Effect of Radix Ginseng Extract on Serum Protein Synthesis

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Intraperitoneal administration of ginseng extract (fraction 3 or 4) to rats was found to
increase the rate of synthesis of serum proteins such as albumin and γ-globulin. The
incorporation rate of 3H-leucine into serum proteins was significantly increased 4 hr after
the injection of fraction 3. The increased rate of protein synthesis reached a maximum
about 8—12 hr after the administration and 46—49% increase in the rate of serum protein
synthesis was observed. It was confirmed by immunochemical precipitation reaction
that the increase in serum protein synthesis was due to the stimulation of albumin and
γ-globulin synthesis. The rate declined to the control level at 20 hr following ginseng
extract treatment.

These observations are similar to the effect of cortisone on the liver. Although the
mechanism of this increase in the rate of serum protein synthesis by ginseng extract is not
known, some differences were observed in effects between fraction 4 and cortisone as follows.
Induction of tryptophan pyrroline and tyrosine transaminase, which is observed with
cortisone, did not occur by fraction 4 administration. These results suggest that the ob-
served effect of ginseng extract would not be due to the elevated concentration of adrenocor-
tical steroids.

From this point of view, this stimulating factor, which we term “prostisol” (protein
synthesis stimulating factor), is active to RNA and protein synthesizes when administered
in vivo.

As reported previously,3—6) treatment of rats with extracts from roots of Panax ginseng
C.A. MEYER produced an increase in the incorporation of labeled precursor into nuclear
and cytoplasmic RNA, and in DNA dependent RNA polymerase activity in the liver. These
effect of ginseng extract would be expected to result in the stimulation of synthesis of serum
protein. Serum albumin is formed largely or entirely in the liver.5,9) Production of γ-
globulin has been observed in various tissues such as the spleen, lymph node, bone marrow,
liver, lung, and intestine.9—11) Therefore, effect of ginseng extract on the rate of protein
synthesis was examined. Administration of ginseng extract was found to increase the rate
of synthesis of serum proteins, such as serum albumin, γ-globulin, β-globulin, and α-globulin,
which examined by DEAE-cellulose column chromatography. It was further confirmed by
immunological technique that the rate of serum albumin and γ-globulin synthesis was acce-
cerated by ginseng extract administration in vivo.

1) This work was presented at the 86th Annual Meeting of the Pharmaceutical Society of Japan, Sendai,
October 1966.
2) Location: 3190, Gofuku, Toyama, 930, Japan.
8) L.L. Miller, H.R. Hanavan, N. Tithasiri, and A. Chowdhury, “Amino Acids and Serum Proteins,”
Liver nuclear preparations of cortisol-treated rats showed a rise in RNA polymerase activity significantly above that of preparations from the control rats\textsuperscript{12-14} and an increase in the rate of RNA synthesis.\textsuperscript{15-17} A variety of acute stresses has been found to induce a similar effect as described above.\textsuperscript{18} However, in contrast to the induction of numerous enzymes by cortisone,\textsuperscript{15} administration of ginseng extract did not affect the activity of tryptophan pyrroline.

From this point of view, the mechanism of this increase in the rate of RNA and protein syntheses by ginseng extract was found to be different from that of cortisone. This stimulating factor, which we term for convenience "prostisol," is active to RNA and protein synthesis when administered in vivo.

Material and Method

Animals—Female Wistar rats weighing about 100 g and female rabbits weighing about 2 kg were used for these experiments. These animals were obtained locally. Rats were fasted for 16–18 hr before the experiments.

Materials—U-\textsuperscript{3}H-L-Leucine (144 mCi/mmol) and U-\textsuperscript{14}C-L-leucine (0.1 mCi/0.0558 mg) were purchased from the New England Nuclear Corp., U.S.A. Bacterial a-amylase (EC 3.2.1.1) of B. subtilis (BoA) was obtained from Nagase Corp. and cortisol acetate was from the Nippon Merk-Banyu Corp. DEAE-cellulose powder produced by Serva Corp. and Sephadex G-100 by Pharmacia Corp. were employed.

Extraction and Partial Purification 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.\textsuperscript{9} 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 obtained in 4% yield from the ginseng powder. Fraction 3 was extracted with 90% 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. Fraction 4 was obtained in 35% yield from fraction 3. Preliminary chemical examination of fraction 4 gave positive Lieberman–Burchard reaction, and it contained 70–75% of hexose by the Tsuchita and Akabori's method.\textsuperscript{10} mp 180–190°.

Incorporation of Labeled Leucine into Serum Proteins—Rats were injected intraperitoneally with fraction 3 or 4 in 0.5 ml of saline. Control animals were treated with the same volume of saline. At the desired time after administration of ginseng extract, the rats received intraperitoneally \textsuperscript{3}H-leucine in saline. Blood samples were collected by heart puncture 1 hr later under ether anesthesia, allowed to clot at room temperature for 4 hr, and then centrifuged. The sera obtained in this manner were dialyzed overnight against 100–200 volumes of 0.0175M phosphate buffer (pH 6.3) in the cold. To fractionate serum proteins, the column of DEAE-cellulose was employed as described by Sober, et al.\textsuperscript{9} Each fraction (3.8 ml) or 0.1 ml of the initial serum was treated with 5% trichloroacetic acid containing Hyflo Super-Cel (20 mg/ml). The suspension was transferred to a filter disc of filter paper which had previously been coated with a thin-layer of Hyflo Super-Cel.\textsuperscript{9} After washing with 20 ml of trichloroacetic acid followed by ethanol and ether, the samples were air dried. The dried disc was then suspended in 0.5 ml of 1M Hyamine in methanol and 10 ml of a scintillation mixture (0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis[2-(5-phenyloxazolyl)]benzene in toluene). The radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer, Model 3003.

Preparation of Rabbit Anti-Rat Serum Albumin and Anti-Rat $\gamma$-Globulin—Isolation of rat serum albumin and $\gamma$-globulin was carried out as follows. Serum globulin was collected by (NH$_4$)$_2$SO$_4$ precipitation at 50% saturation. The supernatant solution was dialyzed against 0.0175M phosphate buffer and chromatographed on a column of DEAE-cellulose.\textsuperscript{90} Rechromatography of the fraction eluted with 0.1M phosphate

\textsuperscript{14} O. Barnabei, B. Roman, and G. Di Bitonio, Arch. Biochem. Biophys., 109, 266 (1965).
\textsuperscript{17} F.T. Kenney, J. Biol. Chem., 237, 1605 (1962).
\textsuperscript{19} A. Tsuchita and S. Akabori, J. Biochem. (Tokyo), 46, 695 (1959).

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buffer (pH 5.8) was carried out under the same condition. Rabbits were immunized with 5 mg of albumin in 1 ml of saline emulsified with 1 ml of Freund's incomplete adjuvant. Three injections were given at intervals of one week. Two weeks after the last immunization, blood was taken and anti-rat serum albumin fraction was precipitated at 33% (NH₄)₂SO₄ saturation from anti-serum.

Rat γ-globulin was prepared from anti-sera of hyperimmunized rats with BaA as follows. Specific anti-BaA was precipitated by addition of an equivalent amount of BaA to the anti-serum. Antigen-antibody precipitate was collected and dissolved in 1N acetic acid. γ-Globulin was separated from BaA by column chromatography over Sephadex G-100 using 1N acetic acid as the eluant. With this γ-globulin (5 mg), rabbits were immunized as described above and anti-γ-globulin was prepared. To determine the proportions of antigen to antibody in the equivalence zone, increasing amounts of serum were added to each 0.5 ml of antibody solution in saline (5 mg/ml). It was found that 2.5 mg of antibody gave a maximum precipitation with 0.5 ml of 500-fold diluted rat serum in albumin-anti-albumin system and with 0.5 ml of 12.5-fold diluted rat serum in γ-globulin-anti-γ-globulin system. To estimate the radioactivity of the antigen-antibody precipitates, 40 mg of each antibody was added to 4 ml of each diluted serum, the precipitate was removed by centrifugation, and its radioactivity counted as described above.

**Assay for Tryptophan Pyrrolase and Tyrosine-a-ketoglutarate Transaminase**—Rats were injected intraperitoneally with a single dose of cortisone (5 mg/100 g body weight) or with fraction 4 (5 or 10 mg/100 g body weight). The steroid was administered as a suspension in 0.5 ml of saline. The same volume of saline was given to the control rats. These rats were sacrificed by decapitation and the enzyme activities in the liver were estimated. Tryptophan pyrrolase was determined by the method of Knox, et al.²¹ Tyrosine-a-ketoglutarate transaminase was assayed as described by Rosen, et al.²²

**Result**

**Rate of Synthesis of Serum Proteins as a Function of Time after Ginseng Extract Administration**

In previous experiments,²³ 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 and cytoplasmic polysomal RNAs. It was found that the incorporation rate was directly dependent on the amount of fraction 4 administered to a rat. Therefore, the rate of synthesis of serum albumin, which is produced largely in the liver, would be increased by ginseng extract treatment. Effect of ginseng extract on the rate of serum protein synthesis is shown in Table I. The administration of ginseng extract increased the rate of serum protein synthesis and the incorporation rate was directly dependent on the amount of fraction 4. As seen in Table II, a significant increase in the incorporation rate of labeled leucine into serum proteins was observed 4 hr after intraperitoneal injection of 5 mg of fraction 3. It reached a maximum level about 8—12 hr after injection and represented 46—49% increase. The rate of synthesis decreased to the control level 20 hr after the administration of fraction 3.

**Table I.** Effects of Fraction 3 and 4 on the Incorporation of Labeled Leucine into Rat Serum

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Material</th>
<th>Dose (mg)</th>
<th>No. of rats</th>
<th>Labeled leucine (cpm/0.05 ml serum)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>control (saline)</td>
<td>5</td>
<td>5</td>
<td>458±16.7</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>fraction 3</td>
<td>5</td>
<td>5</td>
<td>639±16.2</td>
<td>140</td>
</tr>
<tr>
<td>3</td>
<td>control (saline)</td>
<td>5</td>
<td>3</td>
<td>3652±109.0</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>fraction 3</td>
<td>5</td>
<td>3</td>
<td>5127±65.1</td>
<td>140</td>
</tr>
<tr>
<td>5</td>
<td>control (saline)</td>
<td>0.01</td>
<td>4</td>
<td>440±13.3</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>fraction 4</td>
<td>0.05</td>
<td>2</td>
<td>450±17.5</td>
<td>102</td>
</tr>
<tr>
<td>7</td>
<td>fraction 4</td>
<td>0.5</td>
<td>2</td>
<td>614±4.5</td>
<td>140</td>
</tr>
<tr>
<td>8</td>
<td>fraction 4</td>
<td>5</td>
<td>2</td>
<td>743±6.5</td>
<td>169</td>
</tr>
</tbody>
</table>

At 8 hr after treatment, labeled l-leucine was injected (experiment 1, l³H-leucine 4 μCi; experiment 2, l³H-leucine 40 μCi; experiment 3, l³C-leucine 1 μCi per rat). The data are expressed as per cent of the values of untreated control rats which are taken as 100%. The data are presented as mean ± S.E.

TABLE II. Effect of Fraction 3 on the Incorporation of 
\(^3\)H-Leucine into Serum Protein

<table>
<thead>
<tr>
<th>Time after fraction 3 treatment (hr)</th>
<th>No. of rats</th>
<th>(^3)H-Leucine (cpm/0.1 ml serum)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(^a)</td>
<td>5</td>
<td>555 ± 34</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>759 ± 45</td>
<td>137</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>811 ± 39</td>
<td>146</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>826 ± 40</td>
<td>149</td>
</tr>
<tr>
<td>16</td>
<td>3</td>
<td>644 ± 60</td>
<td>116</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
<td>617 ± 26</td>
<td>111</td>
</tr>
</tbody>
</table>

\(^a\) Control rats were sacrificed 8 hr after saline-treatment.

At the desired time after fraction 3 (5 mg) administration, each rat received intraperitoneally 4 \(\mu\)Cl of \(^3\)H-leucine. One hr later, blood sample were taken by heart puncture. The data are expressed as per cent of the values of untreated control rats which are taken as 100\%. The data are presented as mean ± S.E.

An attempt was made to fractionate these labeled serum proteins by the DEAE-cellulose column chromatography which was carried out by stepwise elution using phosphate buffer as described by Sober, et al.\(^a\) Fig. 1 illustrates these results. The elution pattern of control rat sera is shown in Fig. 1 A and that of fraction 3-treated rat sera, which were taken 10 hr after the administration of fraction 3, is shown in Fig. 1 B. The increased labeling of each fraction was observed in fraction 3-treated rats as compared with the control rats. Not only the rate of synthesis of albumin fraction but also the rate of synthesis of other fractions such as \(\gamma\)-globulin, \(\beta\)-globulin, and \(\alpha\)-globulin was found to be increased. About 60, 40, 50 and 70\% increase in the specific radioactivities (cpm per optical density unit at 280 nm) was observed for \(\gamma\)-globulin, \(\beta\)-globulin, albumin, and \(\alpha\)-globulin, respectively. These results suggest that fraction 3 enhanced the rate of synthesis of various serum proteins.

![Fig. 1 A. Elution Pattern of Control Rat Serum from DEAE-cellulose Column](image)

![Fig. 1 B. Elution Pattern of Fraction 3-Treated Rat Serum from DEAE-cellulose Column](image)

**Immunoechemical Characterization of labeled Serum Albumin and \(\gamma\)-Globulin**

To characterize the serum proteins which are formed in an enhanced rate by the administration of fraction 3, immunoechemical precipitation reaction was employed. Anti-rat serum albumin and anti-\(\gamma\)-globulin were prepared from rabbit anti-sera and the proportion
of an antigen (labeled rat serum protein) to antibody in the equivalence zone was determined. Radioactivities of antigen-antibody precipitates obtained in the equivalence zone are shown in Table III. Sera were taken 10 hr after the administration of fraction 3. Counts per minute (cpm) per 0.1 ml of serum were found to be raised by 46% above that of the control. Albumin-anti-albumin precipitates showed 37% increase in the radioactivity and 93% increase in the rate of γ-globulin synthesis was observed at equivalence zone by fraction 3. The results from the column chromatography as above were further confirmed for albumin and γ-globulin by the immunochromatography precipitation reaction.

**Table III. Effect of fraction 3 on the Rate of Synthesis of Serum Protein, Albumin and γ-Globulin**

<table>
<thead>
<tr>
<th>Reciprocal of dilution No. of serum</th>
<th>3H-leucine cpm</th>
<th>(%) increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>cpm in 0.1 ml of serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5/800</td>
<td>4066</td>
<td>6706</td>
</tr>
<tr>
<td>2.0/800*</td>
<td>195</td>
<td>255</td>
</tr>
<tr>
<td>cpm in albumin-anti-albumin ppt.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0/25</td>
<td>398</td>
<td>796</td>
</tr>
<tr>
<td>2.5/25*</td>
<td>447</td>
<td>856</td>
</tr>
<tr>
<td>cpm in γ-globulin-anti-γ-globulin ppt.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rats were injected intraperitoneally with 5 mg of fraction 3 in 0.5 ml of saline. Control groups were treated with the same volume of saline. These rats received intraperitoneally 20 μCi of 3H-leucine 9 hr after fraction 3 administration. One hr later, blood samples were taken and incorporation of 3H-leucine into albumin and γ-globulin were estimated as described in Methods.

Effect of Fraction 4 on Tryptophan Pyrrolase and Tyrosine-α-ketoglutarate Transaminase

These observations on the ginseng extract seem to be similar to the effect of cortisone. Cortisone injection has been found to increase the rate of RNA synthesis and numerous enzyme synthesis in the liver. Activities of tryptophan pyrrolase and tyrosine-α-ketoglutarate transaminase were estimated after the administration of fraction 4 or cortisone to compare their effect. These enzymes were assayed 6 hr after the reagent injection when induction of tryptophan pyrrolase by cortisone is most marked.

**Table IV. Effect of Fraction 4 on Tryptophan Pyrrolase and Tyrosine Transaminase**

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Materials</th>
<th>Dose (mg)</th>
<th>Tryptophan pyrrolase (unit)</th>
<th>%</th>
<th>Tyrosine transaminase (unit)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>control (saline)</td>
<td>5</td>
<td>1.47±0.08</td>
<td>100</td>
<td>392±25</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>fraction 4</td>
<td>10</td>
<td>1.35±0.10</td>
<td>92</td>
<td>485±18</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td>fraction 4</td>
<td>5</td>
<td>1.70±0.13</td>
<td>115</td>
<td>455±33</td>
<td>116</td>
</tr>
<tr>
<td></td>
<td>cortisone</td>
<td>5</td>
<td>5.20±0.29</td>
<td>353</td>
<td>940±44</td>
<td>240</td>
</tr>
<tr>
<td>2</td>
<td>control (saline)</td>
<td>5</td>
<td>1.02±0.08</td>
<td>100</td>
<td>226±10</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>cortisone</td>
<td>10</td>
<td>3.31±0.09</td>
<td>324</td>
<td>949±27</td>
<td>420</td>
</tr>
<tr>
<td></td>
<td>and fraction 4</td>
<td>5</td>
<td>3.32±0.11</td>
<td>325</td>
<td>950±35</td>
<td>420</td>
</tr>
</tbody>
</table>

Rats were treated with a single dose of each reagent. Control rats were injected with the same volume of saline. These enzyme activities were assayed 6 hr after treatment. The data are expressed as per cent of the values of untreated control rats which are taken as 100%. Each value is the mean±S.E. of the results obtained with five rats (expt. 1) or three rats (expt. 2).

As shown in Table IV, tryptophan pyrrolase activity was increased 3.3—3.5 fold by cortisone treatment. In contrast to the effect of cortisone (5 mg/100 g body weight), fraction 4 (5 or 10 mg/100 g body weight) does not appear to alter the level of the enzyme activity. Tyrosine-α-ketoglutarate transaminase seem to increase slightly by treatment with
fraction 4, but increase in tyrosine-α-ketoglutarate transaminase activity is not so clear as the rise by cortisone. Fraction 4 does not affect the activity of tryptophan pyrrolase which is induced by cortisone. On the other hand, as reported previously,"5,4,6" we demonstrated that fraction 3 or 4 (5 mg/100 g body weight) enhances the incorporation rate of labeled precursor into nuclear and cytoplasmic polysomal RNAs at 4 or 6 hr after a single dose injection. These observations suggest that the mechanism of increase in the rate of RNA and protein syntheses in liver by ginseng extract would be quite different from that of increase by cortisone.

Discussion

Injection of several hormones has generally been found to stimulate RNA synthesis in each target organ."23" It was shown in the previous paper,"3-6" that the administration of ginseng extract increased the incorporation rate of labeled precursor into nuclear and cytoplasmic RNA in the liver, and an increase of RNA polymerase activity in the liver nuclei and of heavy polysome content after the treatment had been observed during this series. In an amino acid incorporation system in vitro, stimulatory activities of microsomes and polysomes from treated rat liver are more active than those from normal liver.

On the basis of the present evidence, the enhanced formation of RNA by the ginseng extract appears to increase the rate of serum protein synthesis. As shown in Fig. 1 A and B, administration of fraction 3 enhanced the rate of synthesis of γ-globulin, β-globulin, albumin, and α-globulin (presumably all of serum proteins made in the liver). Serum albumin and γ-globulin, which were synthesized at accelerated rate by fraction 3-treatment, were further confirmed by immunochemical precipitation reaction.

These observations are very similar to alterations in the liver produced by cortisone administration. An enhancement in the rate of RNA and serum protein syntheses by ginseng extract treatment might be due to elevated concentration of adrenocortical steroids. However, this possibility was eliminated by the following observations. Activities of tryptophan pyrrolase and tyrosine-α-ketoglutarate transaminase, which are markedly induced by cortisone injection, were not significantly affected by ginseng extract. Within 4 hr after a single intraperitoneal injection, cortisone has been reported to cause an approximately 3-fold increase in the amount of tryptophan pyrrolase enzyme protein."18" 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."24" Therefore, it was suggested that the observed of ginseng extract are not mediated by the adrenocortical hormone.

We have summarized the sequential stimulation of biochemical effects in the liver nucleus, cytoplasm, and serum protein as a result of a single dose injection of ginseng extract (fraction 3 or 4) in the rat.

The first phenomenon observed recorded a stimulation in the Mg²⁺-activated DNA-dependent RNA polymerase activity beginning 1 hr after administration of ginseng extract in vivo, and reaching a value of 50% increase at 2 hr."9" 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 5.5—6.0 hr after the fraction 4 was given, thus elevating the hepatic polysome content and the protein synthetic activity."6" Finally, in the present work, synthesis of serum protein was gradually stimulated and it reached a maximum level about

24) H. Oura and H. Seno, at the 88th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1968.
8—12 hr after treatment. However, all the effects induced by ginseng extract returned almost to the control level about 24 hr after the treatment (Fig. 2).

Similar phenomena in mammals were shown by the administration of thyroid hormone to the thyroidectomized rats by Tata and Widnell, except for a long period response.

In the present work, administration of fraction 3 or 4 enhanced the rate of synthesis of all the serum proteins. On the basis of these experimental results, we suggest that the action of ginseng extract is the metabolic stimulation or hormone-like action on the RNA and protein biosyntheses. This stimulating factor, which we term for convenience "prostisol" (protein synthesis stimulating factor), is active to RNA and protein syntheses when administered in vivo.

Fig. 2. Summary of Data on the Sequential Stimulation by ginseng Extract (Fraction 3 or 4)

- •, liver nuclear RNA polymerase activity; ▲, nuclear RNA synthesis; ○, polysomal RNA synthesis; Δ, serum protein synthesis. These curves were combined from this series paper.