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Effect of Goitrogen on Serum Concentration of Blood Components in Rats

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Summary

The research was carried out to study the serum concentrations of blood components in the hypothyroidism, using the Wister strain rats. As a goitrogen, methylthiouracil (methiocil; 6-methyl-2-thiouracil) was used. The compound was suspended in 2% of aqueous sol of carboxymethyl cellulose and the suspension was injected into the stomach or injected subcutaneously. In one experiment, the goitrogen disolved partially in aqueous solution of sodium hydroxide was injected subcutaneously to the rats.

The period of administration was 12 hours (4 times of injection, 3 hours interval) or of approximately one week, twice injection a day. The blood was taken at 3 hours following the final administration, and serum of the blood was determined for total protein (TP), albumin (Alb), globulin (Gl), glucose (Glu), blood ureal nitrogen (BUN), cholesterol (Chol), Calcium (Ca), magnesium (Mg), and phosphorus (P).

By the administration of the goitrogen, serum concentrations of BUN, Chol, Mg increased and that of P decreased. Ratio of Alb/Gl in the serum concentration also was high in the group treated with methiocil in comparison with the control group. Oral treatment of methiocil increased only glycine among amino acids in the serum. The changes of concentration of blood serum component following the administration of goitrogen indicates that steer producing the beef with high degree of fat deposit is in a state of hypothyroidism.

Introduction

Goitrogen is a general name given to substances causing goiter. There are many kinds of goitrogens in the natural world [1]. Plants such as cabbage, turnip, brassicae and so on contain goitrogenic substances.

There are many kinds of goitrogenic compounds also. Goitrogen blocks formation or release of thyroxine[15]. Natural goitrogen and synthetic goitrogen respectively induce goiter. The feed back of thyroxine to hypothalamus-pituitary-thyroid axis can not occur because of the lack of secretion of thyroxine in goiter. Accordingly, thyrotropic hormone is secreted continuously. The continuous secretion of thyrotropic hormone causes hypertrophy of the thyroid glands, namely, goiter. Goitrogens including natural one and artificial synthetic one cause a goiter respectively, though it is definite that not all goitrogens act in the same way[10] and potencies are different [9].

Goitrogen increases the fattening rate by decrease of metabolic rate[11] and increases the carcass quality of cattle by the induction of a hypothyroidism [8]. Thus, hypothyroidism is favourable to production of a high quality beef and but not hyperthyroidism.

The effect of hypothyroidal state in the rapid fattening of cattle was reported by a partial thyroidectomy [2]. However in partial-thyroidectomized animals subsequent thyroid hypertrophy soon restored thyroid hormone secretion to normal with the

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rapid lessening of the fattening rate. Surgical partial or half-thyroidectomy was applied to beef cattle for improving beef quality in Japan also only in a short period. The goitrogen used alone or in combination with synthetic estrogen in fattening cattle[3,12,13,14]. Goitrogen stimulates secretion of growth hormone in a small dose, blocks secretion of thyroxine and induces a hypothyroidal condition in a heavy dose. Antithyroidal potency of the goitrogen, and serum factors correlating to fat deposit were studied[12,13], and the relations of these factors to goitrogen and hypothalamus were interesting subject of study.

Methods and Materials

The goitrogen used in the study was a commercial preparation of 6-methyl-2-thiouracil (methiocil). The compound was administered in different routes and doses in the following 4 trials. Male rats of the Wister strain were used in the study. After the administration, they were sacrificed by decapitation. The blood was taken at the decapitating, kept in the room temperature for about 10 hours, centrifuged for separating the serum. The serum obtained was subjected to the procedure determining the concentration of its components, using Vet-Aid, a kind of spectrophotometer (made by Fujihira Industrial Co., Ltd.). The selected blood components determined were total protein (TP), albumin (Alb), globulin (Gl), blood ureal nitrogen (BUN), cholesterol (Chol), calcium (Ca), magnesium (Mg), and phosphorus (P).

Trial I Eighteen male rats, weighing average 267 g in range of 180 g to 350 g, were assigned equally to 3 groups. One group was served as control. The rats of the second group and the third group were administered with the goitrogen suspended in an aqueous sol of 2 % carboxymethyl cellulose (CMC) to the stomach, using a syringe with No. 16 gauge needle the point of which was cut, curved slightly, and connected with the silicon tube slightly smaller inner diameter than outer diameter of the needle. The suspension of goitrogen was injected to the esophagus through the mouth by inserting the silicon tube. The second group were administered with suspension in a dose of 200 mg, twice a day for 8 days and sacrificed on the 9 th day by decapitation. The third group were administered with goitrogen in a dose 400 mg, twice a day for 8 days and sacrificed on the 9 th. This trial was carried out in the middle of March and rats were kept in the room temperature.

Trial II Ten male rats were kept in a temperature of 32 to 35 °C. Six rats of them were assigned to group of control, and 4 rats to group of treatment. The goitrogen was suspended in distilled water in a concentration of 500 mg per ml and pH of the suspension was adjusted to 9.0 with NaOH solution. The suspension was semifluid. The suspension was injected to two rats subcutaneously in a dose of 125 mg/0.25 ml, twice a day. The rats received injections of 5 doses of the suspension in a period of 60 hours. The rest two rats in the treatment group were given the suspension in a dose of 250 mg/0.5 ml to the stomach by using the silicon tube previously mentioned. The rats also received 5 doses of the suspension in a period of 60 hours. The compound administered amounted to 625 mg and 1250 mg per rat. At 12 hours following the final administration, rats were sacrificed by decapitation. Blood was taken at the sacrifice and determined the concentration of the 8 components in blood serum. Thyroid glands were dissected, weighed, and examined for their external appearance.

Trial III Six male rats, weighing average 241 g in a range of 210 g to 270 g, were used in the study. Three rats were assigned to control group and the other three rats to experimental group. Rats of the experimental group were injected subcutaneously the goitrogen suspended in a sol of 2 % CMC in a dose of 48 mg,

4 times at an interval of 3 hours. The rats were sacrificed by decapitation at 3 hours following the final injection. Their sera were asseyed for the 8 components in blood serum.

Trial Ⅳ Fifteen rats were used in this trial, being divided into 3 groups of five rats. One group served as control. The second group were fed methylthiouracil in the feed. Methylthiouracil was made into a paste with carboxymethyl cellulose. The paste of the compound was mixed with commercial pellet of rat chow and dryed. Every morning for five days, rats were given an amount of the feed equivalent to 0.5 g of the compound per animal. After eating up the feed mixed with the compound the commercial pellet was given *ad libitum*. The rats were sacrificed on the fifth day. The blood sera of each 4 rats in both group were analyzed for amino acid composition, using amino acid analyzer.

Results and Discussions

Ⅰ. Effect of goitrogen on concentration of serum component.

Results of the trial Ⅰ to Ⅲ are shown in Table 1 to 3 respectively. A marked effect of thyroxine was reported to accelerate both cholesterol synthesis and the removal of cholesterol from the circulation by the liver. And elevated serum cholesterol levels are observed in hypothyroid animals and administration of thyroxine causes a decline in these levels[14]. The plasma cholesterol levels drop before the thyroxine-induced rise in metabolic rate[14].

Table 1 Serum concentration of blood component in rat treated with goitrogen

	Control	Goitrogen	
No. of rats	6	6	6
Body wt., g	218 ± 35	280 ± 60	303 ± 37
Doses/day	—	200 mg	400 mg
Route	—	Oral	Oral
Period	8 days	8 days	8 days
TP , g/dl	8.1 ± 1.1	8.2 ± 0.5	7.9 ± 0.9
Alb , 〃	3.2 ± 0.3	3.4 ± 0.4	3.5 ± 0.3
Gl , 〃	4.9 ± 0.9	4.8 ± 0.4	4.4 ± 0.6
BUN, mg/dl	11.9 ± 3.0	14.9 ± 1.7	15.6 ± 2.8
Chol , 〃	56 ± 39	56 ± 23	61 ± 26
Ca , 〃	10.0 ± 0.7	10.9 ± 0.8	10.4 ± 0.6
P , 〃	9.6 ± 2.7	8.1 ± 1.5	8.7 ± 2.6
Mg , 〃	2.7 ± 1.0	2.8 ± 1.1	2.2 ± 0.5
Alb/Gl	0.68 ± 0.10	0.71 ± 0.10	0.80 ± 0.05*
Ca/P	1.12 ± 0.31	1.39 ± 0.28	1.28 ± 0.42
Ca/Mg	4.08 ± 1.29	4.28 ± 1.41	5.02 ± 1.24
P/Mg	3.65 ± 0.67	3.05 ± 0.59	4.00 ± 0.65

* $P<0.05$, significantly different to control.

Elevated cholesterol level was observed in Trial Ⅰ, Ⅱ, Ⅲ, but they are not significant statistically. Serum magnesium was elevated in the rats treated with goitrogen. Increase of level of serum magnesium is one of hypothyroidal characteristics. Induction of hypothyroidal state by goitrogen was confirmed again. There were tendencies of increase in serum concentration of calcium and of decrease in serum concentration

Table 2 Serum concentrations of blood component in rat treated with goitrogen

	Control	Methylthiouracil
No. of rats	6	4
Body wt., g	150 ± 23	163 ± 10
Dose	—	a)
Route	—	sc + oral
Period	60 hrs	60 hrs
TP , g/dl	6.1 ± 0.6	6.3 ± 0.4
Alb , 〃	2.7 ±0.1	2.9 ± 0.3
Gl , 〃	3.4 ± 0.6	3.4 ± 0.3
BUN, mg/dl	16.4 ± 4.1	30.9 ± 13.3
Chol , 〃	59 ± 13	84 ± 18
Ca , 〃	7.0 ± 0.2	7.2 ± 0.4
P , 〃	7.7 ± 1.0	6.2 ± 0.7*
Mg , 〃	2.9 ± 0.2	3.5 ± 0.3*
Alb/Gl	0.82 ± 0.18	0.88 ± 0.13
Ca/p	0.93 ± 0.11	1.19 ± 0.17*
Ca/Mg	2.42 ± 0.17	2.10 ± 0.29
P/Mg	2.69 ± 0.46	1.79 ± 0.29*
Thyroid wt., mg	8.9 ± 2.8	10.5 ± 1.0
Thyroid colour	normal	pale

Note : a) 125 mg/inj., 5 times inj./day.
250 mg/oral, 5 times oral/day.
* $P < 0.05$, significantly different to control.

Table 3 Serum concentrations of blood component in rat treated with goitrogen

	Control	Methylthiouracil
No. of rats	3	3
Body wt., g	227 ± 21	257 ± 15
Doses/inj.	—	48 mg
Route	—	sc
Period	12 hrs	12 hrs
TP , g/dl	6.8 ± 0.3	6.5 ± 0.2
Alb , 〃	3.0 ± 0.1	2.9 ± 0.1
Gl , 〃	3.8 ± 0.2	3.6 ± 0.2
BUN, mg/dl	14.3 ± 1.9	21.4 ± 9.1
Chol , 〃	76 ± 5	81 ± 2
Ca , 〃	4.4 ± 0.9	5.8 ± 1.2
P , 〃	7.8 ± 2.1	7.7 ± 0.6
Mg , 〃	1.9 ± 0.5	2.3 ± 0.5

Note : sc , subcutaneous four times injection
3 hrs interval.
: Blood was taken at 3 hrs following the final injection.

of phosphorus. This shows that calcium requirment in the tissue is high in hyperthyroidal condition and low in hypothyroidal condition. Accordingly, concentration of serum calcium is high in hypothyroidal condition caused by administration of goitrogen.

There were also tendencies of decrease in serum concentration of globulin and of increase in serum concentration of albumin. As the result, A/G ratio in serum increased generally by the administration of goitrogen.

II. Effect of goitrogen on serum concentrations of free amino acids.

Data of analysis of blood and serum amino acids in the blood of rat fed orally 500 mg of methylthiouracil for 5 days are shown in Table 4. Decrease of total free amino acids was observed. Concentrations of threonine, methionine, tyrosine, and arginine decreased significantly. Concentration of glycine, however, elevated significantly. Glutamine also elevated, but not significantly. Feature of concentration or percentage of amino acids in Table 4 is thought to show a hypothyroidal condition. Elevated glycine in the sera mentioned above is a prove of hypothyroidism[6,16].

Rats on the thiouracil diet showed a depressed and delayed specific action of glycine and glutamic acid, but not for tyrosine, thus substantiating the theory that tyrosine competes with and tends to displace thyroxine from the active center of tissue cells[10]. Elevated level of glycine as well as glutamine is a sign of hypothyroidism[4,5]. It has been proved that glycine varies inversely with thyroid status[6].

Tabl 4 Comparison of effect of methylthiouracil on blood free amino acid in rats

A.A.	Control (N=4)	Methythiouracil (Oral ; N=4)
Tau	0.362 ± 0.094	0.364 ± 0.036
Asp.A	0.102 ± 0.020	0.079 ± 0.018
Hypro	0.043 ± 0.014	0.031 ± 0.011
Thr	0.395 ± 0.044	0.265 ± 0.049**
Ser	0.291 ± 0.035	0.344 ± 0.083
Asp-n	0.099 ± 0.013	0.091 ± 0.016
Glu.A	0.556 ± 0.130	0.450 ± 0.067
Glu-n	0.256 ± 0.037	0.333 ± 0.134
Pro	0.165 ± 0.051	0.104 ± 0.025
Gly	0.382 ± 0.052	0.542 ± 0.116*
Ala	0.507 ± 0.097	0.404 ± 0.132
a-AnBA	0.003 ± 0.000	0.004 ± 0.002
Val	0.134 ± 0.018	0.132 ± 0.018
Met	0.058 ± 0.011	0.038 ± 0.010*
Ileu	0.094 ± 0.018	0.086 ± 0.015
Leu	0.137 ± 0.024	0.139 ± 0.022
Tyr	0.084 ± 0.013	0.059 ± 0.013*
Phe	0.060 ± 0.010	0.052 ± 0.011
Orn	0.061 ± 0.009	0 062 ± 0.031
Lys	0.536 ± 0.056	0.406 ± 0.107
1-M-His	0.037 ± 0.019	0.027 ± 0.005
His	0.061 ± 0.015	0.061 ± 0.012
Arg	0.276 ± 0.033	0.212 ± 0.038*
Total AA[a)]	4.695 ± 0.665	4.240 ± 0.914

Note : a) Except urea and NH_3.
* $P<0.05$, ** $P<0.01$, to control.

Conclusively, in hypothyroidal state, serum level of Ca, cholesterol are elevated and serum level of Mg also is elevated. In amino acids glycine is apparently elevated.

These facts coincide consistently with those observed in serum in finishing cattle[12].

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ラッテの血清成分の濃度に対する Goitrogen の効果

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この研究は低甲状腺機能状態における血清成分の濃度を調べるためにウイスター系ラッテを用いて実施した．goitrogen としては methylthiouracil (MTU) を用いた．この goitrogen をカーボキシル・メチル・セルローズの２％水性ゾルに浮遊させて胃内注入または皮下に注射し，または稀薄な NaOH 水溶液での部分的水溶液を皮下注射した．注射期間は12時間（３時間間隔，４回注射）または７日間（１日２回注射）であった．最後注射後３時間に血液を採り，その血清の全蛋白質 (Tp)，アルブミン (Al)，グロブリン (Gl)，グルコース (Glu)，血清尿素態窒素 (BUN)，コレステロール (Chol)，カルシウム (Ca)，マグネシウム (Mg)，リン (P) の濃度を測定した．

MTU の注射により BUN，Chol および Mg の血清濃度は増加し，P の血清濃度は低下した．対照区に比べ MTU 注射区では血清中の AG 濃度比 (A/G) が大きかった．また MTU の投与により血清アミノ酸中，グリシンのみの濃度が増加した．血清成分濃度のこれらの変化は，筆者らの前報と一致し，ロース心の脂肪交雑度が高い牛肉を生産する牛は低甲状腺機能状態にあることを示すものである．