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Stimulating Effect of *Panax ginseng* Extract on RNA Polymerase Activity in Rat Liver Nuclei[1]

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It was shown that a single intraperitoneal injection of extract (fraction 3 and 4) from roots of *Panax ginseng* C.A. MEYER, increased activity of RNA polymerase (EC 2.7.7.6), assayed *in vitro*, of deoxycholate-lysed nuclei of rat liver. The maximum increase of the enzyme activity was found at 2 hr with a lag period of 30 min after treatment. The increase was still observed 8 hr after the injection of the extract but disappeared after 16 hr. The increase in the activity was found in the presence of high concentration of ammonium sulfate *in vitro* as well as in the absence of the salt.

Three hours after treatment *in vivo*, a little increase of protein synthesis was also observed in liver homogenate or nuclei and abolished by prior administration of puromycin. The increase in the activity of the polymerase due to *ginseng* extract did not disappear even when the synthesis of bulk nuclear protein was greatly inhibited by puromycin.

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 RNA in rat.[3] The synthesized nuclear RNA was identified with ribosomal and DNA-like messenger RNA.[4] It was also observed that a rise in serum protein synthesis due to *ginseng* extract was proceeded by an increase in amount of polysomes (more than hexamer) of cytoplasm in liver.[5] Recently some hormone have been shown to stimulate protein synthesis in target organs as a consequence of prior increase of RNA synthesis.[6] Increase in DNA-dependent RNA polymerase activity was observed as the response of hormone administration.[6] Gorski[7] reported that an increase in RNA polymerase activity due to estrogen treatment was observed and abolished in the presence of high concentration of ammonium sulfate. Breuer and Florini[8] also observed the similar effect of the salt with muscle RNA polymerase treated with testosterone *in vivo*, whereas the polymerase treated *in vivo* with human growth hormone was unaffected by the salt.

In this paper, we will present the evidence that nuclear RNA polymerase activity *in vitro* increased when *ginseng* extract was administered *in vivo*. The increase in RNA polymerase activity was observed in the presence or absence of ammonium sulfate and could not be abolished by the prior administration of puromycin.

Material and Method

Animals and General Materials——Male Wistar rats were used and received water and food freely for one week or so at 25° in all experiments. Rats weighing about 100 g were fasted overnight before experi-

1) This work was presented at the 86th Annual Meeting of the Pharmaceutical Society of Japan, Sendai, October 1966.
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3) H. Oura, S. Hiai, S. Nakashima, and K. Tsukada, *Chem. Pharm. Bull.* (Tokyo), **19**, 453 (1971).
4) H. Oura, S. Hiai, and H. Seno, *Chem. Pharm. Bull.* (Tokyo), **19**, 1598 (1971).
5) H. Oura, S. Nakashima, K. Tsukada, and Y. Ota, *Chem. Pharm. Bull.* (Tokyo), to be published.
6) J.R. Tata, "Progress in Nucleic Acid Research and Molecular Biology," Vol. 5, Academic Press, New York, 1966, p. 191.
7) J. Gorski, *J. Biol. Chem.*, **239**, 889 (1964).
8) C.B. Breuer and J.R. Florini, *Biochemistry*, **5**, 3857 (1966).

ments. *Ginseng* extract (fraction 3 or 4), labeled orotate, labeled leucine, or inhibitor was injected intraperitoneally as saline solution. Doses and time of treatment will be described in part of results.

Four ribonucleoside triphosphates were obtained from Sigma Biochemical Co. ^{3}H-Cytidine triphosphate (3.1 Ci/mM in 50% ethanol) was a product of Schwartz Bioresearch. 6-^{14}C-Orotic acid (32.6 mCi/mM) was purchased from Radiochemical Centre and neutralized with NaOH and then made saline solution. ^{14}C-U-L-Leucine (231 mCi/mM) and ^{3}H-U-L-leucine (200 mCi/mM) were supplied from New England Nuclear Corp. U.S.A. and Radiochemical Centre, England. DNase (beef pancreas, 1 crystalized) was obtained from Worthington Biochemicals. Puromycin hydrochloride was supplied from Nutritional Biochemicals and actinomycin D from Merk Sharp and Dome Research Laboratories.

Extraction and Property of Radix Ginseng——Fraction 3 and 4 were 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 0.05 M Tris-HCl buffer (pH 7.6) in a cold room. The filtrate were brought to 70% satulation 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 lyophilyzed (fraction 3). Fraction 3 was extracted with 99% methanol on a water bath under refluxing, and the methanol solution was concentrated. Fifteen volumes of cold ether was added to the residual solution. The white or slightly yellowish precipitate (fraction 4) was dried *in vacuo*. Preliminary chemical examination of fraction 3 gave positive Liebermann–Burchard reaction, and it contained about 50% hexose (glucose equivalent) by a modified orcinol reaction.[9] Preliminary properties of fraction 4 are as follows: mp 180—190°; Liebermann–Burchard reaction, positive; hexose content, 70—75%.

Preparation of Lysed Nuclear Enzyme——Liver was excised immediately after decapitation of rat and three pooled livers were homogenized in ice cold 0.25 M sucrose containing 0.0033 M $CaCl_2$. Crude liver nuclei were obtained by low speed centrifugation. The crude nuclei were purified by centrifugation at 105 000$\times g$ for 60 min in 2.2 M sucrose.[10] The nuclei were suspended with 0.02 M Tris-HCl buffer (pH 7.4). The suspension was lysed with sodium deoxycholate (final concentration, 2%) by gentle shaking[11] and centrifuged to remove a little insoluble residue at 7000 rpm for 5 min. The clear supernatant was used for RNA polymerase assay and stored at −70° just before assay and a small activity of the polymerase was lost after storage for one week.

Enzyme Assay——The activity of RNA polymerase (EC 2.7.7.6) was measured as incorporation of labeled CMP into acid insoluble material from CTP. The reaction mixture was 0.25 ml of total volume containing 25 μmoles of Tris-HCl buffer (pH 8.0), 2 μmoles of β-mercaptoethanol, 3 μmoles of $MgCl_2$, 5 μmoles of KCl, 63 μmoles of ammonium sulfate, 0.2 μmoles of adenosine, guanosine and uridine triphosphates, 0.02 μmoles of cytidine triphosphate, 1 or 2 μCi of ^{3}H-cytidine triphosphate and the enzyme preparation (about 0.2 mg of protein). After 15 min, unless otherwise stated, of incubation at 37°, 5 ml of chilled 5% $HClO_4$ containing 50 mg of Hyflo Super-cel as carrier was added to stop the reaction, the acid insoluble precipitate by centrifugation were dispersed in 1 ml of ice cold water. 0.15 ml of 1 N KOH was added to the

TABLE I. Characterization of Nuclear RNA Polymerase Reaction

Conditions	^{3}H-CMP incorporated cpm per mg protein	Per cent of control
Expt. 1-complete	1630	100
Omit ATP	353	22
Omit GTP	220	14
Omit UTP	400	25
Omit ATP, GTP, UTP	286	18
Expt. 2-omitA.S.[a]	1210	100
Omit A.S. add pyrophosphate, 50 μmoles	167	14
Omit A.S. add orthophosphate, 50 μmoles	1310	108
Expt. 3-complete	2160	100
Actinomycin 5 μg, added	414	19
Expt. 4-complete	3000	100
DNase, 100 μg, added	506	17

The composition of the complete reaction mixtue was desecribed in method. Incubation was carried out at 37° for 15 min except that in Expt. 4 nuclear enzyme preparation was preincubated without DNase at 37° for 10 min.

a) A.S.: ammonium sulfate

9) A. Tsugita and S. Akabori, *J. Biochem.* (Tokyo), **46**, 695 (1959).
10) K. Tsukada and I. Lieberman, *J. Biol. Chem.*, **239**, 1564 (1964).
11) K. Tsukada and I. Lieberman, *J. Biol. Chem.*, **239**, 2952 (1964).

suspension and then 10 ml of 5% $HClO_4$ was added to the suspension. Precipitates were obtained by centrifugation and washed twice with 5 ml of 5% of $HClO_4$. The precipitates were collected on a filter paper and washed with ethanol, ethanol–ether (1:1), and ether and dried. The dry preparation was solubilized by incubation at 45° for 3 hr with aid of hyamine methanol solution and suspended in a phosphor solution and counted in a Packard Tri-Cab liquid scintillation spectrometer.

Separation of RNA and Determination of Radioactivity——Nuclear suspension was washed with 5% of cold $HClO_4$ three times by centrifugation. The acidified precipitates were dried after washing with ethanol, ethanol–ether (1:1) and ether. The dry powder was incubated with 0.5N KOH at 37° for 16 hr. The hydrolysate was acidified with $HClO_4$. Acidified supernatant was neutralized with KOH and precipitates were removed by centrifugation. Aliquots of the supernatant were counted in the Bray's solution[12] and assayed by the phloroglucinol method of Dische and Borenfreund for RNA.[13]

Determination of Radioactivity of Protein——Nuclear suspension or homogenates of liver were heated in 0.5N $HClO_4$ at 70° for 20 min. Unincorporated leucine and nucleosides were washed and removed with 5% $HClO_4$ by centrifugation. Precipitates were washed with organic solvents and dried. The dry powder was dissolved in 0.1N KOH, aliquots were counted in a scintillation spectrometer and used for protein determination by the Lowry's method.[14]

Result

Characterization of Lysed Nuclear Enzyme Preparation

As shown in Table I, omission of one or three ribonucleoside triphosphates led a decrease in incorporation of labeled CMP into acid insoluble materials by lysed nuclear enzyme. Pyrophosphate (50 μmoles) as reaction product was inhibitory while orthophosphate (50 μmoles) was not in the absence of ammonium sulfate. The reaction thus depends on the presence of all four ribonucleoside triphosphates. The incorporation was inhibited by the addition of actinomycin D or preincubation of the nuclear preparation with DNase (37° for 10 min). Thus it was shown that the reaction depended on endogenous DNA of the nuclear preparation. When $MnCl_2$ (1.0 μmole) was added to the complete reaction mixture which contained $MgCl_2$ and ammonium sulfate, 20% of increase in incorporation was observed. Thus the lysed nuclear preparation is similar to the liver "aggregate" RNA polymerase described by Weiss.[15]

Stimulating Effect of *Ginseng* Extract on RNA Polymerase Activity

RNA polymerase activity (EC 2.7.7.6) was determined 3 hr after *ginseng* extract (fraction 3) treatment *in vivo*. As shown in Table II, an increase in RNA polymerase activity due to *ginseng* extract was observed by lysed nuclear enzyme preparation. Incorporation of labeled CMP into RNA was about two fold greater in the presence of high concentration of ammonium sulfate (0.25 M, pH 8.0) than in the absence. The increase per cent in the absence of ammonium sulfate was of the same order as that in the presence of the salt. The stimulating effect of *ginseng* extract was confirmed when graded doses of fraction 4, purified

Table II. Increasing Effect of *Ginseng* Extract on RNA Polymerase Activity in the Presence or Absence of High Concentration of Ammonium Sulfate

Reaction mixture	Control cpm per mg protein	Treated cpm per mg protein	Increase % of control
Complete	4570	6210	36
Omit A.S.[a]	2490	3310	33

Rat received a single intraperitoneal injection of 5 mg of *ginseng* extract (fraction 3) in saline solution and control rat received saline only. Nuclear enzyme from three pooled livers was prepared 3 hr after treatment *in vivo*. Incubation was carried out at 37° for 20 min.
a) A.S.: ammonium sulfate

12) G.A. Bray, *Analytical Biochem.*, **1**, 279 (1960).
13) Z. Dische and E. Borenfreund, *Biochim. Biophys. Acta*, **23**, 639 (1957).
14) O. H. Lowry, M.J. Rosenbrough, A.L. Farr, and R.L. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
15) S.B. Weiss, *Proc. Natl. Acad. Sci. U.S.A.*, **46**, 1020 (1960).

Table III. Effect of Graded Dose of Fraction 4 on RNA Polymerase Activity

Material	Dose mg	^{3}H-CMP incorporated cpm per mg protein	Per cent of control
Control	—	2240	100
Fraction 4	0.1	2660	119
Fraction 4	0.5	2760	123
Fraction 4	1.0	2910	130
Fraction 4	5.0	3410	152

Treatment of rat was the same as described in Table II. Polymerase activity was determined in the absence of ammonium sulfate.

from fraction 3, were administered to rats (Table III). RNA polymerase activity in the absence of ammonium sulfate was dependent on the amount of fraction 4 and the administration of 1.0 and 5.0 mg of fraction 4 indicated increase of 30% and 52% at 3 hr after treatment, respectively, over the control rate. The increase in the enzyme activity was consistent with previous results that *ginseng* extract increased the rate of incorporation of labeled orotic acid or orthophosphate into nuclear RNA *in vivo*.[3,4]

When *ginseng* extract was added to the reaction mixture for assay of the polymerase activity, however, we could not observe any effect on the RNA polymerase activity. For liver slices, or liver nuclei, we failed to observe an increasing effect of *ginseng* extract *in vitro* on the incorporation of orotate into RNA under the condition tested.

We could not find any significant change in RNase activity between control and treated nuclear preparation when labeled RNA was added to the reaction mixture omitted four nucleoside triphosphates. So the rise of the polymerase activity seems unlikely due to a drop of RNase activity in the polymerase preparation. As in previous report[3] pool size of endogenous acid-soluble purine nucleotides did not change by the treatment with *ginseng* extract.

Time Course Experiment on the Increase in RNA Polymerase Activity

As shown in Figure 1, when *ginseng* extract was administered *in vivo* an increase in the enzyme activity was observed in the presence of high concentration of ammonium sulfate after varing time of treatment. The maximum increase was 50% at 2 hr with a lag period of 30 min after the treatment. The increase was still 45% and 30% at 3 and 8 hr after the treatment, respectively, but was not significant after 16 and 24 hr. In previous report we showed that the rate of RNA synthesis of liver nuclei also increased as a response of *ginseng* extract treatment and that its rate reached maximum after 4 hr of the treatment.[4] The increase in the RNA synthesis, therefore, is likely to depend on the rise on RNA polymerase activity and to be preceded by the increase in the polymerase activity.

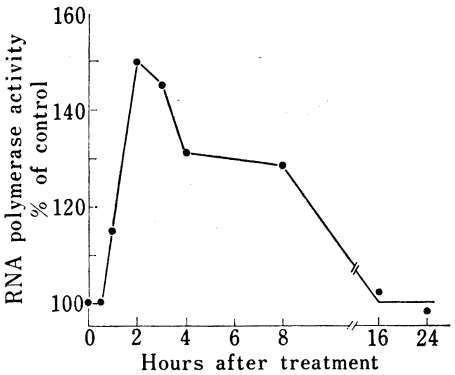

Fig. 1. Increase in the RNA Polymerase Activity of Liver Nuclei as a Function of Time after *Ginseng* Extract Treatment

Rats received a single dose of 5 mg of fraction 3 and were sacrified after varing time of the treatment. Nuclear enzyme was prepared from three pooled livers. Values shown in the figure were mean of two determinations for every enzyme preparation. As for control and 2, 4 and 8 hr treatment, eight determinations were carried out with four enzyme preparations. Incubation was carried out at 37° for 15 min in complete medium containing high concentration of ammonium sulfate.

Effect of Actinomycin D and Puromycin on Synthesis of Nuclear RNA and Protein *in vivo*

It was interesting to examine whether or not actinomycin D inhibits the rise in the RNA synthesis in the extract-treated liver in order to study the mode of action of *ginseng* principle. Actinomycin D or puromycin was injected to rats and after 30 min the extract (fraction 3) was administered to them. Incorporation of orotate into RNA of purified nuclei was determined after 3 hr of the treatment with *ginseng*. As shown in Table IV, actinomycin D (10 μg per rat) and puromycin (3.5 mg per rat) caused 14% and 4% of inhibition, respectively, in the rate of RNA synthesis for untreated groups of rats. For the *ginseng* treated, the rate in RNA synthesis increased 1.65 fold over control. When the rat was administered the small amount of the inhibitors and then treated with *ginseng* extract, the rate was still 1.39 fold increase for actinomycin and 1.46 for puromycin. Thus the increase over control of the RNA synthesis due to *ginseng* extract was still observed for tested dosage of the inhibitors. Higher dose of actinomycin D (125 μg per rat) caused 45% of inhibition with or without treatment of *ginseng* extract.

TABLE IV. Effects of Actinomycin D and Puromycin on the Rise in Nuclear RNA Synthesis Dependent on *Ginseng* Extract Treatment

Treatment	Inhibitor	^{14}C-Orotate incorporation cpm per mg protein	Per cent of control
None	none	46300	100
	actinomycin, 10 μg	39600	86
	puromycin, 3.5 mg	44600	96
Treated	none	76300	165
	actinomycin, 10 μg	64600	139
	puromycin, 3.5 mg	67400	146

Fraction 3 (5 mg) was injected to rat 3 hr before sacrificing. Actinomycin D or puromycin was injected 30 min before *ginseng* treatment. Each rat was pulse-labeled with ^{14}C-orotate (2μ Ci) for 20 min.

TABLE V. Effects of *Ginseng* Extract and Puromycin on Nuclear Protein Synthesis

Expt. No.	Ginseng treatment	Puromycin	Leucine incorporated cpm per mg protein	Per cent of control
1	control nuclei	−	166	100
		+	110	66
	treated nuclei	−	200	120
		+	114	69
2	control nuclei	−	90	100
		+	20	22
	treated nuclei	−	113	126
		+	25	28
	control homogenate	−	81	100
		+	17	22
	treated homogenate	−	95	117
		+	22	27

Ginseng extract (fraction 3, 5 mg) was injected 3 hr before sacrifice. In Expt. 1, 10 mg of puromycin was administered by a single intraperitoneal injection 30 min prior to fraction 3 treatment. ^{3}H-Leucine, 4 μCi, was pulsed 2 hr before decapitation of rat. In Expt. 2, 5 mg of puromycin was administered 30 min before and 60 min after the fraction 3 treatment. 1 μCi of ^{14}C-leucine was injected 2 hr before sacrifice.

Table V shows the small but reproducible increase of incorporation of leucine into protein of liver nuclei or liver homogenate after 3 hr of the treatment. When administered a single dose of puromycin before the extract- or saline-treatment, inhibition of protein synthesis in liver nuclei was 30 per cent (Expt. 1). Also, injection of two doses of puromycin,

before and after treatment with the extract, caused 70—80% reduction on protein synthesis in liver nuclei and homogenate (Expt. 2). Both *ginseng*-treated and control groups showed the similar degree of inhibition by puromycin.

Effect of Puromycin on RNA Polymerase Activity

RNA polymerase activity was determined with aliquots of the same purified nuclei as used in experiments shown in Table V. Table VI shows that RNA polymerase of the rat treated with the extract had approximately 1.4 fold greater activity than that of controls. Puromycin could not block the increase of RNA polymerase activity due to *ginseng* extract, while protein synthesis was greatly inhibited by puromycin (Table V).

TABLE VI. Effects of Puromycin on the Rise of RNA Polymerase Activity due to *Ginseng* Extract

Expt. No.	Group	Puromycin	^{3}H-CMP incorporated cpm per mg protein	Per cent of control
1	control	−	3340	100
		+	3750	115
	treated	−	4810	144
		+	5410	162
2	control	−	4460	100
		+	5230	117
	treated	−	6230	140
		+	6450	145

Treatment of rats was the same as described in Table V. Polymerase activity was determined as in methods. Each value was average of two determinations.

Puromycin treatment *in vivo* caused about 15% of increase in the polymerase activity. Similar increase, but not refered to it, was seen in the results of Noteboom and Gorski.[16] The cause of the increase is not clear but it might have connection with the reported effects of puromycin on the cyclic AMP phosphodiesterase.[17]

Discussion

In vitro incorporation of ^{3}H-CMP into RNA was determined using a deoxycholate-lysed nuclei of rat liver. The character of the lysed nuclear enzyme preparation was similar to that of the "aggregate" RNA polymerase described by Weiss[15] (see Table I). Activity of RNA polymerase in the lysed nuclear preparation of rat liver was determined after varing time of *ginseng* extract treatment *in vivo*. A rise in RNA polymerase activity was observed in the absence of ammonium sulfate (Table I). These *in vitro* results suggest to couple with the rise in RNA synthesis by liver nuclei *in vivo*.[3] Maximum rate of synthesis of bulk nuclear RNA was at 4 hr after *ginseng*-treatment.[4] Since the maximum rise of RNA polymerase activity occured at 2 hr after the treatment (Figure 1), it seems likely that the increase in the synthesis of bulk nuclear RNA is preceded by the rise in RNA polymerase activity and depends on the rise in RNA polymerase activity when *ginseng* extract is administered.

Present increase in the enzyme activity was also found in the presence of high concentration of ammonium sulfate and the increase due to *ginseng* was of the same order as in the absence of the salt (Table I). The corresponding results were also reported by Breuer and Florini[8] for RNA polymerase when porcine growth hormone was administered.

When *ginseng* extract was added to the incubation medium for the assay of RNA polymerase, no stimulation of the enzyme activity was observed. The effect of *ginseng* extract,

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17) M.M. Appleman and R.G. Kemp, *Biochem. Biophys. Res. Comms.*, **24**, 564 (1966).

therefore, may be different from the stimulating effect of ammonium sulfate.[18] The present rise in the polymerase activity is unlikely due to a drop of RNase activity in the polymerase preparation obtained from the treated rat since we could not find significant difference between control and treated one.

Gorski[7] found that RNA polymerase activity of nuclear preparation from rat uteri was increased after estradiol treatment *in vivo* and the difference due to estrogen was abolished in the presence of high concentration of ammonium sulfate. Pegg and Korner[19] and Breuer and Florini[8] also reported similar results for rat liver enzyme treated with human growth hormone and muscle enzyme treated with testosterone *in vivo*. The results suggested that the hormone caused an increase in the activity of the enzyme but not an increase in the amount of the enzyme.[7] Our result was different from these results, that is, the increase in the polymerase activity due to *ginseng* treatment was not abolished in the presence of the salt. If the converse were true, the present increasing effect of *ginseng* principle on the RNA polymerase activity would be a consequence of an increase in amount of the enzyme.

On the other hand, Breuer and Florini[8] studied the effect of ammonium sulfate with a system of bacterial soluble enzyme and muscle chromatin from castrates treated with testosterone. They clearly showed that administration of testosterone increased the priming activity of chromatin and that the difference in the activity between control and androgen-treated chromatin was masked by exposure to the salt during preparation. Chambon, *et al.*[20] reported that the activity of RNA polymerase, measured in the presence of ammonium sulfate, was only related to the amount of RNA polymerase previously bound to DNA in reconstituted deoxynucleohistone and soluble enzyme (purified *E. coli* or partially purified liver enzyme). It was also shown that presence of ammonium sulfate caused an inhibition of the RNA polymerase which is already complexed with DNA and newly synthesized (complementary) RNA. Goldberg[18] also observed the similar inhibition with HeLa "aggregate" enzyme (higher than about 0.3 M of ammonium sulfate). On the other hand, as for soluble enzyme (unbound with DNA)[21,22] RNA polymerase reaction was greatly inhibited by addition of high concentration of amonium sulfate which stimulated maximally the RNA polymerase activity of "aggregate" enzyme (see also the results of Anthony, *et al.*[23] with bacterial enzyme). Thus it is suggested that the stimulating effect of ammonium sulfate on "aggregate" enzyme activity was a balance due to two opposite effects: 1) a stimulation by removing histone from DNA-histone complex; 2) an inhibition on the RNA polymerase-DNA complex.[20] If this were the case the present increasing effect of *ginseng* extract on the enzyme activity would be a consequence of an increase in amount of enzyme and/or an increase in amount of DNA-enzyme complex.

As shown in Table IV, the dose of actinomycin D and puromycin which inhibited slightly the synthesis of nuclear RNA in control rat, could not abolished the increase in the RNA synthesis due to *ginseng* extract when the inhibitors were administered prior to *ginseng* treatment. Administration of large amount of puromycin before *ginseng* treatment greatly inhibited protein synthesis and abolished the increase in protein synthesis due to *ginseng* principle in liver nuclei and homogenates. For the same treated preparation of nuclei, the response to the *ginseng* extract in RNA polymerase activity was still observed with or without puromycin treatment. In connection with the effect of ammonium sulfate discussed above, the increase in polymerase activity may be due to an increase in amount of enzyme-DNA complex, slowing down of possible degradation of the enzyme. Another posibility is increase of the

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19) A.E. Pegg and A. Korner, *Nature*, **205**, 904 (1965).
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23) D.D. Anthony, E. Zeszotek, and D.A. Goldthwait, *Proc. Natl. Acad. Sci. U.S.A.*, **56**, 1026 (1966).

enzyme protein while synthesis of most other nuclear proteins was inhibited. However, the increase in the amount of the enzyme seems to be less probable.

The context of the discussion described above is whether the activation is translational or transcriptional. Increased activity of the polymerase due to *ginseng* extract may, at least, not be translational. In connection with the activation, results on cyclic AMP and catabolite gene activator protein in *E. coli*[24] are full of interest.

We found the *ginseng* extract affected on the metabolism of sugar and lipid[25] as well as nucleic acid and protein. The increased activity of the nuclear RNA polymerase due to *ginseng* extract is an early response and it will support that the principle acted on the synthesis of serum protein in rat by regulating transcription of genetic message. However, the response of RNA polymerase activity might be one of many, direct or indirect, responses due to the principle. The earliest, primary action of *ginseng* principle is still uncertain and the mode of action remains to be clarified.

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