THE EFFECT OF ACUTE AND CHRONIC GINSENG SAPONINS TREATMENT ON ADRENALS FUNCTION: BIOCHEMICAL AND PHARMACOLOGICAL ASPECTS

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The pharmacology of Ginseng, particularly focused to anti-stress, adaptogenic and some metabolic properties, has been studied by several authors who have usually employed preparations of unknown content. Experiments made with pure saponin preparations were generally performed so as to check selected biochemical aspects. Data so obtained make it very difficult to evaluate the real mechanism of action of Ginseng components and above all their true therapeutic efficiency.

Furthermore, many researches were carried out with high Ginseng extract doses, much larger than those given to human beings; in these cases the product was generally administered by parenteral route in order to evidence a prompt and marked action. On the contrary, very little data concerning the responses of a long term Ginseng oral treatment on experimental animals are available; this type of treatment would approach the conditions applied in human therapy.

The active components of Ginseng are considered to be a mixture of saponins belonging to steroid ones and it is almost certain that these substances possess hormone-like activities. We think therefore that it is important to verify what happens after a prolonged administration of this plant extract. Infact, very often the effects of hormones differ depending whether these substances are given in high single doses or chronically at low dosage.

In agreement with traditional medicine the most common preparations employed in therapy up to now (excluding of course, adulterated or diluted ones), are the crude generic hydroalcoholic extracts obtained from *Panax ginseng* roots, their age varying between 4 and 6 years, cultivated in Korea, in Japan or in Northern China. The chemical characterisation of this kind of extracts is based only on the ratio extract to root, with the obvious consequences of having completely erratic saponins content and qualitative composition. This fact is probably the reason why the pharmacological and clinical data reported, are not homogeneus and not reproducible in different laboratories.

Furthermore, if it is true that in saponins mixture are present two fractions showing, the former a stimulative effect, the latter sedative action, and the drug is regarded to be balancing the mental and physical equilibrium, a valid analytical method for the characterisation of the extract is absolutely necessary. In our researches on this plant, the first problem, that we have took into consideration was the standardisation of the product to use for pharmacological and clinical experiments.

First of all, we have set up a qualitative and

quantitative analytical method, based on gas-chromatographic mass spectrometric determination of the main components present in 6 years old roots of the best quality; successively we have tested hundreds and hundreds of Ginseng samples of various origin and denomination, in order to lay down a ratio among the ginsenosides and to fix the average content in total saponins into extracts.

In Fig. 1 and 2 a typical gas-chromatogram of hydroalcoholic extract and the calibration

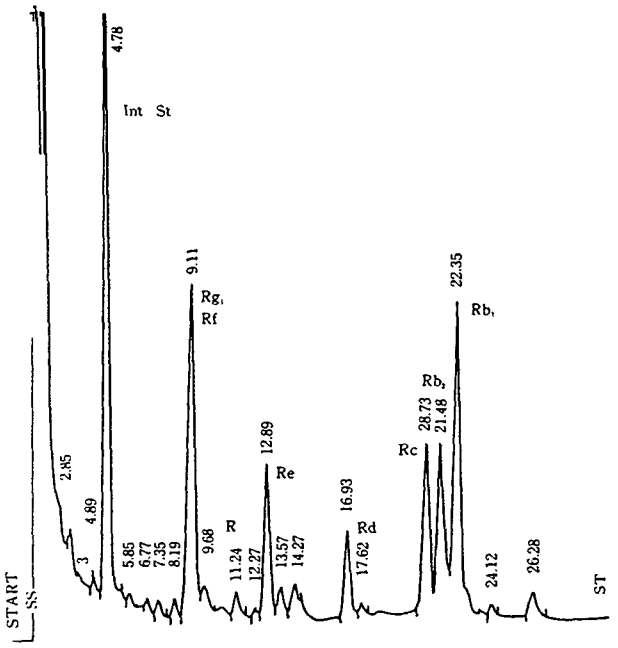

Fig. 1. GLC of *Panax ginseng* curved roots (TMS DER.)

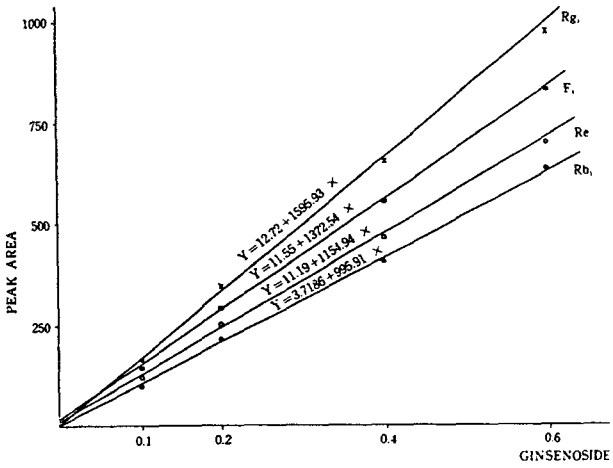

Fig. 2. Calibration curves of ginsenosides Rg_1, Re, Rb_1 and F1. (External standard).

curves of the reference standard are reported. From a practical view point, we analyse the extracts by using stachiose as an internal standard and preparing a calibration curve for each constituent. The ratio of the area of each Ginsenoside peak to the area of stachiose peak is calculated and the obtained value plotted versus the total amount of the relative Ginsenoside contained in a standard sample. The standard curve was computer-estimated by a least squares fit. The amount of the Ginsenosides in the unknowns is calculated from the standard curve.

The program calculated the averaged results, the mean calibration error and confidence limits using a 95 per cent confidence level. In Tab.

Table 1. Ginsenoside composition of some ginseng root samples

Species	Origin	Remarks	$Rg_1 \pm Rf$	Re	Rd	Rc	Rb_2	Rb_1
			% ± S.D.	% ± S.D.	% ± S.D.	% ± S.D.	% ± S.D.	% ± S.D.
Korean Ginseng (*Panax ginseng*)	Korea (Sam Geom San)	White	0.394±0.020	0.317±0.009	0.073±0.003	0.275±0.003	0.324±0.013	0.713±0.036
	Korea	Straight	0.184±0.009	0.052±0.002	0.002±0.001	0.154±0.005	0.187±0.009	0.318±0.016
	Korea	Red	0.180±0.009	0.082±0.003	0.017±0.002	0.198±0.007	0.246±0.012	0.330±0.017
	Korea	Curved	0.196±0.010	0.076±0.003	0.038±0.002	0.207±0.007	0.243±0.013	0.400±0.020
	Korea (Kiboshi)		0.280±0.014	0.700±0.006	0.030±0.002	0.170±0.006	0.181±0.011	0.501±0.025
	Korea	Sliced roots	0.260±0.013	0.150±0.010	0.010	0.190±0.009	0.200±0.015	0.460±0.023
	Korea	Slender tails	0.520±0.026	1.38 ±0.042	0.421±0.017	2.410±0.072	2.42 ±0.097	3.450±0.172
	Korea	Fasern	0.126±0.006	0.312±0.009	0.096±0.040	0.276±0.012	0.322±0.012	0.439±0.025
American Ginseng (*Panax quinguefolium*)			0.200±0.010	1.132±0.034	0.381±0.016	0.409±0.015	—	3.888±0.195

Table 2. Percent ginsenoside distribution and ginsenoside ratio of some ginseng extracts

Sample	Rg_1 + Rf	Re	Rd	Rc	Rb_2	Rb_1	Total
1	1.46 ± 0.12	1.50 ± 0.03	0.64 ± 0.03	1.68 ± 0.20	1.94 ± 0.10	3.46 ± 0.10	10.72
2	2.10 ± 0.12	1.30 ± 0.03	0.46 ± 0.04	1.38 ± 0.11	1.62 ± 0.11	3.56 ± 0.11	10.48
3	1.88 ± 0.06	1.30 ± 0.06	0.62 ± 0.06	1.16 ± 0.06	1.48 ± 0.29	3.36 ± 0.52	9.80
4	1.40 ± 0.06	1.70 ± 0.24	0.62 ± 0.07	1.24 ± 0.08	1.48 ± 0.20	3.84 ± 0.30	10.26
5	2.22 ± 0.06	1.62 ± 0.06	0.54 ± 0.06	1.50 ± 0.06	1.94 ± 0.20	4.06 ± 0.26	11.88
			Ginsenoside ratio				
1	0.42	0.43	0.19	0.48	0.56	1	
2	0.58	0.36	0.16	0.38	0.45	1	
3	0.56	0.38	0.18	0.34	0.44	1	
4	0.36	0.44	0.16	0.32	0.38	1	
5	0.55	0.40	0.13	0.37	0.48	1	

I and 2 the quantitative analysis of some samples of roots and extracts are reported. For our pharmacological trials we have chosen an hydroalcoholic standardized extract produced by us having the composition in agreement with that above mentioned. The aim of our biological study, as we had a Ginseng extract of well defined and reproducible composition, was to test its antistress activity in acute and subacute experiments and evaluate the toxicological aspects of a prolonged treatment in animals. The product was given by intraperitoneal or oral route to rats and mice.

Apart from the doubts raised against it, the natatory exhaustion test remains one of the most valid ones in the screening evaluation of Ginseng and we have used it in order to verify the activity of the extract and the dose response relationship.

In this test (table 3) our extract showed a consistent antifatigue activity after an acute parenteral treatment: this effect was accompanied by a reduced level of α-HBDH activity and consequently by a lesser lactic acid production in quadriceps muscle. The same action was seen also after a subchronic oral treatment; a statistically significant effect appeared with a daily dose of 37.5 mg/kg of Ginseng total saponins.

A more complex view about the antistress activity came from the cold exposure stress in normal or adrenalectomized rats (tables 4 and 5). A single Ginseng dose antagonized significantly the body temperature decline, without affecting blood glucose, plasma NEFA and corticosterone levels.

Under these experimental conditions its action resembles that of hydrocortisone by facilitating catecholamines activity while seems in contrast with that of ACTH which depletes adrenal catecholamines stores, without influencing their effect on peripheral circulation. As stated by Hiai, Sasoky and Oura, Ginseng activity on the

Table 3. Natatory exhaustion test in mice

Treatment mg/kg	Mode	NATATORY EXHAUSTION TIME sec			MUSCLE HBDH mU/mg
		BASAL	AFTER TREATMENT	Δ	
Saline 0.1 ml/10 g	Acute	512.2	590.8	70.9	789.8
Ginseng tot. sap. 7.5	1 day ip	521.8	1026.3	501*	618.1*
Ginseng tot. sap. 37.5		517	910.1	393.1*	640.6*
Saline 0.1 ml/10 g	Sub-acute	558.7	678	119.3	—
Ginseng tot. sap. 7.5	15 days	541.1	716.6	175.5	—
Ginseng tot. sap. 37.5	os	557.9	789.9	232*	—

*: $p < 0.05$ versus controls-Student t test.

Table 4. Cold stress (4 h at 2° C) in normal rats.

Treatment mg/kg/ip	Body temperature Δ° C	BLOOD Glucose mg/100 ml	PLASMA NEFA μEq/l	PLASMA Triglycerides mg/100 ml	PLASMA Corticosteroids μg/100 ml
Saline 0.5 ml/100g	−0.65	110.7	665	30.9	77.6
Ginseng tot. sap. 3.75	−0.24*	108.6	611.6	27.6	81.9
Saline 0.5 ml/100 g	−0.62	115.3	608.6	28.2	—
ACTH (Synth.) 5	−1.28*	126.6*	719.4*	55.8*	—
Saline 0.5 ml/100 g	−0.47	110.4	576.4	25	—
Hydrocortisone emisucc. 10	−0.32	116.9	448.3*	24.8	—

*: $p < 0.05$ versus saline group. Student t test.

Table 5. Cold stress (4 h at 2° C) in adrenalectomized rats

Treatment mg/kg/ip	Body temperature Δ° C	BLOOD Glucose mg/100 ml	PLASMA NEFA μEq/l	PLASMA Triglycerides mg/100 ml
Saline 0.5 ml/100 g	−4.63	61	571.2	39
Ginseng tot. sap. 3.75	−5.99	46.5	500.6	42.7
Saline	−4.68	44.4	328.3	—
Hydrocortisone emisucc. 10	−1.24*	70.6*	433.5*	—

adrenocortical function is indirect and this was confirmed by us too in a series of experiments in adrenalectomized animals (table 5). Rats treated with Ginseng lost completely their ability to antagonize the cold induced temperature fall, while hydrocortisone treated animals were partially protected by the hypothermic effect. The corticomimatic activity of Ginseng extract was also shown in young intact rats (table 6).

A dose response relationship (in thymus involution test) was shown starting from the intraperitoneal dose of 1.5 mg/kg of Ginseng total saponins.

The same Ginseng doses by intraperitoneal route did not modify blood systolic pressure of normal conscious rats in a subacute (four days) experiment.

The eating behaviour and growth of mice of both sexes was studied in a 15 days Ginseng treatment by intraperitoneal route (table 7). A slight decrease of food and water comsumption was found together with a not significant reduction in body growth of male mice treated with doses

Table 6. Thymus involution in weanling rats

Treatment mg/kg	Route	THYMUS wet weight mg
Saline 0.5 ml/100 g	ip	372
Ginseng tot. sap. 0.06	ip	372
1.5	ip	334*
3	ip	301*
30	ip	281*
DOCA 5	im	270*

*: $p < 0.05$ versus controls. Student t test.

over 3.75 mg/kg of saponins. The same results, but not the effect on body growth, were found also in female animals. A possible CNS sedation leading to a reduced feeding habit stands as the possible explanation of these results.

No liquid and salts retention was seen in these mice (table 8). The reduced urine volume found in males follows the decrease in water consumption. The decrease in body growth observed in mice took place also in male rats after 30 days intraperitoneal Ginseng administration (fig. 3) at the doses of 3 mg/kg and 30 mg/kg. The effect

Table 7. Subacute ginseng treatment of normal mice.

Sex	Treatment mg/kg/die/ip	Body weight per cent variation	Total Food consumption g	Total Water consumption ml
	Saline 0.1 ml/10 g	+26.9	370.7	536.2
Male	Ginseng tot. sap. 0.75	+18.3	366.7	476.2
	3.75	+19.9	337.7	487.5
	18.75	+17	334.2	450*
	Saline 0.1 ml/10 g	+5.5	342.7	478.7
Female	Ginseng tot. sap. 0.75	+1.7	301.2	401.2*
	3.75	+4.8	345	443.7
	18.75	+7.3	318.5	390*

*: $p < 0.05$ versus controls. Student t test.

Table 8. Subacute ginseng treatment of normal mice.

	Treatment	URINE			
Sex	mg/kg/die/ip	Volume ml/5 h	Sodium mEq/5 h	Potassium mEq/5 h	Chlorides mEq/ 5 h
	Saline 0.1 ml/10 g	1.85	0.10	0.15	0.23
Male	Ginseng tot. sap. 0.75	1.26	0.11	0.14	0.22
	3.75	1.32	0.12	0.11	0.21
	18.75	1.20*	0.12	0.11	0.21
	Saline 0.1 ml/10 g	1.45	0.16	0.10	0.23
Female	Ginseng tot. sap. 0.75	1.62	0.17	0.14	0.29
	3.75	1.47	0.17	0.10	0.27
	18.75	1.30	0.15	0.11	0.26

Note: Mean of four pooled samples per group. Each pool obtained from 5 mice.
*: $p < 0.05$ versus controls. Student t test.

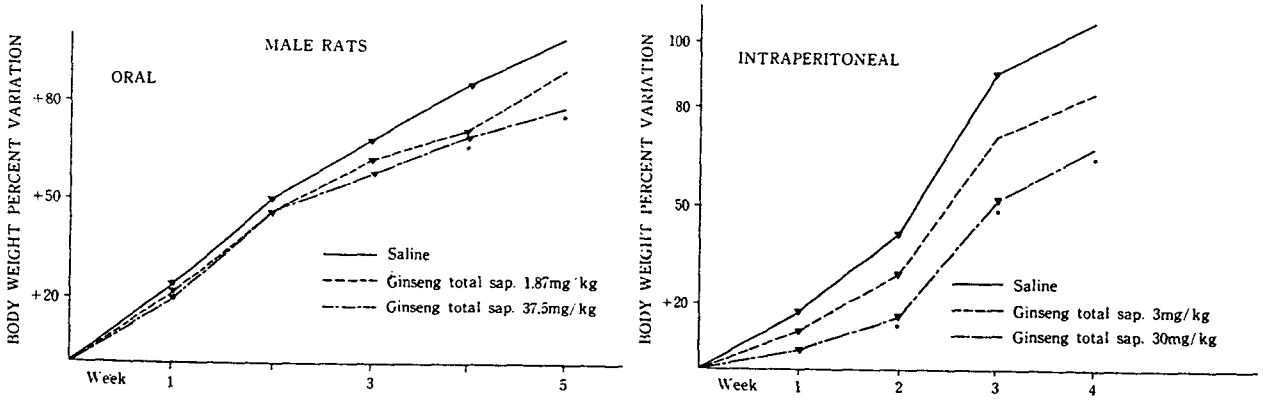

Fig. 3. Body growth of male rats treated subchronically with Ginseng.

was marked at the dose of 30 mg/kg of Ginseng total saponins.

A long term oral treatment of rats was done with two quite different Ginseng doses; the lesser one (1.87 mg/kg as total saponins) was in the order of human dosages.

The 5 weeks oral treatment produced a smaller body growth retardation but no dose response relationship was seen. Female rats revealed a comparable phenomenon to a lesser degree and only after the intraperitoneal treatment. (fig. 4).

Biochemical evaluations in liver and adrenals of these rats (table 9) evidenced, in male animals, a reduction of liver total proteins and total lipids

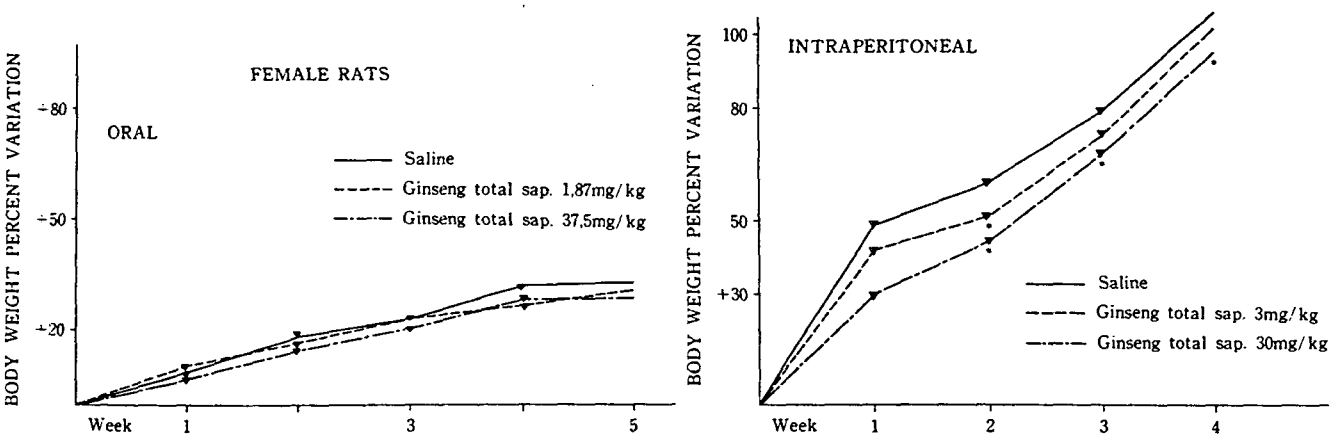

Fig. 4. Body growth of female rats treated subchronically with Ginseng.

Table 9. Subacute ginseng treatment of male rats.

Treatment mg/kg/day	Route	LIVER Proteins g/100 g	LIVER Total cholesterol g/ 100 g	LIVER Total lipids g/100 g	LIVER Triglycerides g/100 g	ADRENALS Total cholesterol g/100 g
Saline 1 ml/100 g		19.3	0.22	3.04	—	3.76
Ginseng tot. sap. 3	intrape	12.1*	0.22	2.61*	—	3.34
Ginseng tot. sap. 30		13.8*	0.26*	2.85	—	4.11
Saline 1 ml/100 g		17.8	0.34	—	1.17	1.21**
Ginseng tot. sap. 1.87	oral	16.6	0.32	—	0.76	1.49**
Ginseng tot. sap. 37.5		14.9	0.34	—	1.09	1.09**

*: $p < 0.05$ versus controls. Student t test.
**: enzymatic determination.

Table 10. Subacute ginseng treatment of female rats.

Treatment mg/kg/day	Route	LIVER Proteins g/100 g	LIVER Total cholesterol g/100 g	LIVER Total lipids g/100 g	LIVER Triglycerides g/100 g	ADRENALS Total cholesterol g/100 g
Saline 1 ml/100 g		15.7	0.31	3.40	—	2.75
Ginseng tot. sap. 3	intrape	14.1	0.30	3.13	—	3.60*
Ginseng tot. sap. 30		13.3*	0.29	2.75*	—	3.39
Saline 1 ml/100 g		14.9	0.32	—	1.73	1.39**
Ginseng tot. sap. 1.87	oral	12.7	0.33	—	1.67	1.35**
Ginseng tot. sap. 37.5		11*	0.30	—	1.21	1.26**

*: $p < 0.05$ versus controls. Student t test.
**: enzymatic determination.

after intraperitoneal Ginseng treatment. This effect did not take place after oral administration. The cholesterol content of adrenals was not modified.

In female animals (table 10) liver proteins had a lower concentration also in animals receiving a large Ginseng dose orally.

The blood picture (table 10) of rats treated orally for five weeks with Ginseng showed a modest blood glucose reduction and a more marked triglycerides decrease in male animals receiving the highest dose of the product. This effect on serum triglycerides was seen also in female rats. The lowering of neutral lipids was not accompanied by serum cholesterol variation.

The white cell formula of oral Ginseng treat-

Table 11. Subchronic oral ginseng treatment of male and female rats.

Treatment mg/kg/day/os	Sex	BLOOD		SERUM			
		Glucose	BUN	GOT	Triglycerides	CHOLESTEROL	
						Total	High dens. lip.
		mg/100 ml	mg/100 ml	U/l	mg/100 ml	mg/100 ml	mg/100 ml
Saline 1 ml/100 g		72.2	16.1	67.8	89.6	40	23.6
Ginseng tot. sap. 1.87	male	75.2	15.6	67.3	73.5	50	24.4
Ginseng tot. sap. 37.5		64.3*	15.4	76.5	63.4*	41.2	25.2
Saline 1 ml/100 g		66.1	18.4	107.9	49.7	45.3	29.7
Ginseng tot. sap. 1.87	female	68.7	16.7	101.1	34.1*	44.3	31
Ginseng tot. sap. 37.5		76.5	19.2	104.2	39.1	48.3	33

*: $p < 0.05$ versus controls. Student t test.

ed rats evidenced a striking fall of eosinophils count both in female and in male animals. To explain some of those Ginseng effects we have viewed microscopically the organs of rats treated for four weeks by intraperitoneal route.

The histological examination of supra-optic and paraventricular nuclei of hypothalamus showed signs of hyperfunction (fig. 5) in rats receiving 8 mg/kg of Ginseng saponins; the cells presented, namely, a great number of vacuols and the nuclei were located peripherally. In the adenophypophysis of the same animals we found a remarkable increase of haematoxilin stainable, basophilic cells which produce ACTH (fig. 6).

In the adrenals of Ginseng treated rats a hyperplasia of the zona fasciculata was evident (fig. 7). This phenomenon was not dose dependent and appeared much more marked in female

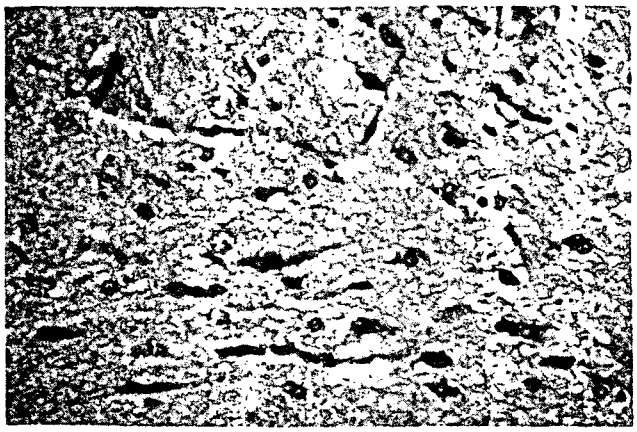

Fig. 5. Hypothalamic tissue of a Ginseng treated male rat (8 mg/kg i.p.). Haematoxylin-eosin; magn. 220 ×.

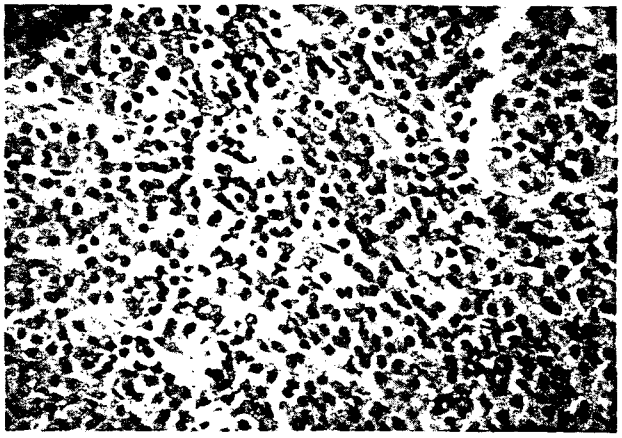

Fig. 6. Adenohyphophyseal tissue of a Ginseng treated male rat (8 mg/kg i.p.). Haematoxylin-eosin; magn. 220 ×.

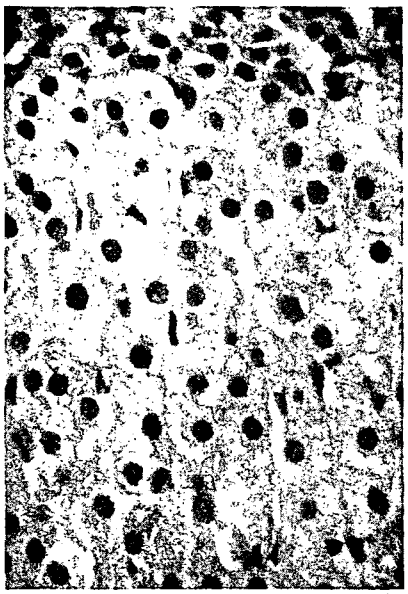

Fig. 7. Adrenocortical tissue of a Ginseng treated male rat (8 mg/kg i.p.). Haematoxylin-eosin; magn. 360 ×.

animals.

Others organs such as liver, thymus and spleen presented modifications characteristic of prolonged steroid treatment.

We hope that some elements which can be extrapolated by our study, that was carried out with a standardized preparation of Ginseng saponins, will spread more light on the mechanism of action of these plant derivatives.

We share the opinions of Oura and coworkers, according to whom Ginseng activity would take place by inducing ACTH release from hypophysis. Our histological findings represent for us an evident demonstration that Ginseng action involves the hypothalamus-hypophysis and adrenal glands axis.

Besides, the hyperplasia of adrenals zona fasciculata we found in rats subchronically treated with parenteral Ginseng, is a typical sign of repeated ACTH administrations. The difference existing between Ginseng activity and exogenous ACTH activity after an acute treatment consists in the magnitude of the relative effect.

A single acute ACTH dose depletes the glucocorticoid stores of adrenals reducing the duration of their bioavailability, while Ginseng seems to act by modulating the hypophysis release of ACTH and consequently the adrenocorticoids production according to the physiological requirements.

The results of our acute stress experiments and the data of Hiai on cyclic AMP concentration in the adrenals of hypophysectomized rats are consistent with our hypothesis.

Chairman: Now the time is open to discussion.

Yamamoto: Thank you very much for your very interesting lecture. Your data about plasma lipid and hepatic lipid entirely correspond to our data which I will talk tomorrow. I'd like to ask a question. Your ideas correspond quite closely to what I discussed and also on hypothalamus. The problem is in finding proof of level of hypothalamus. The difficulty with the histological study is the controls because it causes any changing behavior of the animal or even the stress of laboratory conditions will cause changes in the hypothalamic level. So what kind of controls did you use in your histological studies?

Bombardelli: Yes, Of course we have examined a lot of animals in the histological point of view, and are trying other techniques not only the biochemical but also ecological change. It is possible to see the variation in your stimulation.

Yamamoto: Could you be a bit more specific?

Bombardelli: I think the problem is not the histological and also now we have progressed other studies such as long treatment animals.