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Effect of Radix Ginseng Extract on Serum Protein Synthesis[1]

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Intraperitoneal administration of *ginseng* extract (fraction 3 or 4) to rat was found to increase the rate of synthesis of serum proteins such as albumin and γ-globulin. The incorporation rate of ^{3}H-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 pyrrolase and tyrosine transaminase, which is observed with cortisone, did not occur by fraction 4 administration. These results suggest that the observed effect of *ginseng* extract would not be due to the elevated concentration of adrenocortical steroids.

From this point of view, this stimulating factor, which we term "prostisol" (protein synthesis stimulating factor), is active to RNA and protein syntheses 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.[7,8] 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 accelerated by *ginseng* extract administration *in vivo*.

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).
4) H. Oura, S. Hiai, and H. Seno, *Chem. Pharm. Bull.* (Tokyo), **19**, 1598 (1971).
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Liver nuclear preparations of cortisol-treated rats showed a rise in RNA polymerase activity significantly above that of preparations from the control rats[12–14] and an increase in the rate of RNA synthesis.[15–17] A variety of acute stresses has been found to induce a similar effect as described above.[18] However, in contrast to the induction of numerous enzymes by cortisone,[15] administration of *ginseng* extract did not affect the activity of tryptophan pyrrolase.

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-^{3}H-L-Leucine (144 mCi/mM) and U-^{14}C-L-leucine (0.1 mCi/0.0558 mg) were purchased from the New England Nuclear Corp., U.S.A. Bacterial α-amylase (EC 3.2.1.1) of *B. subtilis* (BαA) was obtained from Nagase Corp. and cortisone 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.[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 obtained in 4% yield from the *ginseng* powder. 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*. Fraction 4 was obtained in 35% yield from fraction 3. 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.[19] 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 ^{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.*[20] 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.[3] 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 γ-Globulin——Isolation of rat serum albumin and γ-globulin was carried out as follows. Serum globulin was collected by $(NH_4)_2SO_4$ precipitation at 50% saturation. The supernatant solution was dialyzed against 0.0175M phosphate buffer and chromatographed on a column of DEAE-cellulose.[20] Rechromatography of the fraction eluted with 0.1M phosphate

12) N. Land and C.E. Sekeris, *Z. Physiol. Chem.*, **339**, 238 (1964).
13) N. Land and C.E. Sekeris, *Life Sci.*, **3**, 391 (1964).
14) O. Barnabei, B. Roman, and G. Di Bitonio, *Arch. Biochem. Biophys.*, **109**, 266 (1965).
15) P. Feigelson, M. Feigelson, and O. Greengard, *Rec. Progr. Horm. Res.*, **18**, 491 (1962).
16) P. Feigelson and O. Greengard, *J. Biol. Chem.*, **237**, 3714 (1962).
17) F.T Kenney, *J. Biol. Chem.*, **237**, 1605 (1962).
18) C. Majumdar, K. Tsukada, and I. Lieberman, *J. Biol. Chem.*, **242**, 700 (1967).
19) A. Tsugita and S. Akabori, *J. Biochem.* (Tokyo), **46**, 695 (1959).
20) H.A. Sober and E.A. Peterson, *Federation Proc.*, **17**, 1116 (1958).

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% $(NH_4)_2SO_4$ saturation from anti-serum.

Rat γ-globulin was prepared from anti-sera of hyperimmunized rats with BαA as follows. Specific anti-BαA was precipitated by addition of an equivalent amount of BαA to the anti-serum. Antigen-antibody precipitate was collected and dissolved in 1N acetic acid. γ-Globulin was separated from BαA 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 400-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-α-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 administrated 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-α-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,[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 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

Expt. No.	Material	Dose (mg)	No. of rats	Labeled leucine (cpm/0.05 ml serum)	%
1	control (saline)		5	458±16.7	100
	fraction 3	5	5	639±16.2	140
2	control (saline)		3	3652±109.0	100
	fraction 3	5	3	5127±65.1	140
3	control (saline)		4	440±13.3	100
	fraction 4	0.01	2	450±17.5	102
	fraction 4	0.05	2	540± 1.6	123
	fraction 4	0.5	2	614± 4.5	140
	fraction 4	5	2	743± 6.5	169

At 8 hr after treatment, labeled L-leucine was injected (experiment 1, ^{3}H-leucine 4 μCi; experiment 2, ^{3}H-leucine 40 μCi; experiment 3, ^{14}C-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.

21) W.E. Knox and V.H. Auerbach, *J. Biol. Chem.*, **214**, 307 (1955).
22) F. Rosen, H.R. Harding, R.J. Milholland, and C.A. Nichol, *J. Biol. Chem.*, **238**, 3725 (1963).

Table II. Effect of Fraction 3 on the Incorporation of ^{3}H-Leucine into Serum Protein

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

At the desired time after fraction 3 (5 mg) administration, each rat received intraperitoneally 4 μCi 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.

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

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.*[20] 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 γ-globulin, β-globulin, and α-globulin was found to be increased. About 60, 40, 50 and 70% increase in the specific radioactivities (cpm per optical density unit at 280 mμ) was observed for γ-globulin, β-globulin, albumin, and α-globulin, respectively. These results suggest that fraction 3 enhanced the rate of synthesis of various serum proteins.

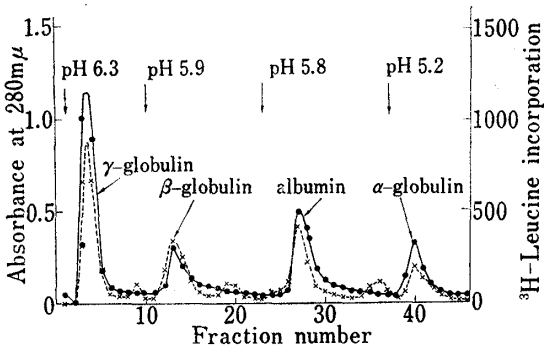

Fig. 1 A. Elution Pattern of Control Rat Serum from DEAE-cellulose Column

One ml of dialyzed control rat serum (38.8 optical density units at 280 mμ, 5460 cpm) was applied on the column of DEAE-cellulose and eluted with 0.0175 M phosphate buffer (pH 6.3), 0.04 M phosphate buffers (pH 5.9), 0.1 M phosphate buffer (pH 5.8) and finally with 0.4 M phosphate buffer (pH 5.2), as described by Sober, *et al.*[20] Each fraction has been reported to correspond to γ-globulin, β-globulin, albumin and α-globulin, respectively. absorbance at 280 mμ (—●—): ^{14}C-leucine incorporation (---×---)

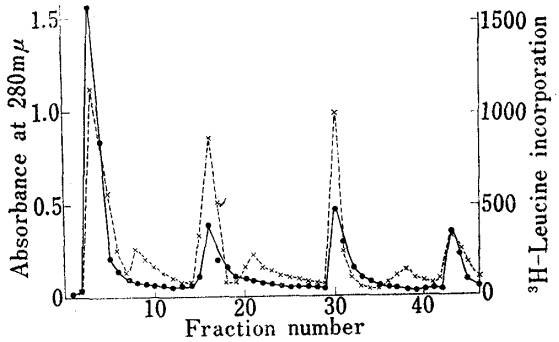

Fig. 1 B. Elution Pattern of Fraction 3-Treated Rat Serum from DEAE-cellluose Column

One ml of dialyzed fraction 3-treated rat serum (42.8 optical density units at 280 mμ, 9480 cpm) was put on the DEAE-cellulose column. Elution was carried out as described in Fig. 1 A. absorbance at 280 mμ (—●—); ^{3}H-leucine incorporation (---×---)

Immunochemical Characterization of labeled Serum Albumin and γ-Globulin

To characterize the serum proteins which are formed in an enhanced rate by the administration of fraction 3, immunochemical precipitation reaction was employed. Anti-rat serum albumin and anti-γ-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 immunochemical precipitation reaction.

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

	Reciprocal of dilution No. of serum	^{3}H-leucine cpm		(%) increase
		Control	Experiment	
cpm in 0.1 ml of serum		4606	6706	46
cpm in albumin-anti-albumin ppt.	1.5/800	195	255	31
	2.0/800[a]	201	276	37
cpm in γ-globulin-anti-γ-globulin ppt.	2.0/25	398	796	100
	2.5/25[a]	447	856	93

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 ^{3}H-leucine 9 hr after fraction 3 administration. One hr later, blood samples were taken and incorporation of ^{3}H-leucine into albumin and γ-globulin were estimated as described in Methods.
a) at equivalence zone

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.[15] 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

Expt. No.	Materials	Dose (mg)	Tryptophan pyrrolase (unit)	%	Tyrosine transaminase (unit)	%
1	control (saline)		1.47±0.08	100	392±25	100
	fraction 4	5	1.35±0.10	92	485±18	124
	fraction 4	10	1.70±0.13	115	455±33	116
	cortisone	5	5.20±0.29	353	940±44	240
2	control (saline)		1.02±0.06	100	226±10	100
	cortisone	5	3.31±0.09	324	949±27	420
	cortisone and fraction 4	5 10	3.32±0.11	325	950±35	420

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,[3,4,6] 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.[16] 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 Mg^{2+}-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.[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 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

23) J.R. Tata, "Progress in Nucleic Acid Research and Molecular Biology," Vol. 5, Academic Press, Inc., New York, N.Y., 1966, p. 191.
24) H. Oura and H. Seno, at the 88 th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, Aplil 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).

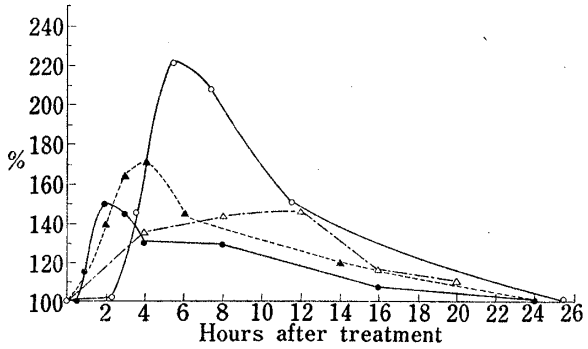

Fig. 2. Summary of Data on the Sequential Stimulations by *ginseng* Extract (Fraction 3 or 4)

●, liver nuclear RNA polymerase activity; ▲, nuclear RNA synthesis; ○, polysomal RNA synthesis; △, serum protein synthesis. These curves were combined from this series paper.[4-6]

Similar phenomena in mammals were shown by the administration of thyroid hormone to the thyroidectomized rats by Tata and Widnell,[25] except for a longperiod 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" (*pro*tein synthesis *sti*mulating factor), is active to RNA and protein syntheses when administered *in vivo*.

25) J.R. Tata and C.C. Widnell, *Biochem. J.*, **98**, 604 (1966).