude drug. Expt. 1, ^{14}C-orotic acid 2.5 μCi; Expt. 2, ^{3}H-orotic acid 4.0 μCi; Expt. 3 and 4, ^{3}H-orotic acid 7.0 μCi were injected per rat respectively. The data are expressed as percent of the values of untreated control rats which are taken as 100%. The data are presented as mean±S.E.

Scientific and Japanese name of each crude drug: *a*) *Rehmannia glutinosa* (Gaertn) Libosch forma *hueichingensis* (Chao et Schih) Hsiao; Kaikei-jiö. *b*) *Lycium chinense* Miller; Kukoshi. *c*) *Cnidium officinale* Makino; Senkyu. *d*) *Panax ginseng* C.A. Meyer; Ninjin. *e*) *Panax ginseng* C.A. Mayer; Ninjin. *f*) *Panacis japonici* C.A. Meyer; Chikusetsu-ninjin. *g*) *Glycyrrhiza uralensis* Fisch et DC.; Tohoku-kanzo. *h*) *Cuscuta chinensis* Lamark; Toshishi. *i*) *Bupleurum falcatum* L.; Saiko. *j*) *Scrophularia ningpoensis* Hemsl; Genjin. *k*) *Lycium chinense* Miller; Jikoppi. *l*) *Atractylodis lancea* DC. var. *chinensis* Kitamura Sojyutsu. *m*) *Ophiopogon japonicus* Ker-Gawler; Bakumondō. *n*) *Asparagus cochinchinenensis* Merill. Tenmondō. *o*) *Cistanche salsa* Benth. et Hook fil; Nikujyuyō. *p*) *Schizgandra Chinensis* Baillon. Gomishi; *q*) *Dioscorea batatas* Decaisne; Sanyaku. *r*) *Alisma plantago-aquatica* L. subsp. *orientale* Samuelsson; Takusha.

assayed, and its specific radioactivity was determined. Table I shows that the administration of extract of radix Ginseng, produced in Korea and Japan, increased 50—60% over the incorporation rate into liver nuclear RNA from the control level. Semen Cuscutae and radix Bupleuri produced in China, respectively increased the specific radioactivity of the nuclear RNA by 28 and 23%. In these experiments, on the other hand, radix Rehmanniae, rhizoma Atractyloidis, and Herba Cistanches showed a slight decrease ranging from 12 to 15%.

Effect of Graded Doses of Radix Ginseng Extract on the Incorporation of ^{14}C-Orotic Acid into Nuclear RNA

In order to confirm the stimulating activity of ginseng extract on nuclear RNA, an attempt was made to determine the dose-response relation of graded doses of the extract (fraction 1), and the incorporation of ^{14}C-orotic acid into liver nuclear RNA is shown in Table II. There is a range in nuclear RNA response to different doses of the extract, and a 40% increase over the control value was indicated by the dose of 15 mg (correspond to Ginseng powder). Furthermore, the administration of 600 mg increased the incorporation rate by 85% over that of the control animals.

Table II. Effect of Graded Doses of Fraction 1 of Radix Ginseng Extract on the Incorporation of ^{14}C-Orotic Acid into Liver Nuclear RNA

Material	Amount corresponding to radix ginseng powder (mg)	No. of rats	^{14}C-Orotic Acid (cpm/mg nuclear RNA)	%
Control (saline)	—	6	38100±1400	100
Fraction 1	15	3	53400	140
Fraction 1	60	3	58700	154
Fraction 1	150	3	61300	161
Fraction 1	600	3	70550	185

^{14}C-crotic acid, 2.5 μCi/rat. At 4 hr after administration of fraction 1 orotic acid was given intraperitoneally 20 min prior to killing the animals by decapitation.

Table III. Effect of Several Doses of Radix Ginseng Extract on the Incorporation of ^{14}C-Orotic Acid into Rat Liver Nuclear RNA

Material	No. of rats	^{14}C-Orotic acid (cpm/mg nuclear RNA)	%
Control (saline)	3	29600	100
Fraction 1	3	54700	185

Fraction 1, 0.5 ml (corresponding to 60 mg of ginseng powder) or saline was injected intraperitoneally at zero, 8, 16, and 24 hr. Five hr after the last injection, ^{14}C-orotic acid (2.5 μCi) was administered, and 20 min later, the animals were killed by decapitation.

In the experiment of the 4 time administration of fraction 1 with an 8 hr intervals, there was a striking 85% increase over control in the incorporation rate into nuclear RNA (Table III).

Partial Purification of Ginseng Extract and Effect of Each Fraction on the Liver RNA

Fraction 1 (60 mg dose) increased the incorporation rate of the rapidly labeled nuclear RNA by 50% over that of the control animals, and its increased activity was not lost by heat treatment at 100° for 10 min (Table IV). Also this fraction enhanced by 48% the incorporation rate of ^{32}P-phosphate into cytoplasmic polysomal RNA over the control level at 6 hr after treatment (Table V).

Table IV. Effect of Each Fraction isolated from Radix Ginseng on the Incorporation of ^{14}C-Orotic Acid into Rat Liver Nuclear RNA

Material	Dose (mg)	No. of rats	^{14}C-Orotic acid (cpm/mg nuclear RNA)	%
Control (saline)	—	18	36370±900	100
Fraction 1	60[a]	6	54500±2500	150
Fraction 1 (100°, 10 min)	60[a]	3	56800	156
Fraction 2	5	3	55500	153
Fraction 3	5	3	58500	161
Fraction 4	0.01	3	40600	112
Fraction 4	0.05	3	47000	129
Fraction 4	1	3	54700	151
Fraction 4	5	3	84800	234
Actinomycin D	0.25	3	4350	12

a) Corresponding to radix Ginseng powder.

The data are presented as mean± S.E., and are expressed in terms of percentage of the values of untreatedcontrol rats which are taken as 100%. ^{14}C-Orotic acid (2.5 μCi/rat) was given intraperitoneally. Treatment to rats was the same as described in Table II.

Table V. Effect of Each Fraction Isolated from Radix Ginseng on the Incorporation of Labeled Prescursors into Cytoplasmic Polysomal RNA

Expt. No.	Material	Dose (mg)	No. of rats	^{32}P-Phosphate or ^{3}H-orotic acid cpm/OD unit of polysome	%
1	control (saline)	—	3	2438	100
	fraction 1	60[a)]	3	3595	148
2	control (saline)	—	3	3285	100
	fraction 3	5	3	5234	159
3	control (saline)	—	3	145	100
	fraction 4	0.005	3	158	109
	fraction 4	0.05	3	187	129
	fraction 4	0.5	3	214	148
	fraction 4	5	3	334	230

a) Corresponding to radix Ginseng powder.

In Expt. 1 and 2, at 6 hr after administration of each fraction, saline solution of ^{32}P-phosphate was administered to a rat intraperitoneally 2 hr prior to killing the animals, under ether aneasthesia, by exsanguination after cutting the abdominal aorta. Each animal received 0.5 ml in total amount containing 2.5—3.0 mCi of ^{32}P-phosphate. In Expt. 3, at 5.5 hr after treatment, ^{3}H-orotic acid of 20 μCi was administered to a rat intraperitoneally 1.5 hr prior to killing and the animals were killed by decapitation.

In order to know the approximate molecular weight of the active substance, fraction 1 was directly dialyzed. Although the result is not shown in Table IV, the outside solution had a weak activity in increasing the incorporation rate of ^{14}C-orotic acid into nuclear RNA, but 5 mg of fraction 2 of inside solution increased the rate by 53% over the control value. Therefore, purification of the active substance was carried out by the precipitation with $(NH_4)_2SO_4$. The effect of partially purified fraction 3 on the incorporation rates is shown in Tables IV and V. Increased rates on these biological assay were 61% in nuclear RNA and 59% in polysomal RNA over the control level.

The rate increase of fraction 3 was not lost by heat treatment (100°, 10 min) and Pronase P (Kaken Kagaku Co., Tokyo) digestion.[13)] These experimental results suggest that the stimulating effect is not due to a protein-like substance. Preliminary chemical examination of fraction 3 gave positive Liebermann-Burchard reaction, and it contained about 50% of hexose by the Tsugita and Akabori's method.[14)]

Therefore, the saponin-like substance was assumed as the stimulating factor, and ether precipitated powder (fraction 4) was prepared from MeOH extract of fraction 3. The effect of the dose-response of fraction 4 is shown in Table IV and V. 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 to 1.0 mg of fraction 4 increased the incorporation rate by 51% in nuclear RNA at 4 hr and 48% in polysomal RNA at 5.5 hr after treatment over the control level. Also the administration of 5 mg of fraction 4 showed 134% increased rate in nuclear RNA and 130% in polysomal RNA over the control animals.

These purification procedures, however, are not complete with respect to the quantitative recovery of the biological activity. Fraction 4 gave positive Liebermann-Burchard reaction. Hemolysis was not seen in the serum by the administration of 5 mg of fraction 3 and 4. Further characterization of the stimulating substance is necessary to clarify the biological activity.

Specific Radioactivities of Pyrimidine Nucleotides from Liver Acid-Soluble and RNA Fractions after Treatment

The possibility was considered that these increased rates of incorporation might result from a change in the specic activities of the labeled RNA precursors rather than from a rise

13) H. Oura, S. Hiai, S. Nakashima, and K. Tsukada, unpublished observation.
14) A. Tsugita and S. Akabori, *J. Biochem.* (Tokyo), **46**, 695 (1959).

in the rate of RNA formation. To test this possibility, using ^{3}H-orotic acid, the pyrimidine nucleotides from both the acid-soluble fraction and from acid hydrolysates of RNA were examined for control animal and 4 hr rat after treatment with fraction 3. Table VI shows that 54—68% increase in the specific radioactivity of UMP from 4 hr RNA sample was not accompanied by a similar increase in the specific activity of the nucleotide from the acid-soluble pool. CMP was not detected in the acid-soluble fraction and a negligible radioactivity of the RNA was present in this nucleotide.

Table VI. Specific Radioactivities of UMP from Liver Acid-Soluble and RNA Fractions after Treatment with Ginseng Extract

Expt. No.	Material	Acid-soluble UMP (cpm/OD)[a]	%	RNA (UMP) (cpm/OD)[a]	%	RNA (cpm/mg RNA)	%
1	control	17575	100	450	100	2488	100
	fraction 3	16974	97	754	168	4160	167
2	control	15060	100	357	100	1895	100
	fraction 3	15900	105	548	154	2954	156

a) cpm per absorbance unit at 260 mμ
At 4 hr after administration of fraction 3 (5 mg/rat) ^{3}H-orotic acid (7 μCi) was given intraperitoneally 20 min prior to killing the animals by decapitation.

Discussion

The nucleus is an organelle of extremely varied and complex function. Not only does it maintain its own protein synthesis, but it is intimately involved in the regulation of protein synthesis in the rest of the cell. Also the nucleus is considered to be the site of synthesis of all types of RNA.

In this paper we have described the partial purification of the stimulating substance and the stimulating effect of Ginseng extract on the incorporation of labeled precursors into rat liver nuclear and cytoplasmic RNA.

Loeb and Gelboin[15] reported that methylcholanthrene-treated rat liver caused an increase in the uptake of orotic acid into nuclear RNA which suggested increased RNA synthesis, and the RNA content of isolated nuclei is almost the same as in the control, at 4 hr after treatment. However at 16 hr after treatment, there is a significant increase in nuclear RNA content of the isolated nuclei as shown by an increase in the ratio of RNA to DNA. On the other hand, the administration of thioacetamide[16] did not produce a rapid increase in nuclear RNA content after the initial dose, and the incorporation of orotic acid into nuclear RNA showed a decrease in labeling of RNA at 3—6 hr after the first dose. The nuclear content of RNA, however, was increased 2- to 3-fold after 9 days of daily administration of thioacetamide.

Many, but not all, hormones stimulate the incorporation of radioactive precursors into rapidly labeled RNA recovered from total target tissues or their nuclei.[17] In the few studies in which levels of nuclear RNA have been measured, there is no significant accumulation of RNA for any length of time after hormone administration.[18] This suggests that the additional

15) L.A. Loeb and H.V. Gelboin, *Proc. Natl. Acad. Sci. U.S.*, **52**, 1219 (1964).
16) *a*) H.R. Adams and H. Busch, *Cancer Res.*, **23**, 576 (1963); *b*) W.J. Steele, N. Okamura, and H. Busch, *J. Biol. Chem.*, **240**, 1742 (1965).
17) J.R. Tata, "Progress in Nucleic Acid Research and Molecular Biology," Vol. 5, Academic Press, Inc., New York, N.Y., 1966, p. 191.
18) *a*) J.R. Tata and C.C. Widnell, *Biochem. J.*, **98**, 604 (1966); *b*) G.P. Talwar, S.L. Gupta, and F. Gros, *Biochem.J.*, **91**, 565 (1964); *c*) D.L. Greenman, W.D. Wicks, and F.T. Kenney, *J. Biol. Chem.*, **240**, 4420 (1965).

nuclear RNA synthesized in response to the hormone is either transferred into the cytoplasm or degraded in the nucleus.

The present experiments demonstrated that each fraction isolated from ginseng root enhances the incorporation rate of labeled precursor into rapidly synthesized nuclear RNA at 4 hr and into cytoplasmic polysomal RNA at 5.5—6.0 hr after a single dose injection. Furthermore, the incorporation rates were directly dependent on the amount of fraction 4 obtained by partial purification. In the experiment of pool size of the acid-soluble nucleotide, a change in pyrimidine nucleotide was not detectable, and only an increase in the specific radioactivity of RNA was observed. Therefore, the increased specific radioactivities of the nuclear and cytoplasmic RNA are not due to change in the endogenous pool size of RNA precursor. Thus it is suggested that the stimulating effect of Ginseng extract is due to the two possibilities; (1) a decrease in the rate of nuclear RNA degradation, and (2) an increase in the rate of RNA synthesis.

Each fraction isolated from Ginseng root enhanced the incorporation rate of labeled precursors into cytoplasmic polysomal RNA. Also the increase of the specific radioactivity of nuclear RNA isolated by phenol extraction,[19] of RNA polymearse activity in liver nuclei[20] and of heavy polysome content[21] after treatment had been observed during this series.[1] These experimental results would seem to suggest that the increased specific radioactivity of liver RNA shows an increase of RNA synthesis in the nucleus and its transfer into the cytoplasm.

Hydrocortisone administration to adrenalectomized rats caused increase of nuclear RNA synthesis and enzyme activities of tryptophan pyrrolase and tyrosine-α-ketoglutarate transaminase.[22] The effect of Ginseng extract on the incorporation rate of orotic acid into nuclear RNA showed the enhancement 4 hr after administration to intact rats, but fraction 3 and 4 showed no increase of these hepatic enzyme activities.[23] On the other hand, the administration of Celite, a nonspecific metabolism stressing agent, induced a marked increase of these enzyme activities.[24] These experimental results suggest that the action of Ginseng extract is not due to the consequence of stressing the animals to produce an increased secretion of steroid hormones. Although 5 mg of fraction 4 was administered to rats every day for 2 weeks intraperitoneally, the usual decrease of body weight associated with toxicity in the experimental group was not observed when compared with the control group of saline treatment.

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