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Studies on the Depressive Factors in *Heterochordaria abietina* affecting the Blood Cholesterol level in Rats

I. Fractionation of Effective Substances from *Heterochordaria abietina*

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Summary

To determine the nature of the substances responsible for the hypocholesterolemic activity, a brown alga called Matsumo, *Heterochordaria abietina*, was fractionated into three portions of ether-soluble, water-soluble and residual, and further the ether-soluble part was separated into four fractions of unsaponifiable substances, volatile, saturated and unsaturated fatty acids. Rats were fed on a diet of each fraction supplemented at a level equivalent to 5 per cent of the dried sample for 8 weeks.

The residual and ether-soluble parts were very effective in lowering blood cholesterol level, but water-soluble part was ineffective. Both fractions of saturated and unsaturated fatty acids exerted a significant hypocholesterolemic effect on the rats. The unsaponifiable matters, however, were insignificant and feeding of the volatile fatty acids resulted in the elevation of the blood cholesterol concentration in rats.

It has long been generally believed in Japan that dietary seaweed is effective in preventing human hypertension. In 1960 Kameda (1) first demonstrated that tang (*Laminaria sp.*) was efficacious in depressing human hypertension and that the substances responsible for it were soluble in water but not in ether. In the following year, he (2) found that the blood pressure of rabbits used as test animal was not only lowered but a hypocholesterolemia was also induced by this alga. Since then several investigations of this problem have been made by some workers the seaweeds examined were found to be more or less antihypercholesterolemic. The junior author (3) presented a brief review of the principal features of the works at the Sixth International Seaweed Symposium at Satiago de Compostela in September, 1968.

There is a small brown alga called Matsumo, *Heterochordaria abietina*, in northern Japan where it is often used as a side dish. Tsuchiya and his co-workers (4) have attempted to investigate the effect of this seaweed on the blood cholesterol level in rats.

The present paper deals with the studies conducted by the authors on the comparative hypocholesterolemic activities of fractionated components of *Heterochordaria abietina* in rats.

Experimental and Results

Feeding of animals

The animals used in this experiment were male rats of the Wistar strain that had been fed with a basal stock diet for a week from the time of weaning. They were housed in individual cages which were automatically maintained at a temperature of 22°C and relative humidity of 60 per cent. Food and water were supplied *ad lib.* The composition of the basal diet is listed in Table 1. In the diet, cholesterol was supplemented at a level of 1 per cent and each algal fraction was equal to the amount included in 5 per cent of Matsumo. Sucrose was reduced the quantity by the addition of algal substance. The rats were weighed individually at weekly intervals for 8 weeks.

TABLE 1. *Composition of Experimental Basal Diet*

Components	Percentage of diet
Sucrose	64.01
Casein	22.0
Salt mixture*	4.0
Choline chloride	0.25
Vitamin mixture**	0.5
Bile salts	0.25
Cottonseed oil	5.0
Cellulose***	4.0

* McCollum salt mixture, No. 185

** The vitamin mixture was supplied with the following vitamins in grams per casein 44 g: P-aminobenzoic acid 1, inositol 1, tocopherol acetate 1, ascorbic acid 1, thiamine 0.5, Ca-pantothenate 0.4, niacin 0.4, B_{12} 0.003, riboflavin 0.2, pyridoxine 0.1, folic acid 0.02, menadione 0.05, biotin 0.01, and vitamin A 20000 and vitamin D 2000 I.U..

*** Toyo Roshi cellulose powder D (100 mesh).

Isolation of Algal Components

Matsumo, *Heterochordaria abietina*, was dehydrated by a vacuum drier, and ground to a fine powder. It was fractionated into three portions of ether-soluble, water-soluble and residual as indicated in Figure 1. The ether-soluble part was separated into three fractions of volatile and non-volatile fatty acids and unsaponifiable substances, and the non-volatile acids were further fractionated into two portions of saturated and unsaturated fatty acids. The yield of each fraction is summarized in Table 2.

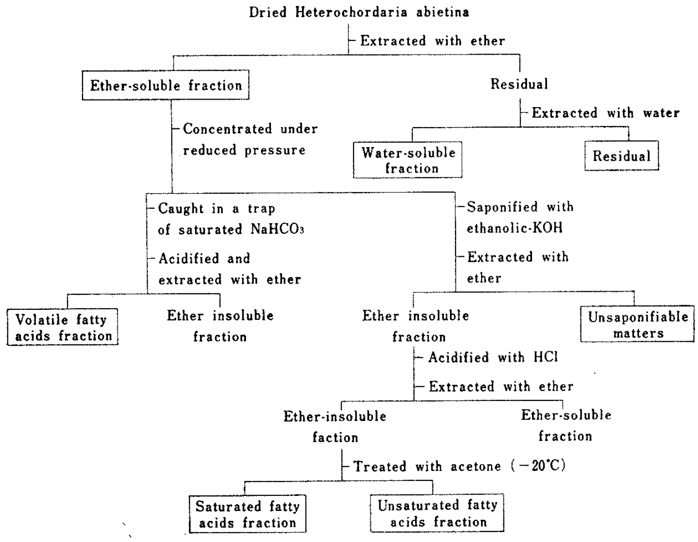

FIG. 1 Diagrammatic Representation of Constituents of *Heterochordaria abietina.*

TABLE 2. *The Yield and Protein Content of Fractions Separated from Heterochordaria abietina**

Fraction	Yield (%)	Protein (%)
Residual	76.3	28.1
Water soluble	18.4	4.9
Volatile fatty acids	0.026	—
Unsaponifiable matters	0.73	—
Saturated fatty acids	1.16	—
Unsaturated fatty acids	2.14	—

* The general composition of *Heterochordaria abietina* was 4.2 of moisture, 25.9 of protein, 4.3 of lipid, 40.1 of cabohydrate, 11.0 of crude fiber and 14.5% of ash.

Fatty Acid Analyses

The fatty acid compositions were determined by the following method. The samples were dissolved in 1 ml of ether and converted into methyl esters by diazomethane. The analyses of them were made with a Shimazu GC–2B gas chromatograph with a flame ionization detector. The column of 3 m in length and 4 mm in diameter was packed with Diasolid L (80 to 100 mesh) and then coated with 10 per cent diethylene glycol succinate polyester. The operation was conducted with the flow rate of nitrogen adjusted to 60 ml per minute and the temperature at 190°C. The composition of both saturated and unsaturated fatty acids fractions is summarized in Table 3. It indicates that there were a very large amount of palmitic acid (81.3%) and a moderate amount of myristic acid (9.5%) in the saturated fatty acids fraction. On the other hand, the unsaturated fatty acids fraction consisted of octadecatetraenoic and eicosapentaenoic acids involving

TABLE 3. *Fatty Acid Composition of Heterochordaria abietina-Oil* (%)

Fatty acid	Saturated fatty acid fraction	Unsaturated fatty acid fraction
C_{14}: 0	9.5	3.8
C_{15}: 0	1.1	trace
C_{16}: 0	81.3	1.6
1	trace	3.8
2	—	trace
C_{17}: 0	3.0	—
C_{18}: 0	0.7	—
1	1.0	13.2
2	0.5	10.5
3	—	1.1
4	0.9	22.9
C_{20}: 0	0.7	—
1	0.5	12.2
2	—	0.8
3	0.5	11.8
4	trace	1.8
5	0.5	16.6

a total of around 40 per cent, with a small amount of oleic, linoleic, eicosamonoenoic and eicosatrienoic acids.

Determination of Cholesterol

At the end of the feeding, the rats were starved for 12 hours prior to the collection of blood in order to avoid any short term change in plasma lipid due to recent ingestion of food. Then the blood was obtained using heparin as an anticoagulant from their hearts by cardiac puncture, while they were under light ether anesthesia. The total cholesterol concentration of the blood was determined by the method of Abellet *et al.* (1952) (5).

The results of the feeding experiments are summarized in Table 4. It should be noted that normal growth was obtained on all diets used in this experiment. The water-soluble fraction shows a higher rate of growth than with other diets in the first experiment. Both the ether-soluble and residual fractions were significantly reduced in cholesterol concentration. Furthermore, it is noticed that the cholesterol depressant power of the ether-soluble fraction was slightly lower than that of cholesterol-free diet. The water-soluble fraction was insignificant, showing a higher concentration of blood cholesterol than the control.

In the second experiment, group 1 was fed a diet containing 1 per cent cholesterol and groups 2 to 5 were fed a cholesterol diet plus volatile, saturated, unsaturated fatty acids and unsaponifiable matters fractions, respectively.

The blood cholesterol analyses showed that both diets containing saturated and unsaturated fatty acid fractions retained a hypocholesterolemic potency. It was also observed that the volatile fatty acids and unsaponifiable substances had no effect on blood cholesterol level, and that the former was higher than the control.

TABLE 4. *Effect of Heterochordaria abietina on Blood Cholesterol Level in Rats*

Group	No. in group	Weight gain (g/rat)	Whole blood* cholesterol (mg/dl)
Experiment 1**			
1. Basic diet	7	176.1	155.4± 2.9
2. Basic+1% cholesterol	7	170.7	173.0± 3.5
3. Basic+1% cholesterol +ether sol. fraction	7	169.0	152.3± 6.5
4. Basic+1% cholesterol +water sol. fraction	7	192.0	185.1±11.6
5. Basic+1% cholesterol +residual fraction	7	175.1	165.0± 4.4
Experiment 2***			
1. Basic+1% cholesterol	7	193.5	170.3± 5.7
2. Basic+1% cholesterol +volatile acid fraction	7	198.0	201.0±20.0
3. Basic+1% cholesterol +sat. fatty acid fraction	7	191.2	153.5±10.5
4. Basic+1% cholesterol +unsat. fatty acid fraction	7	213.7	152.2± 9.4
5. Basic+1% cholesterol +unsaponifiable matters	7	198.3	175.8±12.8

* Mean±stand. dev. $\sigma = \sqrt{\frac{\Sigma d^2}{n}}$

** The average inetial body weight of experiment 1 was 96 g (range: 72 to 112 g). Experiment 1. Group 1 was fed a cholesterol-free diet; group 2, diet containing 1% cholesterol; groups 3–5, the cholesterol diet plus ether soluble, water soluble and residual fraction, respectively.

*** The average initial body weight of experiment 2 was 53 g (range: 41 to 66 g).

Discussion

Of the three fractions of *Heterochordaria abietina* in Experiment 1, both the ether-soluble and residual fractions promoted a great depression of blood cholesterol level in rats. In regard to the residual portion, it is conceivable that the hypocholesterolemic activity is caused by the presence of either polysaccharide or by the iodine-bound protein in it.

As is reported by Fahrenbach *et al.* (6) alginic acid is potent in preventing the development of hypercholesterolemia in white leghorn cockerels. Also previous studies in our laboratory (7) demonstrated that both Na-alginates which were 417 and 226 in average degree of polymerization number were effective in lowering the plasma cholesterol levels in rats, while the sample which was 13 in the degree of polymerization was insignificant. If we consider that the algal polysaccharide interfered with the absorption of cholesterol by the gut in rats, one of the hypocholesterolemic activities of *Heterochordaria abietina* in this experiment is due to the presence of the polysaccharide which is mostly composed of alginic acid and is also found in the residual part of *Hetrochordaria abietina.*

The cholesterol-lowering effect of high protein diets in chickens has been reported by Kokatnur *et al.* (8), Stamler *et al.* (9) and March *et al.* (10). Nath *et al.* (11) presented some data which would suggest that the type of protein may be of importance in the regulation of serum cholesterol levels in rats. The highest level of dietary casein produced the lowest serum cholesterol level, and gluten had a more pronounced hypocholesterolemic effect at all levels of protein than did casein. However, the residual part of Matsumo is so low in protein content and generally indigestible, that it is hardly necessary to think about the effect of high protein diets in this experiment.

It is well known that the thyroid hormone has a hypocholesterolemic effect in man as well as in animals (12, 13, 14). Grande and Schultz (15) reported that daily injections of L-thyroxine of approximately 1.4 μg per 100 g body weight in thyroidectomized dogs significantly lowered serum cholesterol at the time of the first sampling 1 week after injection. More recentry, Raheja and Snedecor (16) found that serum cholesterol in chicks was lowered maximally by triiodothyronine and thyroxine. The iodine-bound protein of brown seaweed is similar in iodoamino acids composition to the thyleoglobulin. The marine brown algae generally contain a small amount of iodoamino acids. Tsuchiya (3) summarized the results of investigations on the iodoamino acids in them. It is noteworthy that both triiodothyronine and thyroxine were the most physiologically active for rats and were predominant in *Heterochordaria abietina.*

Since Peterson's (1951) (17) first description of anticholesterolic action of soy sterols in chicks, many reports involving chicks (18, 19), rats (20) and mice (21) have confirmed his observations. However, it was seen in this experiment that a depressant factor was not present in the unsaponifiable material of *Heterochordaria abietina* oil.

Both saturated and unsaturated fatty acids fractions exerted a significant effect for rats as shown in the second experiment. The fatty acid analyses showed that the amount of octadecatetraenoic, eicosatrienoic and eicosapentanoic acids reached approximately fifty percent in unsaturated fatty acids as in Table 3. Peifer *et al.* (22) reported that highly unsaturated fatty acids, or precursors of such polyenoic acids, produced a marked and rapid depression of plasma cholesterol in hypercholesterolemic rats. Therefore it is supposed that the depressive power of the algal unsaturated fatty acids fraction concerned is at least due to the presence of these highly unsaturated fatty acids in *Heterochordaria abietina.* However, it is surprising that the saturated fatty acid fraction, where palmitic acid is predominant, had a depressing effect on the cholesterol level of rat. Consequently the authors in 1966 (23) suggested that the substance responsible for the hypocholesterolemic activity in rats might be plant pigment such as chlorophyll derivatives present in the saturated fatty acids fraction. First they determined the content of chlorophyll in several marine algae and found that there occurred a fair amount of chlorophyll in *Heterochordaria abietina* that was superior to other brown seaweeds and as much as green alga, *Enteromorpha sp.*. Therefore the absorption of cholesterol by the gut in rats may have been interfered with by the chlorophyll derivatives phaeophytin or phaeophorbide present in above fraction. We shall report in detail on this problem elsewhere.

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