MITOKU

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August 18,2007

Mr. Robert Pooler
Agricultural Marketing Specialist,
National Organic Program,
USDA/AMS/TMP/NOP,
1400 Independence Ave., SW,
Room 4008-So., Ag Stop 0268,
Washington, DC 20250.
Docket number AMS-TM-07-0062

Dear Mr. Robert Pooler,

I am writing to against the interim final rule and recommend the inclusion of Kombu seaweed (*Laminaria japonica, Laminaria japonica* var. *ochotensis, Laminaria angustata,* and *Laminaria angustata* var. *longissima*) to the section of Non-organic agricultural substances allowed in or on processed products labeled as "organic", 205.606. I would like to request your review on Kombu seaweed as we have similar situation with Wakame seaweed (*Undaria pinnatifida*), which was added to 205.606 of the National List.

Please find our petition for the addition of Kombu seaweed (*Laminaria japonica, Laminaria japonica* var. *ochotensis, Laminaria angustata,* and *Laminaria angustata* var. *longissima*) to be added to the section of Non-organic agricultural substances allowed in or on processed products labeled as "organic", 205.606.

CBI copy and CBI deleted copy are enclosed, respectively. I claim the CBI treatments, as I do not want to disclose our contracted suppliers for competitive reasons. In the case the request for CBI treatment of enclosed petition is being denied, please kindly contact me by e-mail before accepting this petition.
Thank you very much for your attention.

Sincerely,

Asuka Shiono

Asuka Shiono
Mitoku Co.,Ltd.

CBI-Deleted
COPY

Subject: Request on Kombu seaweed.(*Laminaria japonica*, *Laminaria japonica* var. *ochotensis*, *Laminaria angustata*, and *Laminaria angustata* var. *longissima*) for addition to the National list under, 205.606

ItemA

Kombu seaweed (*Laminaria japonica*, *Laminaria japonica* var. *ochotensis*, *Laminaria angustata*, and *Laminaria angustata* var. *longissima*) to be added to the section of Non-organic agricultural substances allowed in or on processed products labeled as "organic", 205.606.

ItemB

1. **The substance's chemical or material common name.**

Kombu seaweed (*Laminaria japonica*, *Laminaria japonica* var. *ochotensis*, *Laminaria angustata*, and *Laminaria angustata* var. *longissima*)

2. **The manufacture's or producer's name, address, and telephone number**

(**CBI Deleted**)

3. **A list of the types of products for which the substance will be used and a description of the substance's function in the product.**

Currently, Kombu is used as an ingredient to make stock for Instant Miso Soup and Yuzu Ponzu. These products used to be labeled as "Organic" before 9 June 2007. Since Kombu is integral to the preparation of most Japanese traditional foods as stock, we intend to use the ingredient for our new organic Japanese cuisines in the future.

4. **A list of the crop, livestock or handling activities for which the substance will be used. If used for handling(including processing), the substance's mode of action must be described.**

As mentioned above, Kombu is used to make stock for Japanese traditional foods.

5. **The source of the substance and a detailed description of its manufacturing or processing procedures from the basic component to the final product.**

Kombu is harvested from the ocean. After the crop is harvested, it is usually being sun-dried by the sea. In general, the preparation of stock for Japanese traditional food, dried Kombu is being boiled in water for certain amount of time, and the broth is used for many Japanese traditional foods.

Our Instant Miso Soup manufacture purchases Kombu extracts for their product.

Production Process of Kombu extracts:

Kombu as ingredient – Extracting by hot water – Condensing- Sterilize by heating – Filtering – Inspection - Filling and Weighing – Packaging - Final Product

Yuzu shoyu manufacture purchases dried kombu and makes the Kombu extract(stock) on their own by simply boiling the dried Kombu.

6. **A summary of any available previous reviews by State or private certification programs or other organizations of the petitioned substance.**

Kelp is already included in the National list under 205.606 for use only as thickener and dietary supplement.

Also, Kombu itself is listed on materials list 2007 being published by Global Organic Alliance as use only as thickener and dietary supplement. http://www.goa-online.org/standards.html.

7. **Information regarding EPA, FDA, and State regulatory authority registrations, including registration numbers.**

To the best of petitioner's knowledge, no such information is available.

8. **The Chemical Abstract Service(CAS) number or other product numbers of the substance and labels of products that contains the petitioned substance.**

To the best of petitioner's knowledge, no CAS number or relevant information is available.

The labels of Organic Instant Miso Soups are attached (Attachment 1). Although we do not currently use these labels for NOP certified product after 9 July 2007.

For Yuzu Ponzu, no retail label is available, since it is only shipped as bulk from the manufacture.

9. **The substance's physical properties and chemical mode of action including:**
   a) **Chemical interactions with other substances, especially substances used in organic production;**

   It is 100% natural ingredient. No chemical substance is added to the ingredient.

b) Toxicity and environmental persistence;

The crop is a traditional Japanese food and being included in Japanese diet for centuries. To the best of petitioner's knowledge, it is environmentally persistent.

c) Environmental impacts from its use and /or manufacture;

To the best of petitioner's knowledge, no significant environmental impacts from use and/or manufacture of Kombu .

d) Effects on human health;

Please refer to the Attachment 2.

Reference: SEAWEEDS KAISO by Japan Seaweed Association.

e) Effects on soil organisms, crops, or livestock

To the best of petitioner's knowledge, it is not used on soil organisms, crops or livestock.

10. Safety information about the substance including a Material Safety Data Sheet(MSDS) and a substance report from the National Institute of Environmental Health Studies.

To the best of petitioner's knowledge, no such information is available.

11. Research information about the substance, which includes comprehensive substance research reviews and research bibliographies, including reviews and bibliographies which present contrasting positions to those presented by the petitioner in supporting the substance's inclusion on or removal from the National List. This information item should include research concerning why the substance should be permitted in the production or handling of an organic product, including the availability of organic alternatives.

General information of *Laminaria* spp. is described in Wikipedia at http://en.wikipedia.org/wiki/Laminaria

Please refer to the Attachment 3.

Reference: SEAWEEDS KAISO by Japan Seaweed Association.

As the result of search on the word "Laminaria" at USDA's website, no relevant information which present contrasting positions to those presented by the petitioner in supporting the substance's inclusion on or removal from the National list was found. The search result is attached as Attachment 4.

12. Petition Justification statement

- **A comparative description on why the non- organic form of the substance is necessary for use in organic handling.**

Kombu is integral to the preparation of most Japanese traditional foods as stock. Kombu, which is abundant in glutamic acid makes a good stock and provides nice flavor to the food. Attached table1 shows high glutamic acid content of Kombu species.

Mitoku has been exporting Japanese traditional foods including organic certified products (Or the equivalent for the years before organic policies are established) since 1969. We would like to continue exporting organic certified Japanese traditional foods including the ones contain Kombu and increase our varieties of products in the future. Kombu stock is essential to high quality Japanese cuisines.

In the modern times, many food industries, even many households, use monosodium glutamate to replace stock made from natural ingredients such as Kombu. As a company, which wish to offer organic, natural and traditional Japanese foods to the market, we feel it is necessary for one of the most important ingredient in Japanese traditional food, Kombu, to be permitted to use in "Organic" labeled product.

Several types of fish are also common to use as stock in Japan.
In this point of view, Kombu stock is beneficial to the people, who are on certain types of diet and do not eat fish or meat. Kombu, which is rich in glutamic acid provides flavors for vegetarian dishes. Most importantly, Kombu contains important nutrients to vegetarians'diet. Kombu is a good source of a wide range of essential vitamins and minerals, including calcium, iron, iodine, and vitamins A, B1, B2, C, B6, B12, and niacin.

As the attachment 2 shows, many health benefits by eating Kombu has been reported.

- **Current and historical industry information/ research/evidence that explains how or why the substance cannot be obtained organically in the appropriate form, appropriate quality, and appropriate quantity to fulfill an essential function in a system of organic handling.**

For the past few years, we have been contacting several Kombu suppliers to obtain organic certified Kombu to use for our organic certified products. Up to now, we have not been able to source organic certified Kombu. (Affidavit for non-availability of organic Kombu from Kombu supplier is attached as Attachment 5) . As the result of Internet search by petitioner, there was organic certified *Laminaria digitata* from the Atlantic available in powdered form.

However, our contracted instant miso soup manufacturer claims that it is necessary to use the species of Kombu they currently use in order to keep the same flavor and quality of their products. Also, the manufacture was not able to find organic certified Kombu on their search. Comment from instant miso soup manufacture is attached as Attachment 6. The manufacture reported that they use Kombu called Kushiro (place in Hokkaido Prefecture, Japan) Kombu and Hidaka (place in Hokkaido Prefecture, Japan) Kombu. Petitioner researched the distribution of Kombu species and found that Kushiro Kombu is the species of *Laminaria angustata* and *Laminaria angustata* var. *longissima,* and Hidaka Kombu is the species of *Laminaria angustata.*

Species of *Laminaria japonica*, *Laminaria japonica* var. *ochotensis, Laminaria angustata,* and *Laminaria angustata* var. *longissima* are known to be used for making stock.
As the data for glutamic acid contents in the table 1 shows, glutamic acid contents vary according to the species and harvested region. Even among Kombu species, which are growing in Japanese seawater, there are species being known as not preferable to make stock. Each species of Kombu has different characteristics such as flavors, softness, etc. Manufactures are choosing species of Kombu, which is desirable as stock in their organic processed products.

Yuzu Ponzu manufacture claims that the use of Kombu is necessary for their Yuzu Ponzu product as the product is characterized by Kombu stock. They also were not able to find organic certified Kombu. Comment from the manufacture is attached as Attachment 7.

Petitioner intends to use organic Kombu if we could source organic certified Kombu of species *Laminaria japonica*, *Laminaria japonica* var. *ochotensis, Laminaria angustata,* and *Laminaria angustata* var. *longissima* are available. Petitioner and manufactures will keep searching for organic certified *Laminaria japonica*, *Laminaria japonica* var. *ochotensis, Laminaria angustata,* and *Laminaria angustata* var. *longissima* as we have been for the past few years. Meanwhile, we request that non-organic Kombu limited to the species of *Laminaria japonica*, *Laminaria japonica* var. *ochotensis, Laminaria angustata,* and *Laminaria angustata* var. *longissima* to be allowed to use for "Organic" labeled products. Alternatively, petitioner request to allow the use of Kelp as a stock in addition to Kelp used as a thickener and dietary supplement for "Organic" labeled products.

Industry information on substance non-availability of organic sources regarding

commercial availability.

The information is included in the above section.

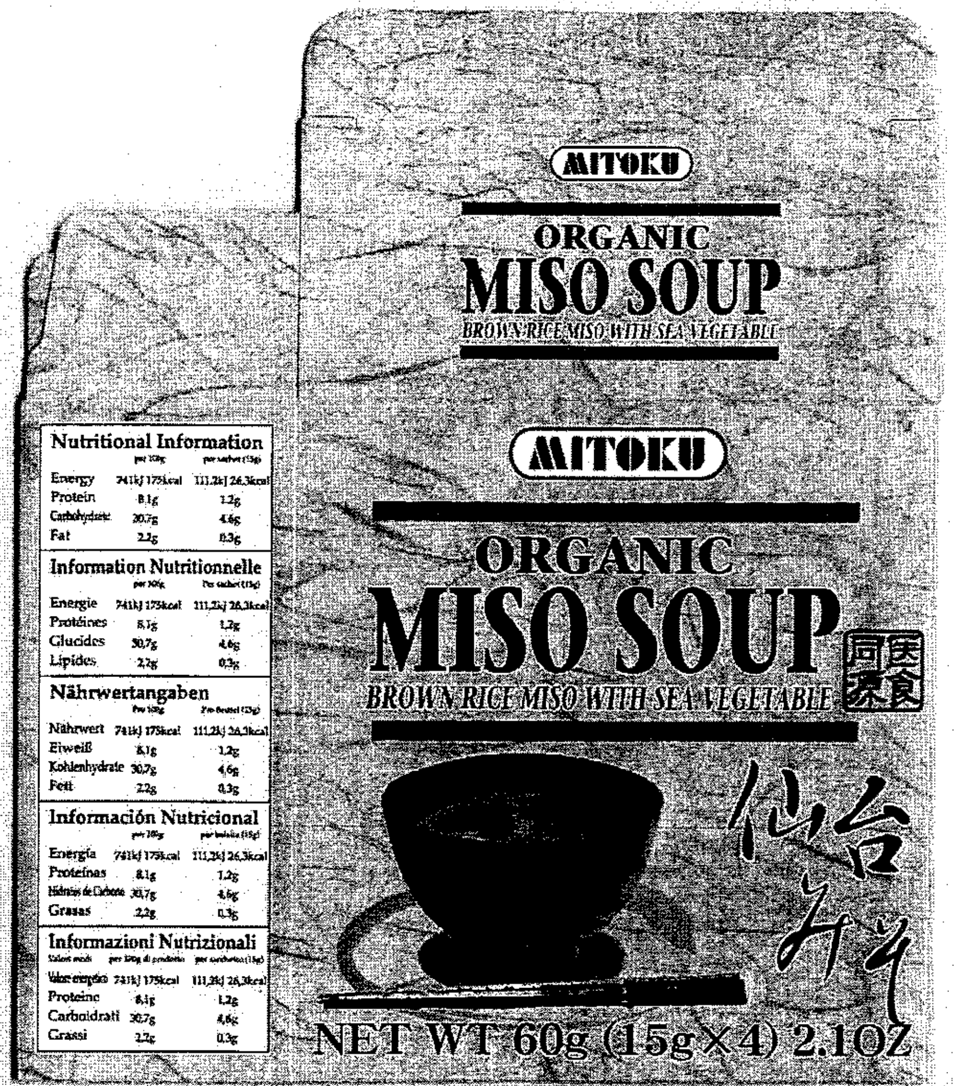
Table.1

Gulutamic Acid contents of different species and growing district of Kombu

Japanese Common Name	Scientific Name	Growing District	Gulutamic Acid content
MA KOMBU	*Laminaria japonica*	Osatsube in Hokkaido	2,470mg
		Nishitoi in Hokkaido	1,740mg
		Ishizaki in Hokkaido	2,370mg
		Oma in Aomori	2,400mg
		Omoe in Iwate	1,560mg
MITSUISHI KOMBU	*Laminaria angustata*	Chikafue	970mg
		Esan	1,910mg
NAGA KOMBU	*Laminaria angustata* var. *longissima*	Hamanaka in Hokkaido	1,590mg
		Akkeshi in Hokkaido	2,790mg
		Ochiishi in Hokkaido	1,010mg
		Habamai near Hokkaido	1,830mg
RISHIRIKEI ENAGAONI KOMBU		Rausu in Hokkaido	4,690mg
RISHIRI KOMBU	*Laminaria japonica* var. *ochotensis*	Funadomari in Hokkaido	1,840mg

References:
Reference for Gultamic acid contents data:Nihon Konbu Kyokai
Reference for Scientific Name: COLORED ILLUSTRATIONS OF THE SEAWEEDS OF JAPAN

Attachment 1



Product of Japan
Store in a dry, cool place

Produit du Japon
Conserver à un endroit frais et sec

In Japan hergestellt
Kühl und trocken aufbewahren

Producto de Japón
Mantener en un lugar seco y fresco

Prodotto in Giappone
Conservare in luogo fresco e asciutto

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www.mitoku.com

Certified Organic by ECOCERT SA, F-32600

EAN
4 539093 100763

UPC
0 21009 10619 7

60g (15g×4)

Preparation: Empty the contents of one sachet into a bowl. Add one cup of hot water, stir, and allow to sit for a minute.

Ingredients: Johsen Brown Rice Miso*(59%) (whole soybeans*, brown rice*, sea salt, water, Koji (Aspergillus oryzae)), water, Mikawa Mirin*(sweet rice*, water, rice*, Koji (Aspergillus oryzae)), Johsen Shoyu*(whole soybeans*, whole wheat*, sea salt, water, Koji (Aspergillus oryzae)), sea salt, sea vegetable (1.5%)(Wakame, Kombu)
*organically grown

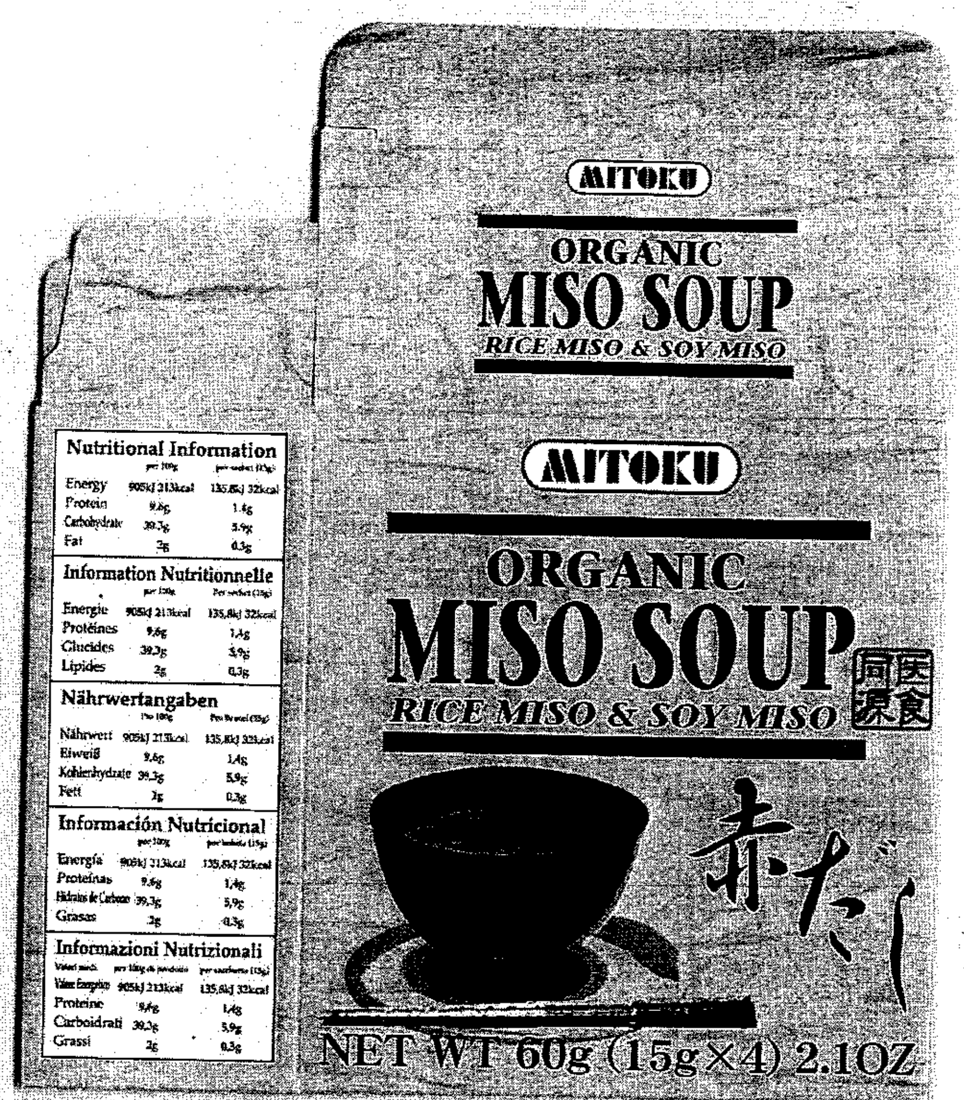
F Soupe instantanée au Miso de riz complet et algues marines - Préparation: versez le contenu d'un sachet dans un bol, ajoutez une tasse d'eau bouillante et mélangez. Laissez reposer une minute avant de boire.
Ingrédients: Miso Johsen de riz complet*(59%)(fèves de soya entières*, riz complet*, sel marin, eau, Koji (Aspergillus oryzae)), eau, Mirin Mikawa*(riz doux*, eau, riz*, Koji (Aspergillus oryzae)), Shoyu Johsen* (fèves de soya entières*, blé complet*, sel marin, eau, Koji (Aspergillus oryzae)), sel marin, algue marine (1.5%)(Wakame, Kombu)
*issu de l'agriculture biologique contrôlée

D Miso Suppe mit Reismiso und Meeresalgen - Zubereitung: Den Inhalt eines Portionsbeutels in eine große Tasse geben. Mit heißem Wasser aufgießen, umrühren und kurz ziehen lassen.
Zutaten: Johsen Reismiso*(59%)(Sojabohnen*, Vollkornreis*, Meersalz, Wasser, Koji (Aspergillus oryzae)), Wasser, Mikawa Mirin*(Süßer Reis*, Wasser, Reis*, Koji (Aspergillus oryzae)), Johsen Shoyu*(Sojabohnen*, Vollkornweizen*, Meersalz, Wasser, Koji (Aspergillus oryzae)), Meersalz, Meeresalgen (1.5%) (Wakame, Kombu)
*aus ökologischem Anbau

E Sopa de Miso Instantánea con Genmai Miso y Algas - Preparación: verter el contenido de una bolsita en una taza con agua caliente y dejar reposar durante 1 minuto.
Ingredientes: Genmai Miso Johsen*(59%)(Granos de Soja*, Arroz Integral*, Sal Marina, Agua, Koji (Aspergillus oryzae)), Agua, Mikawa Mirin*(Arroz dulce*, Agua, Arroz*, Koji (Aspergillus oryzae)), Shoyu Johsen*(Granos de Soja*, Trigo Integral*, Sal Marina, Agua, Koji (Aspergillus oryzae)), Sal Marina, Alga (1.5%)(Wakame, Kombu)
*de cultivo ecológico

I Zuppa di Miso Istantanea alle alghe con miso di riso - Modo d'uso: Versare in una tazza o in una scodella il contenuto di una bustina, aggiungere una tazza di acqua calda e mescolare. Far riposare per circa un minuto prima di bere.
Ingredienti: Miso di riso integrale Johsen*(59%)(fagioli di soia*, riso integrale*, sale marino, acqua, Koji (Aspergillus oryzae)), acqua, Mirin Mikawa*(riso dolce*, acqua, riso*, Koji (Aspergillus oryzae)), Shoyu Johsen*(fagioli di soia*, frumento integrale*, sale marino, acqua, Koji (Aspergillus Oryzae)), sale marino, alghe marine (1.5%)(Wakame, Kombu)
*da agricoltura biologica

Best before End: A consommer de préférence avant: Mindestens haltbar bis: Consumir preferentemente antes de: Da consumarsi preferibilmente entro:
07, 02, 2007


MITOKU
ORGANIC
MISO SOUP
RICE MISO & SOY MISO
MITOKU
ORGANIC
MISO SOUP
RICE MISO & SOY MISO
医食同源
赤だし
NET WT 60g (15g×4) 2.1OZ
Nutritional Information
Energy 905kJ 213kcal 135,8kJ 32kcal
Protein 9,6g 1,4g
Carbohydrate 39,3g 5,9g
Fat 2g 0,3g
Information Nutritionnelle
Energie 905kJ 213kcal 135,8kJ 32kcal
Protéines 9,6g 1,4g
Glucides 39,3g 5,9g
Lipides 2g 0,3g
Nährwertangaben
Nährwert 905kJ 213kcal 135,8kJ 32kcal
Eiweiß 9,6g 1,4g
Kohlenhydrate 39,3g 5,9g
Fett 2g 0,3g
Información Nutricional
Energía 905kJ 213kcal 135,8kJ 32kcal
Proteínas 9,6g 1,4g
Grasas 2g 0,3g
Informazioni Nutrizionali
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Producto de Japón
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Certified Organic by ECOCERT S.A. F 32600

EAN
4 539093 100756

UPC
0 21009 10618 0

60g (15g×4)

Preparation: Empty the contents of one sachet into a bowl. Add one cup of hot water, stir, and allow to sit for a minute.

Ingredients: Johsen Brown Rice Miso*(31%)(whole soybeans*, brown rice*, sea salt, water, Koji (Aspergillus oryzae)), Yaemon Soybean Miso*(20%)(whole soybeans*, water, sea salt, roasted barley flour*, Koji (Aspergillus oryzae)), water, Mikawa Mirin*(sweet rice*, water, rice*, Koji (Aspergillus oryzae)), Barley Malt*, sea salt, Johsen Shoyu*(whole soybeans*, whole wheat*, sea salt, water, Koji (Aspergillus oryzae)), sea vegetable (Wakame, Kombu)
*organically grown

F Soupe instantanée au Miso de riz complet et Miso de Soja - Préparation: versez le contenu d'un sachet dans un bol, ajoutez une tasse d'eau bouillante et mélangez. Laissez reposer une minute avant de boire.
Ingrédients: Miso Johsen de riz complet*(31%)(fèves de soya entières*, riz complet*, sel marin, eau, Koji (Aspergillus oryzae)), Miso Yaemon de soya*(20%)(fèves de soya entières*, eau, sel marin, farine d'orge torréfiée*, Koji (Aspergillus oryzae)), eau, Mirin Mikawa*(riz doux*, eau, riz*, Koji (Aspergillus oryzae)), malt d'orge*, sel marin, Shoyu Johsen* (fèves de soya entières*, blé complet*, sel marin, eau, Koji (Aspergillus oryzae)), algue marine (Wakame, Kombu)
*issu de l'agriculture biologique contrôlée

D Miso Suppe mit Soja- und Reismiso – Zubereitung: Den Inhalt eines Portionsbeutels in eine große Tasse geben, mit heißem Wasser aufgießen, umrühren und kurz ziehen lassen.
Zutaten: Johsen Reismiso*(31%)(Sojabohnen*, Vollkornreis*, Meersalz, Wasser, Koji (Aspergillus oryzae)), Yaemon-Sojamiso*(20%)(Sojabohnen*, Wasser, Meersalz, geröstetes Gerstenmehl*, Koji (Aspergillus oryzae)), Wasser, Mikawa Mirin*(Süßer Reis*, Wasser, Reis*, Koji (Aspergillus oryzae)), Gerstenmalz*, Meersalz, Johsen Shoyu*(Sojabohnen*, Vollkornweizen*, Meersalz, Wasser, Koji (Aspergillus oryzae)), Meeresalgen (Wakame, Kombu)
*aus ökologischem Anbau

E Sopa de Miso instantánea con Soja y Miso de arroz - Preparación: verter el contenido de una bolsita en una taza con agua caliente y dejar reposar durante 1 minuto.
Ingredientes: Genmai Miso Johsen*(31%)(Granos de Soja*, Arroz Integral*, Sal Marina, Agua, Koji (Aspergillus oryzae)), Miso de Soja Yaemon*(20%)(Granos de Soja*, Agua, Sal Marina, Cebada tostada*, Koji (Aspergillus oryzae)), Agua, Mikawa Mirin*(Arroz dulce*, Agua, Arroz*, Koji (Aspergillus oryzae)), Sirope de Cebada*, Sal Marina, Shoyu Johsen*(Granos de Soja*, Trigo Integral*, Sal Marina, Agua, Koji (Aspergillus oryzae)), Alga (Wakame, Kombu)
*de cultivo ecológico

I Zuppa di Miso istantanea con miso di riso e soia - Modo d'uso: Versare in una tazza o in una scodella il contenuto di una bustina, aggiungere una tazza di acqua calda e mescolare. Far riposare per circa un minuto prima di bere.
Ingredienti: Miso di riso integrale Johsen*(31%)(fagioli di soia*, riso integrale*, sale marino, acqua, Koji (Aspergillus oryzae)), Miso di soia Yaemon*(20%)(fagioli di soia*, acqua, sale marino, farina di orzo tostato*, Koji (Aspergillus Oryzae)), acqua, Mirin Mikawa*(riso dolce*, acqua, riso*, Koji (Aspergillus oryzae)), malto di orzo*, sale marino, Shoyu Johsen*(fagioli di soia*, frumento integrale*, sale marino, acqua, Koji (Aspergillus Oryzae)), alghe marine (Wakame, Kombu)
*da agricoltura biologica

Best before End: / A consommer de préférence avant: / Mindestens haltbar bis: / Consumir preferentemente antes de: / Da consumarsi preferibilmente entro: 13.02.2007

Attachment : 2

CHAPTER 3 Dietary Fibers

(1) General

Modern diets today, especially in the U.S. are high in refined and processed foods and are often lacking in the necessary dietary fiber. The healing and therapeutic qualities of a diet high in both soluble and insoluble fiber are manifold. Dietary fiber is necessary for clearing the digestive system. Constipation elevates blood pressure so algal dietary fiber can help to lower blood pressure. Algal dietary fiber, in addition to assisting in clearing the digestive system, also protects the surface membrane of the stomach and intestine from potential carcinogens and absorbs various substances such as sodium ions and cholesterol, ultimately to be eliminated. Recent research on experimental animals shows that certain algal acidic polysaccharides do prevent the proliferation of implanted cancer cells, possibly by enhancing the immune system (1 ~3).

(2) Dietary fibers of seaweeds

Dietary fibers which are high in the absorption of water are found aplenty in edible marine plants such as konbu, wakame. These seaweeds contain a sulfated polysaccharide known as fucoidan. In addition, brown algae such as konu and wakame contain alginates, a polyuronic acid which is also a water-soluble dietary fiber. These types of acidic dietary fibers are generally not present in terrestrial vegetables. Terrestrial plants generally contain a uronic acid such as pectin and plant gums which are different from that of alginates. Cellulose and hemicellulose or similar polysaccharides occur in both land and sea plants but these are neutral polysacchnides. Seaweeds contain more of the acidic polysacchrides which are soluble in water or dilute alkali.

When seaweeds such as konbu and wakame are soaked in water for a while, a viscous substance like mucin (mucopolysacchanide of saliva) comes out. This viscous dietary fiber of brown seaweeds consists mainly of alginate and fucoidan along with a small amount of protein. The protein serves to bind these soluble fibers. Konbu and wakame contain these acidic fibers from 25 to 35% on a dry weight basis. It is this all important dietary fiber in its muscilage form that assists with elimination and coats the digestive tract to protect the walls from inflamation, hence is used for the treatment of ulcers in Japan and protects the digestive tract by envelping carcinogens. The alginates and fucoidans of the brown seaweed correspond to the pectins in terrestrial plants. An apple, which is a rich source of pectin, however, contains roughly 12% insoluble fiber.

Dietary fiber in nori is composed mainly of porphyran, a sulfated galactan, in rough amount of 30% of dry weight. The substance is not found in land plants. It is similar in biological funcution to the alginates and fucoidan of the brown seaweeds. Other edible red algae have characteristic sulfated polysaccharides, mainly of galactan. Several forms of carrageenans, in varying amounts are found in *Chondrus crispus* (Irish moss, carageen moss, "tochaka" in Japanese) and *Eucheuma muricatum. E. amakusaensis,* kirinsai and in the Philippines: *E. cottonii and E. spinosum* and *Kappaphycus striatum,* "ookirinsai" in Japan.

The red algae *Gelidium* (cow hair, stone flower or 'tengusa" in Japanese) and *Gracilaria* (Sea noodle, thin dragon beard plant or "ogonori" in Japanese) contains similar water soluble sulfated galactans called agar, so are an excellent source of dietary fiber. Agar differs from carrageenan mainly in chemical structure but both are classified as galactans. Agar contains less sulfate, and when dissolved can form a gel. It serves as gelatin for vegetarians. Agar is used a mild purgative in Japan.

Some green algae, e.g. *Ulva* and *Enteromorpha* have been known to contain other kinds of sulfated heteropolysaccharides (mostly belonging to complex rhamnan sulfate) than the mentioned above. Most of them are a kind of heparinoid and work as dietary fiber when they are uptaken as food (2, 3).

CHAPTER 4 Antihypertensive Substances of Seaweeds

(1) Relationship between hypertension and saltuptake

Hypertension, simply stated, is when the blood pressure is elevated, especially, at the lower reading or diastolic pressure. Normally this reading would be 140/90 mg Hg. There are various reasons why the blood pressure may be elevated but obesity and high levels of sodium (salt) may aggravate this condition. Epidemiologic statistics and research experiments show that a large intake of salt can result in hypertension which can lead to arteriosclerosis and eventually to a stroke or cardiac infarction. In the United States, the Food and Nutrition board recommends an amount lower than 6 grams of salt per day. In Japan the amount is higher, being 10 grams per day. Since ancient times, it has been known in Japan that a diet of marine plants had the effect or lowering blood pressure. In recent laboratory experiments results show that incorporating marine algae to the diet of rats effectively lowers blood pressure (1~2).

(2) Blood pressure caused by rennin; inhibitive factors against angiotensin converting enzyme

Among other things, blood pressure is regulated by the balance between sodium and potassium ion levels in the blood. With an increase in sodium ions, the autonomic nerves gives orders for vasoconstriction. With the lowering of sodium ions and increasing potassium ions, blood pressure is lowered (Fig. 36).

Most of edible seaweeds and paticurarly polysaccharides isolated from them have been known to show high dietary fiber activities such as somewhat noticeable hypotensive material when they are ingested as food. Here, one of the results from our relating experiments, using rats as experimental animal. A part of them is shown in Figs. (37~38).

In hypertension, it has often been observed that the balance in the reabsorption of sodium and calcium in the kidneys becomes abnormal and there is an increased reabsorption of sodium in the blood stream. This results in a high calcium, low sodium level in the urine. Supplemental calcium might be required. Konbu has a relatively high calcium content (1% of its dry weight). Much of this is in a combined state with alginic acid but a large amount of this is released in the small intestine because of a slightly alkaline state in the blood stream. A portion will be taken into the blood stream and some will be lost as it recombines and is eliminated with the soluble fiber.

Research studies also show that a magnesium deficiency creates vasoconstriction

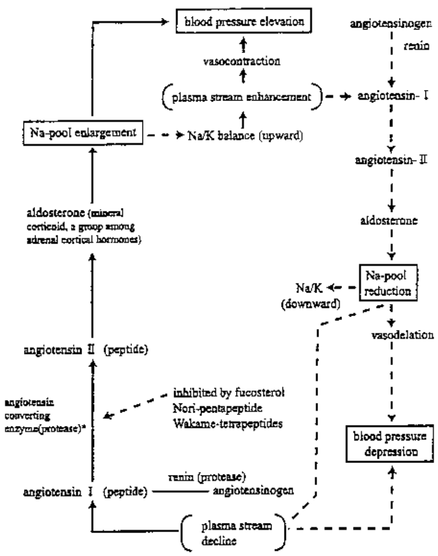

Fig. 36 Changes in blood pressure caused by the renin (Angiotensin-aldsterone system)
⟶ Upward direction,
- - → Downward direction
*EC Number: 3.4.15.1, Systematic name: Peptidyl-dipeptide hydrolase, common name: Angitesin 1-converting enzyme or carboxy-cathepsin

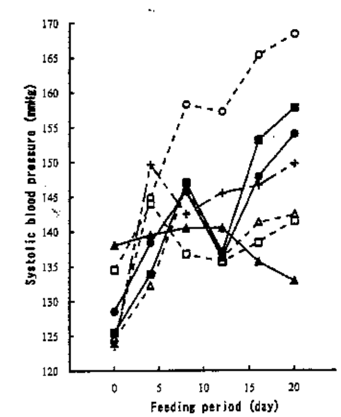

Fig. 37 Effect of algal polysaccharides on systolic blood pressure of feeding rats. (1)

	Body weight gain (g)
○Control	:75.5
■Surfated glucuronoxyrorhamnan (*Monostroma nitidum*)	:92.1
□Fucoidan (*Sargassum ringgoldianum*)	:80.0
△Sodium alginate (*Analipus japonicus*)	:92.9
●Porphyran (*Porphyra yezoensis*)	:81.5
▲Funoran (*Gloiopeltis tenax*)	:77.8
+Agar (Commercial product of W. P. C.)	:83.1

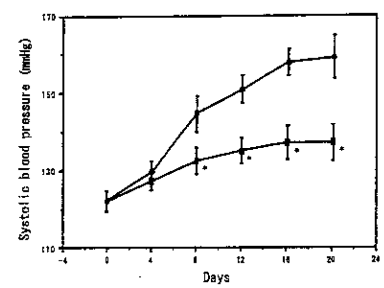

Fig. 38 Antihypertensive effect of rats subjected to funoran treatments for 20 days
● : control, ■ : funoran. Values are mean ± S. D., *p <0.01.

and increases blood pressure. Konbu and wakame are excellent sources for both calcium and magnesium.

All marine algae contain a substance called "fucosterol" but comparatively speaking, brown algae contain the largest amounts. Fucosterol belongs to the same sterol group as cholesterol and sitosterol but it exhibits "antilipemic and hypotensive activity. In other words, its presence helps to reduce fats in the blood stream and lowers blood pressure. Fucosterol is found only in marine plants while sitosterol is abundant in land plants such as spinach and soy beans. Sitosterol functions in a similar way to fucosterol. General hypertension is caused by the changes in the sodium/potassium ratio which brings about an increase in the concentration of angiotensin II, a physiologically active peptide, which is produced by a protease called angiotensinconverting enzyme (ACE). Angiotensinogen, through the action of renin (a protease), forms angiotensin I which then becomes ACE. ACE has a potent vasoconstrictive and hypertensive activity. Fucosterol inhibits the activity of ACE to change the balance in the sodium/potassium ratio (3).

Another substance, laminin, a non-proteinous amino acid derivative is found in marine brown algae, primarily in the Laminariacea (konbu family). Laminin has a weak transitory effect in depressing blood pressure. *Laminaria angusta* (mitsuishi konbu in Japanese) has the highest content of laminin in the brown algae group (4).

(3) Hypotensive new oligopeptides from protein of *Porphyra and Undaria*

Most recently, several oligopeptides which are fractionated from the different kinds of proteolytic digests from *Porphyra yezoensis* protein were tested for their hypotensive capacity on experimental rats and a number of volunteers. (5, 6). Of the various kinds of oligopeptides, five kinds of ones revealed the activity and a pentapeptide, Ala-Lys-Tyr-Ser-Tyr (AKYSY) (PS-1 fraction) had the highest activity. The authors suggested that the result obtained may due to be caused by the possible inhibition of an angiotensin I-converting enzyme by the oligopeptides. This suggestion has been supported by another parallel experiment using a purified preparation of this enzyme (7). The inhibitory patterns by the pentapeptide are shown in Fig. 37. Several tetra-peptides having a similar function to the mentioned above were most recently prepared from a proteorytic digest of a protein fraction extracted from the fronds of wakame (*Undaria pinnatifida*) by ion-exchange chromatography and gel-filtration. The individual peptides showed more or less inhivitory activity against angiotensin I-converting enzyme (ECE)(8). The inhibitory patterns of these peptides are shown in Fig. 39~40.

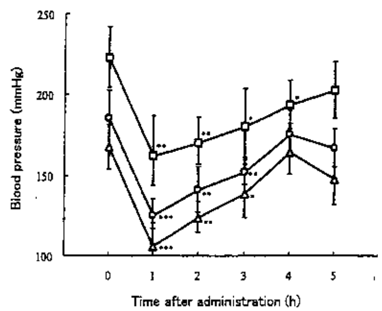

Fig. 39 Effecet of oral administration of the *P. yezoensis* peptides (SP-I fraction) on the blood pressure of SHRs. Vertical bars represent the mean±SD, N=5, Significant differences from blood pressure at 0 h; *P<0.05, **P<0.01, ***P<0.001. □, systolic blood pressure; ○, mean blood pressure; △, diastolic blood pressure. (7)

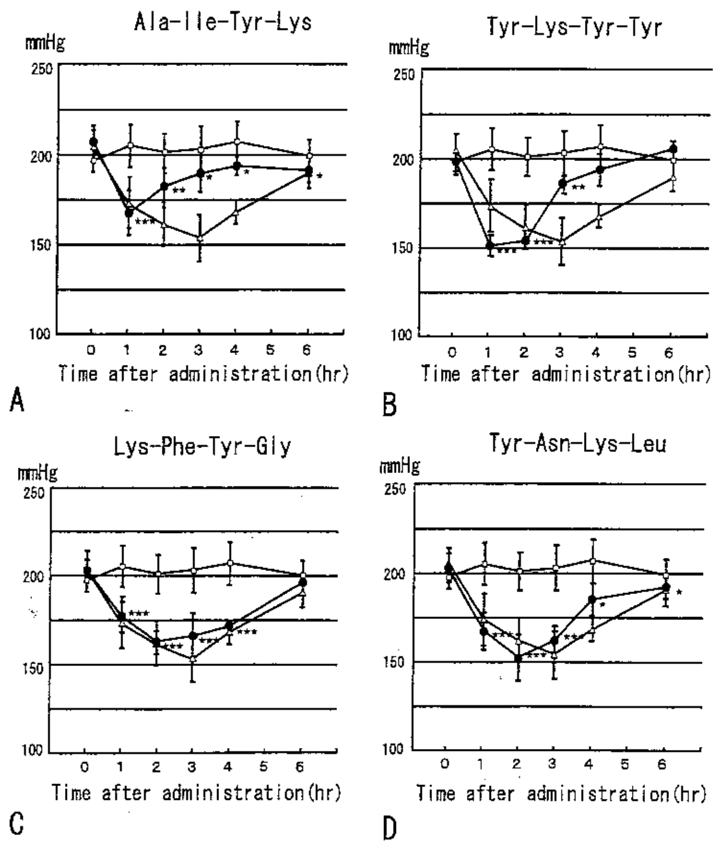

Fig. 40 Antihypertensive effect in spontaneously hypertensive rats of single oral doses of the four tetrapeptides (A-D) with angiotensin I-converting enzyme inhibitory activity isolated from wakame. Each point represents the mean change in systolic blood pressure in five rats: □ control (0.9% saline); △ captopril (oral 10 mg/kg); ● each tetrapeptide (oral 50 mg/kg). Different from control at *P <0.05, **P <0.01, and ***P <0.001. (8)

CHAPTER 5 Cholesterol and Seaweed Diet

(1) General

Cholesterol is a common word in today's vernacular and most people are aware of what their cholesterol levels are. Also known are the facts that when cholesterol levels are high, this can lead to serious heart problems, heart attacks, bypasses, etc. Perhaps less understood is factual information on the role it plays in physiological functions. Cholesterol is an important substance, particularly in the cell membrane in all living orgnisms. It is a complex chemical present in all animal fats, especially in bile, the brain, blood, adrenal glands and nerve-fiber sheaths. It is involved in the synthesis of certain hormones and of the bile acid which is essential to the digestion and absorption of lipids. Studies indicate that an excessive amount of cholesterol can clog arteries and lead to cardiovasular diseases. While too little cholesterol can also lead to serous health problems, in FOOD FOR LIFE. Dr. Neal Barnard writes that on a fruit and vegetable diet, there is enough cholesterol to take care of our needs. Cholesterol attaches itself to lipoproteins. Low density lipoproteins (LDL) carries cholesterol that can build up plaque in the arteries and high-density lipoproteins (HDL) carries cholesterol to the level where the body can get rid of it.

Cholesterol is taken in as food in our diets and is also biosynthesized in the liver. Concentrations of cholesterol between the liver and plasma are interchangable. It, however, must fluctuate within a certain range. Arteriosclerosis, along with cancer, is a leading cause of death in Japan. This statistic can no doubt apply also to the United States, as with many other countries.

Research evidence in Japan show that there is a distinct link between the lowering of blood cholesterol level and the prevention and reduction of plaque buildup in the arteries and the ingestion of certain seaweeds. Research evidence show that most of the edibe seaweeds, for example, aonori (*Enteromorpha*), konbu (*Laminaria*), wakame (*Undaria*), and nori (*Porphyra*) elevated the HDL levels and depressed LDL levels in the blood for the test animals. In particular, a diet containing algal polysaccharides which have a high functbn of dietary fiber showed this remarkable tendency (2). These marine algae have long been used a part of Japanese diet.

Cholesterol is, on the other hand, a vitally very important substance in living organisms. For example, it is a complex compound and occupies roughly 40 % of ordinary cell membrane lipids of mammalian erythrocytes. In addition, most of steroid hormones including sexhormones as well as bile acids are biosynthesized from choresterol. The formers are highly necessarily for keeping various bioreactions in the tissues and organs of the body, and the latter has a role to assist the digestion and absorption of lipids at small intestine. Cholesterol is ingested with

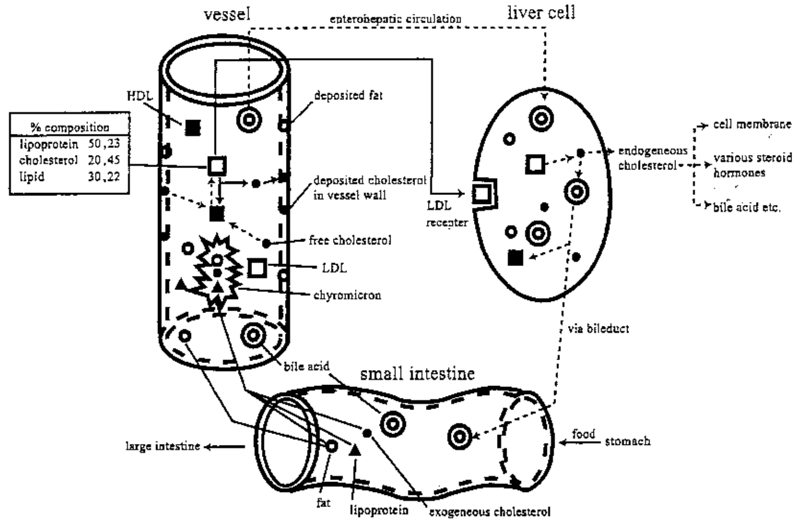

Fig. 41 Diagram of different types of cholesterol intissues and liver and their circulation in body.

other food we eat ordinarily. As shown in Fig. 41, choresterol absorbed is usually combined with some lipoprotein to make chyromicron, a large size lipoprotein and transported via lymph vesssels into blood vessels. From which some of LDL (light density lipoprotein) are finally absorbed into liver through LDL-specific receptor. It must be added here that relatively large amounts of seaweed powder or polysaccharides obtained from them were mixed with the diet.

Besides this kind of cholesterol in exogenous origin, part of cholesterol is biosynthesized in liver and they are always mutually interchanged between blood and liver. The cholesterol concentration in blood, therefore, is changed usually within some extents. This is due to the fact that it must be kept physiologically suitable concentrations which are statistically agreed for normal health. In fact, too little cholesterol concentrations may cause serious physiological damage such as apoplexy while too much ones can cause lesions in the blood vessels resulting in arteriosclerosis or atherosclerosis and other ischemic heart diseases.

It seems reasonable that the biosynthesis of cholesterol may be inhibited by fucosterol which is an isomer of cholesterol and moreover it corresponds to sitosterol in terrestrial higher plants. Fucosterol occurs in relatively large amounts in brown seaweeds such as konbu and wakame. Since sitosterol has been known to use as an hypocholesterolemic drug, it may be assumed that it has the same fiction as sitosterol.

It is well known that thyroid hormones such as thyroxine and related compounds are existent in the brown seaweeds, particurarly in *Laminaria*. These compounds appear to show antilipemic activity in some resach experiments (1). In addition, there is a reported that a brown alga called "fir needle" (*Analipus japonicus*) which has been usually taken as food in Japan, being called *matsumo* in Japanese has the similar activity(l).

(2) Recent animal experiments on hypercholesterolemic activity of seaweeds.

In a most recent investigation of us, the possible effects of seaweeds were carried out on rats by putting on a high cholesterol diet (2-4). The experimental rats were separated in two groups:

Group A was placed on a standard diet mixed with seaweeds meal from 26 species, in which 1 species of green algae, 21 species of brown algae and 4 species of red algae are included, and with cholesterol of 1.5% level. Group B was placed on the standard diet mixed with the same cholesterol level, but without the addition of the seaweed meel. Both Group rats were placed on this diet for 20 days. Except for the seaweed meal, all other treatment conditions remained the same. At the end of 20 days, the total weight gain for each rat was measured, then food was withheld for the next 12 to 14 hours. Then blood from the heart was measured for free cholesterol in the blood, HDL (high density lipoprotein), LDL (low density lipoprotein) and TG (triglyceride: a representative neutral fat). The results seemed very notable and are summerarized as follows(2).

①Changes in body weight for Group A on an algal diet were plus/minus around 10% for more that 20 species and the rest 6 species increased or decreased to extremely large extent.

②Total cholesterol levels in blood were irregularly changed from around plus 200% to around minus 60% by majority of seaweed diet, but changes fluctuated within around plus/minus 10%.

③In contrast, HDL levels in blood were elevated by addition of almost half number of seaweed species. The highest value attained 64% of the control, although the levels elevated by remaining another almost half of seaweeds showed only 20% or below the control value.

④Changes in neutral fat triglycerides levels in blood showed trend to elevate with around 2/3 species of seaweed attaining more than 36% of the control at the highest level while minor changes of plus/minus occurred with the rest of seaweeds.

⑤LDL levels in blood were lowered by around a half seaweeds while those were elevated by nearly the same number of species, but the rates of depression were far higher than those of elevation in general, the highest value of the forming at 57% lower level than the control while that of the latter being at 27% of higher level than the control.

⑥ Atherogenic indices crisis frequency for atherosclerosis which were calculated from an equation (TC-LDL)/HDL were decreased with more than half of seaweeds. The lowest value of reducing index attains around more than 60% lower than that of the control while there were about 1/3 numbers of elevated indices in which extremely exaggerated one was present.

⑦As a whole, it was discovered that more than a half of the 26 species of seaweeds tested tend to repress elevation of blood cholesterol levels, which had been caused by forced hypercholesterolemia of rats. Aonori, konbu, wakame and nori which have been in use as food for a long period in Japan showed more less the repressive activity. In particular, the experiment showed that these seaweeds elevate HDL level while depressing LDL level in blood thereby decreasing the probability for atherosclerosis.

(3) Recent animal experiments on hypercholestetleremic activity of seaweed polysaccharides

From above mentioned results from ① to ⑦, it was posturated that, water-soluble polysaccharides having the high function of dietary fibers in these seaweeds may reveal the antihyperlipidemic activity on experimental rats. Thus, we attempted similar experiments to the seaweed meal using purified polysaccharides extracted from appropriately selected seaweeds: Sulfated glucuronoxylorhamnan from *Monostroma nitidum* (green alga), sodium alginate from *Analipus japonicus* (brown alga), porphyran from *Porphyra yezoensis* (red alga), funoran from *Gloiopetis tenax* (red alga), and agar from commercial products of Wakoh Chem. Md. Ltd. (2).

In this experiment, entirely similar procedures to those with seaweed meal were employed both for rearing rat and analytical methods, and the results obtained are shown in Fig. 42.

In this Figure, the effects of diet on the changes of serum lipid levels are expressed as percentages on the values from control experiment where only the diet without polysaccharides are used. The results obtained revealed almost similar tendency to active seaweed meal experiment, although the each lipid content fluctuates to a considerable extent according to the polysaccaride spieces, whose chemical structure are considerably different.

Upon rough inspectation of the results, it may be obviously pointed out that changes of lipid levels may be separated into two groups. The one group which includes total cholesterol (TC), free cholesterol (FC), low density-lipoprotein (LDL) arid triglyceride (TG) all of which show a tendency to decreases and particurarily higher with LDL. In another group, high-density-lipoprotein (HDL) shows remarkable increases although they fructuate widely according to the species of seaweed polysaccharide added to diet. Concequently, atherogenetic index (AI) for each polysaccharide changes downwards throughout the breeding period.

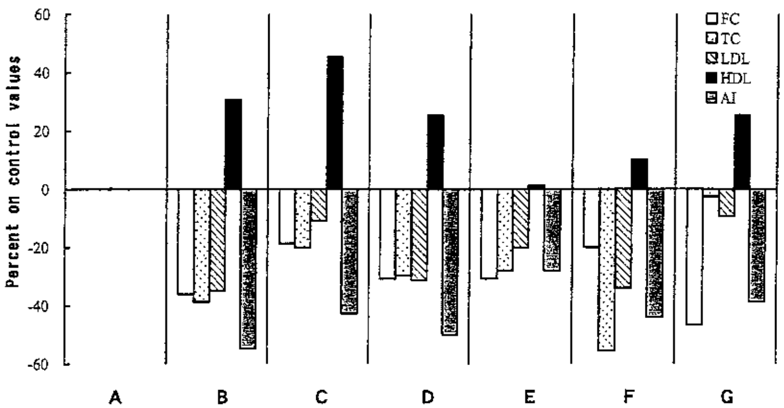

Fig. 42 Hyperchoresterol effect of algal polysaccharide-containing diet on serum lipid level of rat for 20 day-raising. (2)
A : control, B : surfated glucuronoxylorhamnan, C : fucoidan, D : Na-alginate, E : porphyran, F : funoran, G : agar,
FC : free cholesterol, TC : total cholesterol, LDL : light density cholesterol, HDL : high density cholesterol, AI : atheroscrelosis index

Furthermore, it was found that the capacity to decrease AI values of individual seaweed polysaccharides is roughly as follows:

Porpyran = control < (slightly) funoran < agar < sulfated glucuronoxylorhamnan ≪ fucoidan.

Thus, as a whole, it may be concluded that the six kinds of seaweed polysaccharides have a capacity to lower more or less the LDL level of experimental animal while more or less elevate the HDL level.

(4) Why are seaweeds and their polysaccharides useful for lowering cholesterol level in hypercholesterolemia blood.

① Circulation of lipoprotein between various tissues and organs (Fig. 41)

There are two pathways in the cholesterol circulation. One pathway is that the cholesterol synthesized in liver (endogenous one) is released into blood stream and is used mainly as a food into small intestine (exogenous one) is first absorbed into stream and then it is subjected to the same fate as the one synthesized in liver.

The cholesterol in blood is primarily incorporated into lipoprotein, although some of it remains in a free state or esterified with fatty acids. There are mainly four forms of cholesterol depending on the partner with which cholesterol is combined.

The first form is a member of chylomicron which is the largest complex lipid particle containing almost exclusively neutral fat (TG) beside phospholipids and cholesterol of free and ester forms. The second is one of the components of very low density lipoprotein LDL which has a large size next to chylomicron and contains neutral fat and phospholipid as the major ingredient. The third is one of the ingredients of low density lipoprotein LDL which has a medium size and consists of mainly protein and phospholipids as the major component. The last type is one of the components of high density lipoprotein (HDL) which is very small in size but is of high density because protein is the major ingredient and it has the highest amount of phospholipid among the four types of lipoproteins.

When exogenous cholesterol in food is ingested and reaches the small intestine, it is liberated from food and icorporated into chylomicron as fats and lipids and will be absorbed through the villi of the intestinal mucous membrane. Then the cholesterol is conveyed by blood stream to the liver. Most of the cholesterol is present in the form of LDL but small part is in free or esterified form.

Free and esterized form of cholesterol in blood of rats used in the experiment mentioned above occupy roughly 12% and 45% of the total cholesterol in blood. These forms of cholesterol will eventually to be incorporated into any blood lipoprotein mostly into LDL. Cholesterol of LDL is largest amount among blood lipoproteins makes up more than 40% of the total. As HDL is present in very small amounts in the blood, therefore, the cholesterol bound to it is also small in amount.

② Role of LDL and HDL.

Role of LDL and HDL in the circulation of cholesterol is comprehensive and depends upon the quantities found in the bloodstream. The main role of LDL is to transport cholesterol to the necessary sites for the cells. Cholesterol transported to any wound in the arteries, will form an atheroma, a fatty deposit of mucoprotein, phospholipid, fibrin and a protein of LDL. This deposit on the walls of the artery form an atheroma in the endotheial cells and always cause atherosclerosis. When this occurs in a coronary artery it causes an infarction.

An excessive amount of LDL in blood stream can be decreased in a healthy liver. The liver has receptor proteins for LDL on their surface and LDL in blood stream enters the liver after it is absorbed in this receptor, a relationship like "key and lock" as shown in (Fig. 41) Usually, excess LDL is degraded in the liver cells and the exogenous cholesterol is metabolized in the same way as endogenous cholesterol which had been biosynthesized here in liver. Mostly, the cholesterol is converted in liver mainly to bile acid, but it also serves the biosynthesis of various steroid hormones as raw material. An example is that sex hormones are synthesized in gonad and corticosteroids such as aldostrone is made in adrenal cortex and so on.

HDL has densities of 1.003 to 1.21 and these are 1.057 to 1.2138 times those of LDL respectively. That is why they are called HDL because HDL is higher in protein content instead of lower in cholesterol content. HDL also wanders about with blood stream and its main function is to pick up cholesterol sedimented on the vessel wall in contrast to that of LDL. In general, therefore, HDL is understood as "good lipoprotein" while LDL as "bad one". HDL delivers cholesterol to the liver to be finally metabolyzed.

③ Role of seaweeds and dietary fibers.

When seaweeds that are in high dietary fibers are ingested in the intestine, the dietary fibers themselves gradually form a colloidal state in a random network of the polysaccharide molecules. Part of cholesterols whether they are exogenous or endogenous get wrapped up in this network and move through the diogstive tract, finally to be eliminatled. Thus, cholesterol to be absorbed may become lesser. However, it is difficult to explain why the levels of HDL tended to elevate while that of LDL was lowered in the animal experiment mentioned above(Fig. 42) Nevertheless, it should be noted that in other experiments similar to the above mentioned one, researchers have reported some decrease in the cholesterol level in liver. Changes in the cholesterol level in the liver was not examined in the experiment mentioned above.

④ Lowering cholesterol level in blood and liver by loss of bile acid.

As indicated in Fig. 41, bile acid which is essential for lipid absorption is biosynthesized from cholesterol in the liver. It is excreted into intestine via bile duct after temporary storage in gall bladder. It is usually combined with some amino acids such as glycerine and taurine and enhances the detergent activity of the bile acids. The conjugated compounds serve in the fat and lipid digestion by forming a droplet called chyromicron which is mostly composed of neutral lipids stabilzed by a small amount of lypoprotein. Thus a large part of bile acid formed from cholesterol in the liver is secteted into the small intestine, but a large part of its reabsorbed into the blood stream to be returned to the liver by the route of "enterohepatic circulation". Therefore, almost of the bile acids which originated in the liver is reabsorbed in liver via portal vein. However, some remains in the intestine and it is this portion of the bile acids which must be supplemented from the cholesterol in the liver. By this enterohepatic circulation of bile acid, biosynthesis of bile acid in liver is controlled in general. When foods from seaweeds or their polysaccharides are present in the intestine, the net work of these colloidal dietary fibers such as alginates combine not only with part of the cholesterol from food origin but also with the bile acid excreted from the liver, and their absorption is disturbed at last in part. To supplement the amount of bile acid lost in intestine by absorption to dietary fibers, the liver must synthesize an additional amount, thereby sacrificing some of cholesterol present in liver. The total cholesterol in liver is then to be decreased by such events as the additional synthesis of bile acid. The capacity of adsorption may be effected by minor

difference of colloidal property due to the indivisually characteristic molecular structures.

The above speculation may fill in part the explanation for depression of cholesterol level in the liver by a seaweed diet in some cases. But, the cholesterol level in liver is not always lowered by experiment with seaweed diet. The main reason for the uncertainty is that there have been many reports which state that seaweed meal and their polysaccarides depress the serum cholesterol level, but not with liver. In the liver, there are some reports in which the level being depressed, while some being elevated and some appearing ineffective. The reason for these experimental results has not been theoretically cleared at present.

⑤ Fucosterol depresses cholesterol level in blood (3).

In a research experiment using chickens, Group A were fed a standard diet mixed with 1% cholesterol. Group B were fed the same diet with an added 1% fucosterol (a substance found in seaweeds). After 21 days on these diets, the cholesterol concentration in plasmas collected from the two groups were determined. It was found that cholesterol level of B group was 56.8% lower than that of Group A. The result may be explained from an assumption that fucosterol inhibits some stage of biosynthesis of cholesterol which started from acetyl CoA.

Fucosterol is relatively plentiful in the brown seaweeds including wakame and konbu.

⑥ Antilipemic activity in organic iodide compounds from konbu.

Inorganic iodides in seaweeds were mostly in a form of sodium and potassium salts, but various forms of organic iodide compounds are also present. for example, thyroxine, a thyroid hormone and the compounds related to it are found relatively in a large amount in brown seaweeds such as konbu and horsetail (hondawara in Japanese). There is a work in which quantitative determination of organic iodide compounds was made for several marine algae, in which seaweeds being edible but not eaten in common are included (1).

For example, their contents of two kinds of brown algae were selected and shown in Table 2.

Table 2 Contents of organic iodione compounds in two kinds of Japanese brown algae (mg %, dry weight basis) (1)

Organic iodione compound	*Laminaria* sp. (one species of Konbu)	*Sargassum* sp. (one species of horse tangle)
monoiodidotyrosine	1.55	10.61
diiodotyrosine	2.33	4.04
triiodotyrosine	0.91	1.51
thyroxine	1.3	1.9
iodine-bound protein	0.67	5.56
iodine-bound unidetified	2.9	8.72

One group of rats were fed with standard diet containing 1% cholesterol and the other two groups of rats were fed the same diet with addition of 0.01 thyroxine or 2 mg of diiodotyrosine per rat per day. The 3 goups of rats were raised for 6 weeks under the same condition and the cholesterol level in blood of each rat was measured. The result was obtained is that the levels were dropped nearly 17% and 28% of the control group. It may be noted here, the drop by addition of organic iodide compounds to diet seems to be due to some different reason from the drop by acidic polysaccharides diet. The repressive activity may be related to the possible controlling effect on some stage of biosynthetc pathway of cholesterol in either way being directly or indirectly.

⑦ Effect of chlorophylls and partially degraded products.

We are often aware of the data for examination of the possible antilipemic activity of chlorophylls which occur ubiquitously in photosyntheic plants including marine alge. Most experiments have been carried out using diet containing 1% cholesterol plus/minus 20-30 mg chlorophyll or pheophytin and pheobarphide commonly formed in the digestive tracts of rats. The latter two compounds are each chlorophyll degradation product. Pheophytin having lost its magnesium intead of two hydrogen atoms and pheophorbide being lost magnesium and phytol in its molecule. As the result of experiment, pheophyitin and pheophorbide show antilipemic activity in a range of depression between 20% to 30% of the control (1).

CHAPTER 6 Anticoagulents

(1) Blood coagulation

When we get a cut or wound and bleeding occurs, the blood has a protective mechanism by which the blood clots and the bleeding stops, unless of course, the wound is so deep that a vessel has been cut. The bleeding stops because in a series of enzymatic reactions (by a series of proteases called thrombin or fibrinogenase) following the blood coagulation. If there is a lesion in the vessel and the clot or thrombosis is formed in the vessel, the results can be very serious, especially if the clot is in the cerevral or coronary arteries. Close to twenty steps in enzymatic reactions by thrombin and its cofactors take place in blood coagulation. In essence, what happens is that an enzyme, thrombin, catalizes the formation of a water insoluble network of fibrin from the soluble fibrinogen found in the blood streem. Fibrin enmeshes the erythrocytes, leucocytes and platelets to form a blood clot.

On the other hand, there is an enzyme called plasmin which degrades blood clots and improves blood stream as has been before. Plasmin is a kind of serin protease and found in the tissues of the vessel walls and in urine. Plasmin is produced from a precursor called the plasminogen activating enzyme (PAE). Plasminogen is a type of protease. Any protease belongs to a group of protein digesting enzymes

(2) Fucosterol

Fucosterol, a substance which occurs naturally in marine algae, paticcularly in brown algae, has recently been found to activate PAE and accelerate the formation of plasmin (4). It has been noted that fucosterol also plays a role in preventing hypercholesterolemia (Chapter 5) in addtion to repress hypertension (Chapter 4). In this Chapter, one more such an activity of it is reported as it may work to prevent blood clot formation in the vessels by daily intake of it. This fuction of plasminogen activating fucosterol has been deduced from the following experiment. Endotherial walls from bovine (cattle) carotid artery were cultured in the three kinds of media; culture A contained only the standard medium, culture B contained the standard medium plus fucosterol, culture C contained the standard medium plus sitosterol (a sterol similar to fucosterol in function, but is found only in some land plants). When the PAE which formed in the proliferating cells in the culture mediums were checked, the PAE activity of the cells grown on fucosterol medium was nearly 7.5 times higher than that of the control cells. In contrast, the medium containing sitosterol showed a 2.4 times activity of the control. Thus, in these experiments, fucosterol proved to be 3 times higher in the production of PAE than sitosterol. (1)

CHAPTER 7 Thrombosis

Thrombus is the state in which blood coagulation occurs within the blood vessels. The main mechanism for blood coagulation has been discussed in the preceeding chapter. When thrombosis occurs in the brain or coronary artery, serious illnesses such as cerebral and cardiac infarctions are the result.

(1) Preventive property of PUFA (n-3) for thrombosis

Recent investigations show that thrombosis can be prevented by polyunsaturated fatty acids belonging to the n-3 groups besides sulfated polysaccharides (1-3). The so called essential fatty acids belong to the n-6 group. This classification is made according to the number of carbon atoms of fatty acid enumerated from the terminal methyl group, when the first double bond (an unsaturated bond) occurs at the number three carbon atom from the end methyl group of an unsaturated fatty acid, it belongs to the n-3 group and when it occurs at the number six atom, this fatty acid belongs to the n-6 group. For example α-linolenic (C-18), total number of carbon atoms being 18, eicosapentaenoic (EPA) or icosa (IPA) (C-20) and docosahexaenoic (DHA) (C-22) acids etc. belong to the n-3 group while α-linolenic (C-18) and arachidonic acids etc. belong to the n-6 group (Fig. 43).

A large number of investigations show that most prostaglandins (local hormones) are derived from α-linolenic (n-3 group), arachidonic (n-6 group) and eicosapentaenoic (n-3 group) acids, and various prostaglandins, PGH_2 and PGE_2, for example, are formed from the arachidonic acid and from EPA respectively. PGH_2 exhibits its physiological functions, for example by induction for platelet aggregation and vasocontraction, etc. While PGE_2 display an almost opposite function for PGH_2, the inhibition of platelet aggregation and vasodilation, etc. In this respect n-3 type fatty acids have recently been considered to be essential fatty acids for the human body along with the n-6 type ones.

The highly unsaturated fatty acids of the n-3 group are known to be in fish oil such as sardines, saury and mackerel, but for a long time their nutritional and physiological functions in the human body has remained obscure. Recently they have been thrust in the limelight because of the discovery that these oils help in preventing arteriosclerosis in adults. Meanwhile, scientists involved in the research of the nutritonal value of seaweeds have discovered that these plants also contain fatty acids of the highly unsaturated n-3 group, particularly EPA, although the amount is small in comparison to fish.

(2) Content of PUFA (1-3) in several edible seaweeds

The content of fatty acids in the four kinds of marine algae widely used as food in Japan has been carefully investigated and the results are shown in Table 4. About 3% of *Porphyra* (nori) is lipids, of which almost 50% is EPA. *Laminaria* (konbu) contains almost the same amount of lipids as non but the unsaturated fatty acids of the n-3 group is not as large in comparison. However, konbu and wakame contain oleic acid (C18-1) in larger amounts, almost equal to arachidonic acid than those in nori. However, the oleic acid (C18) is changed into higher unsaturated fatty acids of neither n-6 nor n-3 such as arachidonic acid (n-6 group) and EPA (n-3 group) in the human body. In addition, both types of fatty acids are unable to change mutually in

Table 4 Kind and content of the individual fatty acids in four kinds of edible seaweeds (mg % on dry weight basis)

Fatty acid (C-number and double bond number)			*Porphyra yezoensis* (Nori)	*Laminaria japonica* (Konbu)	*Undaria pinnatifida* (Wakame) blade	*Undaria pinnatifida* (Wakame) sporophyll	*Hizikia fusiformis* (Hiziki)
Saturated acid	myristic acid(14.0)		9	209	502	464	79
	palmitic acid(16.0)		655	332	1587	2576	570
	stearic acid (18.0)		20	13	-	-	-
	others		-	13	19	432	24
	sum		684	567	2108	3472	673
Unsaturated acid	hexadecamonoenoic acid (16:1)		74	89	278	96	59
	hexadecadienoic acid (16:2)		-	-			
	oleic acid(18:1)		102	311	342	2040	112
	linoleic acid(18:2)	a)	56	174	202	728	-
	α-linoleic acid(18:3)	b)	8	94	-	28	214
	γ-linoleic acid(18:3)	a)	17	72	-	-	-
	parinaric acid (18:4)	b)	14	202	-	136	73
	eicosamonoenoic acid (20:1)		109	-	-	-	-
	eicosatrienoic acid(20:3)	a)	67	-	-	88	6
	eicosadienoic acid (20:2)	b)	43	-	-	-	-
	alachidonic acid & other eicosatetraenoic acid (20:4)	a)	73	350	74	888	182
	eicosapentaenoic acid (20:5)	b)	1607	220	128	344	92
	others		30	11	458	-	85
	Sum	a	213	596	276	1704	188
		b	1672	516	128	518	379
	Total fatty acid		2884	2090	3590	7820	1496
	Ratio of unsat/sat		3.2	2.7	0.7	1.2	1.2

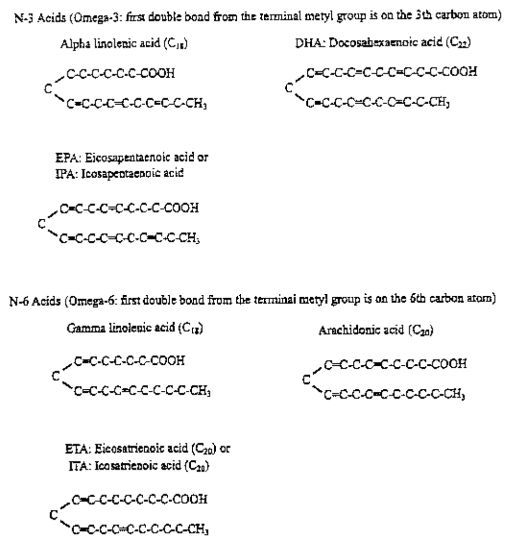

Fig. 43 Diagram showing carbon-skelton and double bond position at typical polyunsaturated fatty acid molecules, relatively abundunt in fatty acids of edible seaweeds.

our body; highly unsaturated fatty acids of both n-3 and n-6 types are interchangeable only within the same type.

Undaria (wakame) contains various fatty acids similar to that of konbu with the exception that it has a same quantity of arachidonic acid and a large amount of EPA. In contrat, the sporophyll part of wakame shows an entirely different pattern of fatty acid content. Of special note is that the wakame sporophyll (mekabu in Japanese) has the highest total lipid content of the edible marine algae amounting to as much of the dry weight (7～8%) while those of most other edible seaweeds are around 3% in general. Consequently, the fatty acid of n-3 type in the wakame sporophyll attains almost four times of wakame thallus. On the other hand, the total amount of n-3 PUFA of nori is nearly 7 times that of n-6 PUFA (net amount being approximately 1.7mg% of dry thallus) (Table 4), (Fig. 43).

The fatty acid pattern for *Hizikia* (hijiki) resembles konbu, except that it has a larger content of alpha linolenic acid. As mentioned above, it is commonly known that fatty acids of the n-3 and n-6 groups are metabolically interchangeable in their individual groups, but fatty acids in the n-3 group never changes to a fatty acid of the n-6 group and vice versa.

Thus, a fatty acid of the n-3 group, for example, α-linolenic acid (C18-3) is able to change EPA (C20-5) and even DHA (C22-6) by the addition of 2 carbon atoms and 2 double bonds (unsaturated) one by one. In contrast, if we ingested γ-linolenic acid

(C18-3) belonging to the n-6 group, it is able to change to arachidonic acid (C20-4) by. the addition of two carbon atoms and a double bond. In both groups of fatty acids, reverse changes do occur in vivo; ie. arachidonic acid (C20-4, n-6 group) to γ-linolenic acid (C18-3, n-6 group) by the loss of two carbon atoms and one double bond while EPA (C20-5, n-3 group) can change into α-linolenic acid (C18-3, n-3 group).

(3) Roles of EPA in preventing arteriosclerosis

Noticeable epidemiological studies were made by the Danish medical scientist, Dyerberg and his co-workers.They researched the correlation between the lipid levels of cholesterol and the neutral lipids in human plasma with coronary atheroschlerosis(4). The studies were carried out on the Danes living in Demark and the Inuits who lived in Greenland. The Inuits who lived in Greenland had larger amounts of EPA in their bloodstream than their counterparts who lived in Denmark. In addition the levels of cholesterol, neutral fat, beta-lipoprotein, both light density and very light density, was lower in the Inuits of Greenland than those residing in Denmark. The main reason for this may be due the diet, in that Inuits in Greenland eat more marine animals than terrestrial one for protein and fat sources. Marine animals, in turn eat marine plankton which carry an abundant supply of EPA. In additon, the Inuits of Greenland consume about 1.5 times more protein than most Danes while their carbohydrate intake is about two third that of the Danes.

It should be mentioned that fatty acids of both the n-3 and n-6 groups are needed to sustain our bodies and the ratio of the amounts is important. An imbalance of either too much or too little of either is detrimental for health and well being. An experiment was devised in which rats were able to get food when they stepped on a lever. They were unable to get food in the darkness. (5). By trial and error, the mice were able to depress a lever, turn on a light and acquire food. The diets of the two groups differed. In one group the diet consisted of more alpha linolenic acid than linoleic acid. In the second group, the balance was reversed. A noticeable result was that the rats on a diet of higher alpha linolenic acid learned to turn the light on sooner than the ones on a higher linoleic acid level. This experiment leads to the conclusion that a diet with more α linolenic acid and less linoleic acid is necessary for nourishing the cerebral and retimal nerves of rats, thus decreasing the learning time.

It is well known that linoleic acid given to experimental animals depresses cholesterol levels in blood at both levels of HDL and LDL, but fatty acids of the n-3 groups such as α-linolenic acid depresses the LDL level more as compared with HDL as mentioned earlier. Most recently, docosahexaenoic acid, DHA (C22-6, n-3) which occurs plentifully in fish oil such as that of tuna, yellowtail, mackerel, sardines, eel and in certain monocellular alga such as *Isochrysis galbana*. When the microalga is cultured in an appropriate medium, DHA production attains 3.8% of its dry weight. Most recently, it was found that DHA ingestion reinforces the cerebral nerve activity in experimental animals (Fig. 43).

Nearly fifteen years ago, very noticeable epidemiological studies on the effectiveness of dietary EPA (6, 7).

In the 1982's, detailed epidemiological studies were made by Yoshida and his associates at Chiba University in Japan on the correlation between the daily dietary intake of EPA and lesions found in the circuratory system such as hyperlipemia and hypertension, etc. for people living in agricultural and fishering villages. Estimation of blood pressure, total blood cholesterol, HDL, neutral fat, EPA, total fatty acid in plasma, inhibitive potency of platelet aggregation, atherogenetic iudex, etc. were carried out in select male and female groups.

In their really wide and precise investigations, they have made a precise epidemiological observation in which moterity rates of patients with ischemic cardiopathy as well as those with ceribrovascular disease are far lower in fishing villages than those in farming villages in Chiba prefecture. The result seemed to be due to difference in the dietary life style of both villages. The results are shown in Fig. 44. Basing this fact, the research group has made further artificial study in which patients (27-42 capita) with hyperlipidemia participated in this study of oral taking EPA of a constant amount during 16 weeks, the result being shown in Fig. 45.

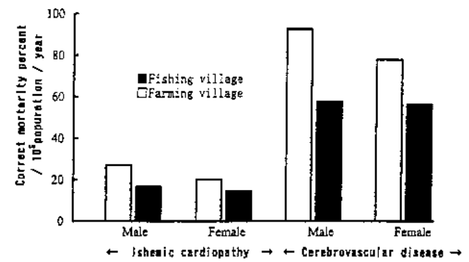

Fig. 44 Comparison of correct mortarity rates of patients with ischemic cardiopathy and cerebrovascular diseases in fishing (Katsuura City) and farming (Kashiwa City) villages (1977-1980). (6)

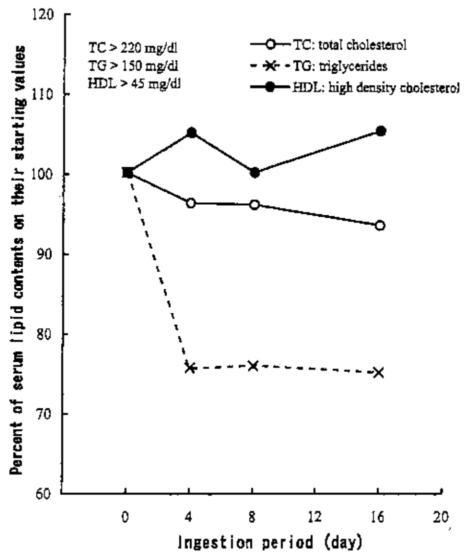

Fig. 45 Changes in serum lipid levels of patients with hyperlipidemia after injection of EPA-E capsules. (7)

According to the features of the Figure, it can be concluded that the hyperlipidemia of patients has been mitigated considerably by intake of EPA for about one month.

Furthermore, it was revealed by the result from an investigation into the death rates between 1977 to 1980 that the main cause in heart deseases were ischemic cardiopathy diseases caused by lack of oxygen due to the retardation of blood flow by coronary atherosclerosis leading to stenocardia and cardiac infarction, but the rate in the fishing residents was nearly 30% lower than the farming groups both in males and females. Similarly, the death rate due to cerebrovascular diseases such as softening of the brain, thrombus in the cerebrovascular system, cerbro-apoplexy, etc. was lower in the fishing village than in the farming one with male and female ratios being about 39% & 29% respectively. These studies suggest that a diet higher in marine plants and animals was more beneficial to cardiovascular health than diets composed of higher terrestrial and animals. Since we have the analytical data of individual fatty acid composition and their chemical structure contained in the main Japanese edible marine algae, we show them already in Table 4.

According to most recent studies, the content of taurine, which is important during mammarian developement, particularly associates with retinal dysfunction and occurs ordinarily in fishes and other marine animals, is found in *Porphyra* (nori) at amounts of 1.0 to 1.3% of its dry weight, this content being nearl equal to that of EPA. Nori, then, is an excellent food source as our daily diet, in considering the fact that it contains other various nutrients such as dietary fiber in good quality, various kinds of vitamins (B_{12} etc) and minerals. It is plausible that the excelent Emperor of Shin of ancient China thought nori (zicai in Chinease), reportedly, to be "the elixir of life" and he had highly esteemed it.

(4) Monthly changes in EPA content in aquacultred *L. Japonica.*

L. japonica contains various kinds of PUFA acids including EPA in relatively high amount, so seasonal variation of individual fatty acids including EPA of this tang were invstigated (8). The results are shown in Table 5. The EPA contents were highest among n-3 group throughout the year and attained 10% of the total FA in summer season being 219mg% of dry thallus. (Table 5).

Table 5 Monthly variation of fatty acid contents in cultivated *Laminaria japonica* (8)

CONTENT \ MONTH	3	4	5	6	7	8	9	10
Total lipid mg %	2,309	2,041	1,905	2,163	2,465	2,304	3,041	3,530
Total fatty acid mg % / Relative content %	2,213	1,772	1,830	1,591	2,172	2,116	2,468	2,689
12 : 0	-	0.1	0.1	-	0.1	0.1	-	0.4
14 : 0	18.2	15.6	20.1	10.6	9.6	11.2	10.6	5.9
14 : 1 (n-9)	-	1.3	-	-	-	0.1	-	-
14 : 1 (n-7)	2.3	-	1	0.6	0.1	-	-	-
15 : 0	0.4	0.4	0.4	0.5	0.4	0.5	0.4	0.5
16 : 0	23.4	21	20.7	19.1	15.3	20.1	27.5	27.6
16 : 1 (n-7)	5.3	5	4.8	5.4	4.1	4.2	3.2	5.2
16 : 1 (n-5)	-	-	-	0.4	-	-	-	-
17 : 0	-	-	-	-	-	-	0.3	0.8
17 : 1 (n-8)	-	-	-	-	-	-	0.2	
18 : 0	0.5	0.5	0.4	0.9	0.6	1.3	2.5	3.7
18 : 1 (n-9)	10	11.6	10.7	15.1	14.3	21	26.8	22.7
18 : 1 (n-7)	-	-	0.1	-	-	-	-	-
18 : 1 (n-5)	-	0.1	-	-	-	0.1	-	0.2
18 : 2 (n-6)	5.9	6	6.4	8.9	8	9	8.2	6.6
18 : 3 (n-6)	0.7	1.1	1.8	2	3.3	2.8	1.8	1.8
18 : 3 (n-3)	6.8	6.8	4.9	4.8	4.3	2.8	1.9	2.8
18 : 4 (n-3)	10.3	13.5	10.3	9.2	9.3	4.1	2.4	3.3
20 : 0	0.1	0.1	0.2	0.3	0.2	0.5	0.7	0.6
20 : 1 (n-7)	-	-	0.1	-	-	-	-	-
20 : 2 (n-6)	-	-	-	0.4	0.2	-	-	-
20 : 3 (n-9)	-	-	-	-	-	0.2	-	0.3
20 : 3 (n-6)	0.4	0.3	0.6	0.5	0.6	0.4	-	0.6
20 : 3 (n-3)	-	-	-	0.1	-	-	-	0.1
20 : 4 (n-6)	7.4	7.6	9.1	8.4	16.1	14	7.6	8.8
20 : 4 (n-3)	0.4	0.5	0.4	0.7	0.6	0.4		0.5
20 : 5 (n-3)	6.6	7.4	5.6	7.9	10.1	6.1	4.5	5.7
22 : 1 (n-9)	-	-	0.1	0.3	-	-	-	-
Others	1.3	1.1	2.2	3.9	2.8	1.1	1.4	1.9

CHAPTER 8 Diabetes

(1) Capacity of acidic seaweed polysaccharides to lower blood glucose level

Diabetes is a complex disorder in metabolism when a person is not able to properly digest sugar, either due to a partial or total lack of insulin or the inability of insulin to carry out its normal funtion. Insulin is a hormone secreted by the Islets of Langerhans in the pancreas. In childhood, if there is damage to the pancreas by autoimmunity for example and insulin cannot be produced, the result is an insulin-dependent diabetes and the individual will need injections of insulin for the rest of his or her life. The second is a more common form, where the insulin cannot work effectively, mostly by genetic factors.

When blood glucose level is elevated in normal body, insulin which is synthesized in the β - cell of the pancreas and controls glucose metabolism for various tissues, is secreted into blood to accelerate glucose degradation in certain tissues (e.g. fat cells) and glycogen formation in other tissues (e.g. liver and muscle). This results in the lowering of glucose levels in the blood. In the blood stream, insulin must be present before the cells can accept glucose. If insulin is not working effectively, the glucose level in the blood stream rises. Recent advice for this type of diabetes is to have a diet low in sugar, fats and/oils and high in complex carbohydrates (heteropolysaccharides etc.) and dietary fibers.

A protein which exhibits similar function to insulin was isolated from the red alga, *Gelidium pacificum* (obakusa) (1). *Gelidium* was boiled in water and the extract consisting mainly of agar and water soluble protein was fractionated on column chromatopgaphy. On intravenous tests with rabbits, the third protein fraction showed an activity like insulin. The algal protein was slower in action in the beginning but its effect lasted much longer than a dose of insulin.

(2) Powder of aqueous extract of a red alga, *Porphyra yezoensis* and a few otheralgal dietary fibers lowers artificially elevated glucose level of diabetic rats

Foods high in dietary fibers, whether of marine or terrestrial origin are effective in lowering glucose levels in the blood stream. Agar and pectin are both known to contribute in lowering blood sugar and controling insulin levels in the blood stream.(2) In recent experiments, it has aslo been found that funoran (a sulfated galactan of the red alga, *Gloiopeltis*. ('funori' in Japanese) lowered blood glucose levels significantly when added to the diet in experiments with mice. In our

CHAPTER 10 Anti-Tumor, Anti-Cancer, Anti-Virus and Immunity Activation

Investigations and research concerning the antitumor activites of seaweeds began in the 1960's and 70's. As early as the 1940's and 50's, investigations were carried out to study the effects of certain polysacchanides isolated from bacteria, fungi and higher land plants. Today, consensus is that many of the β- 1-3 glucose polymer (glucan) and certain complex polysacchanides exhibit an ability to repress the growth of transplanted tumor cells. However, potency is relatively low and it appears that the effectiveness is indirect, and is more of a subsidiary agent, such as in preventing side effects during the course of treatment. However, research continues at present, with hopes that a cure can be found in sea vegetables (7～10). For example a sulfated polysaccharide from a red alga *Schizymenia dubyi* shows an active antiviral actibity (9).

(1) Several examples of recent experiments using tumor carrying animals

Numerous experiments have been carried out during the past 20 years and the results are varied. In early studies in Japan, algal meal which was not highly purified and contained acidic algal polysaccanides were used for the experiments. However, since no decisive results were obtained from these kinds of experiments, researchers have examined the purity of the substances used. As a result, a polysaccharide fraction of fairly high purity was obtained from *Sargassum horneri* (akamoku: a member of the horsetail tangle) which repressed not only the growth of the implanted solid tumors such as sarcomas, but also tumor cells in a suspended state such as Ehrlich's ascites carcinoma, although some toxicity was observed. The explanation made in those days was that the repression of tumor growth was due to the stimulation of the host immune system by the injection of the algal polysaccharide (2～6).

Recently a notable experiment was carried out in Japan (1). For example, the 46 species of marine algae belonging to any of green, brown and red algae were each made into a meal (0.12 mm diameter) after washing enough with deionized water and then dried in air. After several days under raising with ordinary diet, individual seaweed powder of 1600 mg per kirogram of mouse was given per oral with a tube once a day for 14 days, then Ehlrich carcinoma (solid form) was inoculated in an amount of $1 \sim 2 \times 10^6$ cells / 0.2 ml in their back and then sacrified after continuing breed for further 14 days under the same condition as before. Thus, it was found that growth of the inoculated tumour cells is hindered by most of the seaweed powder feeding in varying degrees from -20% to $+60\%$ of the standard one. This increase in the growth of tumour cells is not explainable at present.

(2) Recent investigation of antitumor activity of seaweed polysaccharides.

A series of studies using various saweed polysaccharides in place of seaweed meal were made while maintaining the same condition as in the preceeding investigations, except for the dose (1). Part of the results are shown in Table 6.

Table 6 Antitumor activity of polysaccharides against Meth-A fibrosarcoma by intraperitoneal administration of 40 mg kg^{-1} for seven days (five days for kappa- and lambdacarrageenan) (1)

Polysaccharide	Inhibition rate (%)	No. of toxic deaths
Green-algal sulfated polysacchareide	26.5	0
Sodium alginate	1.2	0
G-fraction	-15.3	0
M-fraction	15.5	0
Fucoidan I	53.4*	3
A fraction	54.5*	1
B fraction	51.4*	0
C fraction	38.6	0
Fucoidan II	78.1*	0
A fraction	32.0	0
B fraction	26.2	0
C fraction	34.7	0
Iota-carrageenan	40.1*	0
Kappa-carrageenan	54.0*	5
Lamda-carrageenan	45.8*	4
Porphyran	58.4*	0

*Significant difference $p < 0.05$.

Among the 16 kinds of polysaccharide samples, fucoidan group, porphyran and part of carrageenan show higher activity against Meth-A fibrosarcoma and those with higher content of sulfate group seem to have higher anitumor potency. G-fraction which is an alginate fraction exhibits rather a tendency of promotion. In contrast, a fucoidan fraction II shows the highest activity close to 80% inhibition. There seemed some relationship between antitumor activity of polysaccharids and their heparinoid activity so far as their sulfate content.

However, most of recent conclusion for these antitumor activities of seaweed polysaccharides is due to the elevation of immunity of animals having cancer. Recent data concerning this matter, such activities are due to elevation of macrophage activity in some cases (2 ～ 4), resulting increase in lymphocyte-activating factors such as various interleukins and interferons. Most recently, it has been reported that reverse transcriptase of virus such as HIV is inhibited by some seaweed polysaccharids, e.g. by uronic acid-containing sulfated galactan of a red alga *Schizymenia pacifica* (5).

A recent paper reports, on the other hand, that fucoxanthin which is main accessory pigment of photosynthesis of seaweeds belonging to the Phaeopyceae shows an inductive effect on apoptosis of human leukemia cell line HL-60 (8). However, inhibition by most seaweed polysaccharides is below 60% which is the proposed threshold for effctiveness in antitumor activity for the transplanted tumors, some were found to be effective ones. Thus, since a fair number of algal polysaccharides exhibited antitumor activity near the threshold values, it is suggested that including seaweeds in the ordinary diet may prevent the possible formation or development of tumors.

(3) Antitumor capacity of three lipid famillies of seaweed origin

Three kinds of lipid fractions: Neutral nitro-, glyco- and phospho-lipids were obtained from eight kinds of lipids of seaweeds (which included green, brown and red algae as listed in Table 8. As shown in this Table, less than half of the twenty four experiments showed inhibition rates of above 35%. However, in a small group of experiments involving the G and P fractions from *Undaria* (wakame) and *Porphyra* (nori) had values close to or higher than the threshold numbers. This investigation showed that the two complex lipid fractions, glycolipid and phospholipid exhibited inhibition while the neutral lipid did not. The one exception was with the neutral lipid from a member of horse tail tangle (*Sargassum ringgoldianum*) (Table 7).

Table 7 Antitumor activity of lipids against Meth-A fibrosarcoma by intraperitoneal administration. Dose in mg kg-1 d-1 for seven days (1)

Sample	Lipid fraction					
	Neutral		Glycolipid		Phospholipid	
	Dose	Inhibition rate (%)	Dose	Inhibition rate (%)	Dose	Inhibition rate (%)
Ulva pertusa	40	3.6	40	33.4	3.2	32.2
Eisenia bicyclis	40	27.0*	40	35.9	40	39.4
Ecklonia cava	‹	26.2	8.9	13.8	4.0**	40.9
Undaria pinnatifida	40	− 5.9	17.1	55.8*	14.3	33.0
Laminaria japonica	40	28.6	40	36.5	6.1	9.8
Laminaria angustata	40	13.0	40***	45.9	6.4	58.0*
Sargassum ringgoldianum	40	42.6*	40	36.1	40	47.1*
Porphyra yezoensis	40	4.7	40	16.0	6.7***	64.0*

* $p < 0.05$. ** Three toxic death recorded at the dosage.
*** One toxic death recorded at the dosage.

Thus it is probable that seaweeds contain substances that can prevent the formation or development of tumors, however, it does not seem that such substances attack tumor cells directly because they are all high in molecular weight, except for the lipids. Hence, the preventative mechanism of the polysaccharides and lipids might differ. It is reasonable to assume that the immune system of experimental animals which have been implanted with tumor cells is stimulated by a minute amount of the absorbed polysaccharide which represses tumor growth.

In contrast, algal lipids are absorbed more easily in the intestine compared to other kinds of lipids, hence, their antitumor activity may differ from the mechanisms of polysaccharides which exhibit stimulation of the immune system to some extent.

It was reported most recently that fucoxanthin (an accessory pigment of photosynthesis) of *Undaria pinnatifida* (wakame) inhibits the proliferation of HL-60 cells (a kind of human leukemia cell) and induces their apoptosis (8).

CHAPTER 11 Antibiotics

(1) Outline

With the introduction of the antibiotic penicillin in the 1940's, a new weapon was found to combat diseases caused by toxic microbes and viruses. Over the years, many new antibiotics have been discovered and produced for use against infectious diseases particularly by bacteria and molds. A relatively recent discovery is that an antibiotic is effective for a specific group of the invaders. For example, penicillin and its derivatives are specifically toxic to "gram positive bacteria" and the kanamycin group covers a wide spectrum from both gram positive to gram negative bacteria. Now it appears that some antibiotics show toxicity for some malignant tumors.

While these antibiotics are being used, mutant bacteria appear which are resistant to the usual antibiotics and a vicious cycle arises. A case in point is that a kind of *Salmonera* (gram negative enterobacterium) called MRSA in abbreviation of methicillin resistant *Staphylococcus* has become a problem in many hospitals because of its resistance against the known antibiotics in use.

Most seaweeds have various antibiotics which appear to serve as protection from marine microbes as well as "agalophagous" (seaweed eating) animals. Hundreds of antibiotics of seaweed origin have been studied chemically and biologically. The antibiotic spectra for each has been determined (1, 2).

(2) Brown algae

Brown marine algae contain relatively large amounts of tannin as compared with the green and the red. Algal tannin is called phlorotannin and is different from the tannin found in terrestrial plants, but the physiological properties are similar. They all have an antioxidant property. Brown algae such as *Ecklonia* and *Laminaria* contain small amounts of tannin (around 0.3% of dry weight) amd certain molluscs and abalone eat them. On the other hand, *Hizikia* contains much more tannin (around 2.5% of dry weight), so molluscs leave them untouched.

In brown algae, similar bromophenols of hydroquinon group also occur in addition to the abundant phlorotannin previously mentioned, particularly in the genus *Ditciota* (edible but not commonly used as food).

(3) Green and red algae

It is acrylic acid that occurs widely, relatively speaking, in common seaweeds such as *Ulva* (sealettuce), *Codium* (spongytang), *Monostroma, Enteromorpha, Laminaria* their toxicity and it appears to be relatively low from data obtained mostly from animal experiments. More precise researches must be carried out concerning the toxicity of marine algal organic arsenic.

CHAPTER 13 Vitamins and Related Substances in Seaweeds

(1) General

Marine algae carry on photosynthesis, therefore it stands to reason they are able to syntheize all of the vitamins that terrestrial plants produce. Just as in land plants, there are species differences in which vitamins are produced in greater quantity. One unique difference between land plants and marine plants is that marine algae, particularly, *Porphyra* contains) (relatively much vitamins B_{12}. B_{12} is normally obtained from meat, fish dairy products and eggs in diets in the United States. Vitamin E is also found in relatively abundant quantites in marine algae. The kinds and quantities of vitamins found in seaweeds, of course, are species specific and also depend upon the month they are gathered. Seasonal changes will affect the total amount present in a particular species. Tables 9, 10 and 11 show analytical data for vitamins found in Japanese seaweeds (1, 2).

Of all of the sea vegetables, *Porphyra* (nori) can be called the great repository of vitamins. It is especially rich in the B-complex family of vitamins: especially choline, lipoic acid, biotin, B_6 and B_{12}. Vitamin B_{12} is not found in terrestrial plants. Among the seaweeds, nori also has the highest content of vitamin A. As the table will show, *Chlorella* has a higher Vitamin A content but compared to the other seaweeds, *Porphyra* has a higher content

(2) Beta-carotene

Beta carotene has the highest efficiency among the carotenoids in forming the provitamin group from which the body synthesizes vitamin A. The carotinoid is converted enzymatically into retinol (a synonym for Vitamin A after it has been absorbed from the intestinal epithelial cells . It takes nearly twice the amount of beta carotene to produce an equivalent amount of retinol.

Recent research shows that Vitamin A (retinol) and the related componds show high anti-cancer activity. It has been elucidated that retinol and related compounds such as retinoic acid exhibit high activity for tumor repression or antitumor activity. It is thought that the reason for this is of such an indirect ones that some carcinogens such as superoxide anion radical (O_2^-) mostly produced in the mitocondria is destroyed by beta carotene in the same manner as vitamin C and E.

Ordinary vegetables contain large amounts of β -carotene, in particular several common ones as carrot (76.0 mg% on diy weight basis), spinach, *Perilla* and *Cryptotaenia* (32.3, 69.6 and *50.8* mg%, respectively). This vitamin content,

Table 9 Vitamin contents of algae on dry basis (1)

Species of algae	A (Provitamin A) I. U./100g	D (Ergosterol) γ/g	E γ/g	B_1 γ/g	B_2 γ/g	B_6 γ/g	NiA γ/g	PaA γ/g	FA mγ/g	Biotin mγ/g	Lipoic acid γ/g	B_{12} mγ/g	Choline γ/g	Inositol γ/g	C mg/100g
Chlorophyceae															
Ulva pertusa				0.90	2.83		7.50	2.35	118	224	420	62.8	61	330	27-41
Monostroma nitidum				1.19	8.46		10.28	4.11	429	115	515	12.6	79	219	75-80
Enteromorpha linza	2900	3		1.50	1.21		28.05		270	198	175	97.5	417	95	10-257
Caulerpa racemosa				0.78	1.97		21.18	5.53	612	131	295	149.4	358	584	
Chlorella ellipsoidea	500000		111-183*	10-23	23-37	0.3-2.5	112-125	3.5-8.5	22000-47000	190-230		42-89	2200-2500	1600-2190	100-320
Phaeophyceae															
Dictyota dichotoma				0.75	6.14		15.20	0.73	521	187	500	10.1	77	125	
SpathogloSsum pacificum				0.36	0.84		25.21	0.25	566	150	653	7.0	87	114	
Dictyopteris prolifera				0.50	4.10		18.02	3.70	170	163	485	17.1	242	151	1005**
Padina arborescens				0.29	0.99		8.86	1.74	542	160	230	4.2	27	690	
Myelophycus caespitosus				0.27	3.06		14.17	3.15	88	162	360	7.6	618	1131	
Colpomenia sinuosa				0.31	5.44		5.13	2.92	46	136	540	76.5	406	116	
Hydraclathrus clathratus				0.34	2.82		3.51	3.07	857	181	330	65.8	49	408	
[illegible]	440			0.86	0.20		30.00					3.0			3-91
Ecklonia cava				1.10	2.52		18.84	0.49		209	90	3.3	262	379	
Hizikia fusiforme	450	16		0.28	2.67		6.80	1.49	218	237	230	5.7	33	328	0-92
Sargassum fulvellum				0.30	5.03		8.65	2.30	634	126	300	25.4	95	60	
S. thunbergii				0.38	5.34		4.46	8.76	308	282	270	46.5	28	566	
S. nigrifolium				0.40	6.21		17.19	0.49	249	159	410	20.7	24	197	
Rhodophyceae															
Porphyra tenera	44500			1.65	23.08		68.33		88	294	790	290.8	2920	62	10-831
Gelidium amansii				1.60	17.95		20.10	1.22	782	61	570	35.9	4885	443	
Chondrococcus japonicus				1.41	10.51		8.15	1.30	97	40	250	219.7	1337	449	
Grateloupia ramosissima				1.38	6.27		24.46	2.32	719	82	530	29.4	1119	55	
Gloiopeltis tenax				2.45	14.60		23.79	5.85	676	37	330	15.2	319	163	
Hypnea charoides				1.42	2.90		21.50	6.82	540	94	355	26.5	636	257	
Gracilaria gigas	800			1.80	1.09		7.81	1.82	304	18	495	212.3	1492	324	
G. textorii				4.60	6.92		33.90	9.73	668	153	985	75.7	230	668	
Chondrus ocellatus				2.19	14.73		29.65	7.04		69	700	89.2	856	111	16
Lomentaria catenata				0.97	3.16		24.24	12.21	220	90	625	25.2	240	263	
Laurencia okamurai				0.53	10.24		39.02	8.97	763	95	300	100.2	1346	89	4

*Scenedesmus

**Dictyopteris divaricata

Table 10 Vitamin B_{12} Content in Seaweeds (2) *

Species	B_{12} content γ / 100g
Green algae	
Enteromorpha linza	3.9
Monostroma nitidum	0.3
Lettersstedtia japonica	0.8
Ulva pertusa	0.4
	0.3
*Chaetomorpha crassa****	1.7
	1.4
Caulerpa okamurai	0.1
Codium divaricatum	0.2
Brown algae	
Ectocarps sp.	0.3
Dictyota dichotoma	0.2
Padina araborescens	0.1
Ishige okamurai	0.2
	0.1
*Colpomenia sinuosa****	0.1
Hydroclathrus cancellatus	(3.0)**
Laminaria angustata	0.3
Endarachne binghhamiae	(1.5)
*Scytosiphone lomentarius****	(1.5)
Eisenia bicyclis	0.3
*Ecklonia cava****	(0.6)
	0.1

Species	B_{12} content γ / 100g††
Brown algae	
*Undaria pinnatifida****	(0.7)(0.1)
	0.1
Hijikia fusiformis	1.2
Sargassum enerve	(1.0)
S. ringgoldianum	0.1
	0.2
S. horneri	(0.4)
S. serratifolium	0.2
S. pilubiferum	0.1
S. thunbergii	(0.4)
Red algae	
Betrachospernum moniliform†	0.2
Gelidium amansii	(4.0)(0)††
Pterocladia capillacea	(1.0)
*Acanthopeltis japonica****	4.0
	0.8
Chondrococcus japonicus	0.9
*Meristotheca papulosa****	0.1
	0.2
Ceramium tenerrinum	4.0
C. rubrum	2.0
Martensia eleganse	1.3

*Arranged in taxonomic order.

**The figures in parentheses show the content on the materials digested with trypsin.

***Data on this species show the contents of different collection dates.

† A freshwater alga.

†† After alkali treatment.

Table 11 Vitamin B_{12} Content in Unicellular Algae, Zooplanktons and Bottom Muds (2)

Species	B_{12} content γ / 100g	Species	B_{12} content γ / 100g
Daphnia pulex	3.8	*Chlorella elipsoidea*	2.5-3.1
Zooplankton	0.9	Eight sorts of dried	(4.2-8.9)
Mysis sp.	0.9	powder prepared from	mean (6.1)
Skeletonema costatum	8.5	Chlorella	
	4.7	Shinobazu-pond	2.5-3.8(10)
Microcystis sp.	4.9(12.3)	Bottom mud Sanshiro-pond	0.8(2.2)
Scenedesmus obliquns	0.2	Ichigaya-pond	2.0(3.8)

The figures in parentheses show the contents on a dry basis.

Notes on samples; Daphnia was cultured in the usual way. Zooplankton and *Mysis* were collected at the Misaki Marine Biological Station, and the former was mainly composed of *Gypridina hilgendorfii* and a small amount of an amphipod. *Skeletonema* was pure-cultured with Matsue's soln. (a modification of Miquel-Allen-Nelson soln.) by the courtesy of Mr. Hirano. *Scenedesmus* and *Chlorella* were pure-cultured in the author's laboratory. The dried *Chlorella* powder was prepared in Prof. Asai's laboratory in this Faculty. *Microcystis* was collected at the Shinobazu Pond nearly in pure state.

however, is generally low in marine algae particularily in those with large and thick blades and thier contents are almost below 10 mg% such as 1.1 mg % and 3.8 mg% in *Laminaria* (konbu) and *Undaria* (wakame), respectively.

It is well known fact that *Dunaliella*, a unicellular green algae, produce beta caroten in extraordinarily high amounts. This algae, therefore, are cultivated in an industrial scale in several countries such as Israel, the USA and Australia etc. The pigment is utilized as supplement of vitamin A for us at present.

In addition, beter carotene has antitumor activity as an oxidant like vitamins C and E. Superoxide anion radicals, called also free redicala, are a potential cause in accelerating the the aging of cells and can cause cells to change and form tumors, therefore, any additional antioxidant we can take in our diet is important for maintaining optimal health.

(3) Choline

Choline another member of vitamin B-complex family, is found in relative abundance in *Gracilaria* and *Porphyra*. Its acetylester (acetylcholine) is well known as a neurotransmitter, in other words, is able to cross the so-called blood-brain barrier, Of the substances digested from food, the fat-soluble substances, water and gas are able to pass into the brain without limitation, while most of the other substances are controlled. Choline is one of the few substances that is able to pass through this barrier and enter directly into the brain cells to produce acetylcholine. This aids with memory and other neuroactivity.

The RDA has not been established for choline, but seems at least 500-900 mg are needed for adults. Choline is a necessary ingredient for the for biosynthesis of cholesterol and helps to eliminate poisons and drugs from the liver. A deficiency of choline may lead to a fatty liver which could lead to cirrhosis (hardening) of the liver, and arteriosclerosis, etc. Thus, including nori in the diet should help protect the normal function of the nervous sytem.

(4) Vitamin B_{12}

A deficiency of B_{12} and cobalt causes anemia. Both contribute to the regeneration of red blood cells. The overall effect is that B_{12} promotes the growth and appetite of children and helps the body maintain a healthy nervous system. It promotes the normal metabolism of fats, carbohydrates and proteins and is important for the nervous sytem because it leads to improvement in concentration, memory and balance.

(5) Availability of nori for the suppliment of B_{12} in human dietary life

It has been believed so far that vitamin B_{12} is not synthesized in plants including algae. Therefore, this vitamin found in marine algae may be originated in the sea water where various kinds of microbes as well as marine Invertebrates and Vertebrates are inhabiting and most of them may excrete it into there (1, 2). Recently it was elucidated that those substances in seaweeds which seem to be vitamin B_{12} identified by the bacterial methods are not necessary true one but more or less include its analogues. Thus, various corrinoids existent in marine algae may not be true B_{12} only. Being due to this fact in part, some living body-tests using several edible seaweeds for children or infants have been resulted in unsuccess as far as availability of vitamin B_{12} of seaweeds for human body concerns (3, 4). In facts, there have been reported that the corrinoids including B_{12} found in certain algae such as *Spirulina* to be mostly analogues (8). However, it has been demonstrated most recently by Japanese researchers that cobaramines of Japanese marine algae such as *Porphyra tenera* and *Enteromorpha* are found to be mostly mixture of CN-, OH- and Ad-cobaramine forms by identification using bioassay and chemiluminescence procedures (6, 7). In addition, the cobaramins of *Porphyra yezoensis* was found to be readly stored in the liver of experimental rats during feeding period for 40 days when the standard diet mixed with fine nori powder at a rate of 13% concentration containing 4.5 μg% of B_{12} (an sample being tested by both the bioassay and chemiluminescence methods) is given *adlibtum* (Table 12) (9, 10).

Table 12 Concentration of B_{12} in the liver of rats breeding with B_{12}-containing feed during 40 days (11)

Group including each 6 heads	Day of breeding	Mean body weight before breeding (g)	Mean body weight on dissection (g)	Mean liver weight on dissection (g)	Content of B_{12} in liver (ng/g-liver)
G-1	0	197.6 + 12.4	183.7 + 9.2	7.9 + 0.6	22.13 + 2.12
G-2	14	197.6 + 7.1	281.7 + 8.9	11.6 + 1.5	17.75 + 3.22
G-3	28	196.0 + 6.7	343.8 + 17.9	13.2 + 1.2	16.33 + 4.34
G-4	40	195.2 + 6.5	398.3 + 18.0	15.2 + 1.1	14.61 + 1.91
G-5	14	198.0 + 7.2	284.5 + 16.3	10.4 + 1.4	125.17 + 21.69
G-6	28	196.7 + 7.1	352.3 + 25.1	13.0 + 1.2	180.63 + 36.12
G-7	40	195.5 + 6.3	398.3 + 18.0	15.4 + 2.3	189.51 + 13.98

Note: The content of B_{12} in sera of rats was almost undetectable at another series of similar experiments.

For this reason, *Porphyra yezoensis* which is aquacultured in a large scale at present in Japan as a marine food may partly be utilized also as a supplemental foodstuff of B_{12}, perticularly useful for strict vegitarian. However, since there is a report in which cobaramin of *Porphyra tenera* is easily converted to analogues during drying process, it must take notice of this point (9, 10).

Attachment: 3

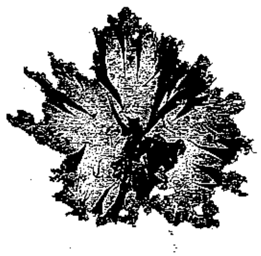

Fig. 6 *Monostroma nitidum*

Fig. 7 *Caulerpa racemosa*

food by being boiled in soy sauce to a concentrated form, and used as a substitute of the same type of Nori-food.

Sea grapes, Iwazuta (*Caulerpa racemosa and C. lentillifera.*) (Fig. 7)

Known as "Ararucip" in Philippines. It is widely distributes over middle and south coasts of the Pacific Ocean, Malay Islands and Philippines etc. It is widely used as food and it began to cultivate recently in this country (*C. racemosa.*) and in Okinawa (*C. lentillifera*). However, some of *Caulerpa* produce anesthetic toxins such as caulerpin and caulerpicin, but they are destroyed on boiling.

Sea lettuce (*Ulva sp*) (Fig. 8)

There are many species Of *Ulva*. *Ulva* is used in soups, salads or dried, made into powder and sprinkled on foods. Besides in warm Japanese coasts, widely distribute in Malaysia Islands, Polynesia, Australia, West coasts of North America and Indian Ocean etc.

Fig. 8 *Ulva pertusa*

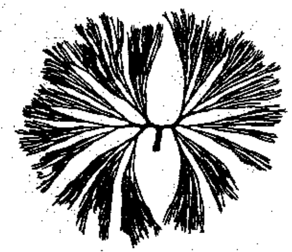

Fig. 9 *Codium fragile*

Spongetang, miru or 'chongak' in Korean (*Codium fragile*) (Fig. 9)

In Korea, *Codium* is sun dried and used as tea. In the Philippines, it is processed for soup stock or used as a salad.

Habitat: Japan, Malaysia, Australia, Pacific Coast of No. America, Atlantic Ocean, Indian Ocean and Bering Sea.

(3) Phaeophyta: Brown algae

Brown Algae are characterized by having large amounts of a brownish carotenoid called fucoxanthin besides alginates and fucoidans. They have chlorophylles a and c but not b. However, they do have some carotenoids similar to those of green algae and terrestrial plants. The dietary importance of fucoxanthin will be discussed in connection with β-carotene. Some brown algae form a very large group, both in species and size. The giant kelps belong to this group. Bathed in seawater, kelps (Laminariales) contain all of the essential minerals our bodies need, along with varying amounts of the essential vitamins. The significance of these pigments, alginates, fucoidans and minerals will be discussed in the section on the pharmaceutical and nutritional properties of marine algae. Brown algae are a very important source for alginates which are used in very large quantities in a variety of commercial products.

Konbu or kelp (*Laminaria* or "sea tangle") (Fig. 10)

There are about ten species of *Laminaria* used as food. Some common ones are as follows: *L. japonica* (makonbu), *L. angustata* (mitsuishi konbu), *L. religiosa* (hosome konbu) *L. saccharina*. Some kelp such as *L. japonica* are cultivated in Japan, Korea and on a much larger scale, in China. In China, roughly 35% of this kelp is used for the manufacturing of alginates. The kelp synthesizes particularly abundant mucilaginous acid polysaccharides such as alginates and fucoidans, those of which are much effective as water-soluble dietary fibers upon taken the blades as food.

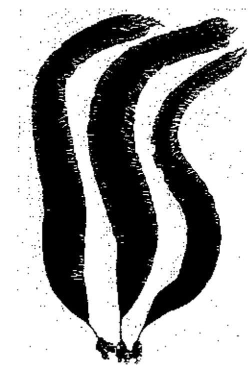

Fig. 10 *Laminaria japonica*
Japan (Hokkaido) cultured,
China (all cultured)
Korea (mostly cultured)

Kelp is sundried on graveled ground near the beach and made into commerical products. All of these dried products are reconstituted in water before using. Konbu (kelp) is also made into a powder. Before it is made into powder, it is boiled to remove the excess iodine. Excessive iodine leads to the same condition as too little iodine. It is important to soak konbu, if large quantities are consumed because of its high iodine content. This probably is a cause for alarm in the U.S. where konbu will not be eaten to the extent that it is in Japan. If seaweeds are included in the diet, it is probably not necessary to use iodized salt. The mature blades of European *Laminaria* are tough and difficult to eat but the juvenile blades are soft and can be used as food. Most of them are used as fodder and manure. Tororo konbu: Dried sheets of konbu are shaved into very thin sheets which are 'ecru' in color. This product can be found in oriental groceries. It can be eaten as is, added to soups, etc.

Wakame (*Undaria pinnatifida*) (Fig. 11)

Wakame is widely used as food in Japan, Korea and China especially for soup and in salads. Some of them are cultivated. Large quantity of wakame are exported from China and Korea to Japan. Wakame in China and Korea are often cooked in oil. Recently, there has been mass production of an instant convenience food called "cut wakame". This product can be added to any prepared soup or salad. Wakame contains, like konbu, abundant acidic polysaccharides effective as watersoluble dietary fibers.

Alaria crassifonia (honeyware, wina kelp. Ainu wakame) (Fig. 12)

Alaria wakame has a softer blade and is used in products such as instant soup, etc. This alga disributes in Bering Sea, and North Pacific coasts of North America and some are in the coast of Hokkaido.

Bladder wrack or yellow tang (*Ascophyllum nodosam*) (Fig. 13)

Large size and perennial alga. Distributes widely in the coasts of the North Pacific Ocean. An alginophyte, and often utilized as fodder for domestic animals. Fresh algal body or its aqueous extract are used as manure for fruit or vegetable cultures. Commonly, a small red alga, *Polysiphonia lacemosa* is found as an epiphyte. This bladder wrack contains much phlorotannin.

Fig. 11 *Undaria pinnatifida* (Wakame) Japan. China. Korea

Fig. 12 *Alaria crassifolia* (Chigaiso) Japan, Pacific coast of Noth America

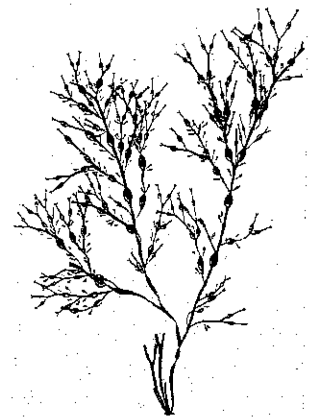

Fig. 13 *Ascophyllum nodosum*

Fig. 14 *Hizikia fusiformis*, Japan Sea. Korea. China

Hijiki (*Hizikia fusiformis*) (Fig. 14)

Hijiki is made from boiling the sundried fronds for a few hours, then redried. Raw hijiki has not only a high content of tannic substances but also inorganic and organic arsenic in a high content which makes it rather toxic to humans and must be precooked. Commercial hijiki has already been properly treated hijiki also has some arsenic compounds in it. While some of the arsenic is removed during processing, (boiling in much amount of water) the amount remaining (mainly arsenic of organic form) has been questioned by Western nutritionists. The Japanese, however, have been eating hijiki for centuries without dire consequences. Packaged hijiki are all precooked and ready for use in various recipes.

Bull Whip Kelp (*Nereocystis Iuetkeana*) (Fig. 15)

Bull whip kelp is found along the North Pacific Coast of North America. Alter a storm, it can be found in huge wracks along the beach. As an edible seaweed, it has great versatility. When fresh fronds are immersed in boiling water, they turn a beautiful green. To remove excess iodine,

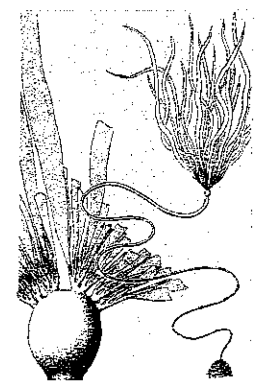

Fig. 15 *Nereocystis luetkeana* (Abott & Hollenberg, 1976)

function. Both hormones accelerate the consumption of energy and leads to the loss of fat and muscle in the body. The hormones also accelerate the growth of young animals. Iodine ingestion with food is necessary for biosynthesis of these hormones. If it is too low in the content, some obstacles such as goitre occurs. But an exessive intake also causes the abnormal function of the thyroid gland, e.g. resemble to the goitre.

U.

Ultraviolet rays (UV): Radiation of wave legnths between 400 nm (lower limit of the visible wave lengths) to around 1 nm is called ultraviolet rays, but the UV ray which affects biolgical function is around 400 to 290 nm. UV rays are usually divided into two categories: the A and B ranges. The A range covers 400 nm to 320 nm(causes sunburn) and the B range covers 320 to 290 nm(causes suntan). Both A and B UV are harmful for the human skin. The B range is especially toxic. Damage such as erythema or sunburn, the degradation of elastic tissue, actinic aging and even various types of skin cancer is the result of too much exposure. In contrast, changes in skin color by the ray with in wave length of A area is almost non-harmful for human skin.

Urea formation: Approximately 50 g of urea is excreted in urine per day in the normal human body. Urea is synthesized in the liver as a result of the detoxication of ammonia which originates in amino acid metabolism combined with the carbonate ion dissolved in the blood as a result of carbohydrate metabolism. The metabolic cycle of urea formation is called the urea-cycle, the Krebs-Henseleit urea-cycle or the ornithine cycle. This cycle involves several steps in enzymatic reactions.

V.

Villi or Villus: Also called villus apophysis or finger-like projections from the intestinal mucous membranes and from the interspaces between the placenta and ovary walls of vertebrates. The internal side is dense with capillary vessels which makes for greater absorption of nutrients.

Virus: Biologically smallest organic particle which consists of single DNA or RNA as genom. The DNA or the RNA is surrounded by single or some layers of protein, and has a few members of enzymes. It reproduces like bacteria in living cell but the former can not reproduce in artificialy prepared organic and inorganic media, this being different from bacteria. Therefore, and it is potent pathogen for our body. Virus somelimes causes normal cell to change tumor cell.

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Page 1. ItemDNumber ?° 3971 D fl0t Scanned Author Diaz-Colon, JD Corporate
Author Report/Article Title Selected Bibliography of the ...
www.nal.usda.gov/speccoll/findaids/agentorange/text/03971.pdf - 2006-09-2
Version

ReniDMHir Author Journal/BookTitlo Year Color Numberof Images ?° [PD
Page 1. ReniDMHir Author Corporate Author United States Department of
Agriculture, in cooperation Report/Article TitlO The Biologic ...
www.nal.usda.gov/speccoll/findaids/agentorange/text/04906.pdf - 2006
- Text Version

Resin-Extractable Phosphorus, Vanadium, Calcium and Magnesium as ... [PDF]
... for haloperoxidase activity in marine brown algae: perification and characteriz
of a vanadium (V)- containing bromoperoxidase from **Laminaria** saccha- rina. ..
www.ars.usda.gov/SP2UserFiles/Place/36450000/Products-Reprints/2... - 2006
Version

In order to show you the most relevant results, we have omitted some entries ve
already displayed.
If you like, you can repeat the search with the omitted results included.

Last Modified: 08/16/2007

Attachment 5

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株式会社　ミトク　御中

To: Mitoku Co., Ltd

（日 / 月 /西暦年号）

Date: 10/08/2007

(会社名

From:) CBI-Deleted

（ご署名）

(Signature:) CBI-Deleted

現在、米国ｵｰｶﾞﾆｯｸ基準（NOP-USA National Organic Program）において認定されているｵｰｶﾞﾆｯｸ昆布は、弊社の知る限りでは存在しておりません。

To the best of our knowledge, we hereby declare that there is no organic Kombu certified under NOP regulation.

Translated to English by Mitoku (Petitioner)

Attachment 6

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(株)ミトク 塩野 様

10 August 2007

平成19年8月10日

To. Mitoku Co., Ltd.

株式会社　ミトク　御中

From.

([CBI-deleted])

CBI-deleted

拝啓　時下ますますご清栄のこととお喜び申し上げます。お問合せ頂きました弊社オーガニックインスタント味噌汁に使用している昆布エキスにつきまして、下記の通りご報告いたしますので、ご確認の程宜しくお願い致します。

We will report about Kombu extract, which is being used for our organic instant miso soup, as below.

敬具

記

1. Product and Supplier

1.商品名　メーカー

( ) CBI-Deleted

2.使用に関する見解　2, Remark

当該商品に使用されております昆布は、北海道日高、釧路産昆布となっております。これらの品質の高さに関しましては広く認知されているところであり、弊社におきましてもその品質の高さが採用理由となっております。また現在のところ、当該商品のメーカーより、オーガニック認証の昆布の取扱いはないとの回答（文書添付）をいただいており、同品質の同等品の入手は困難であると推察されます。従いまして現在のところ当該商品から他商品への切り替えの予定はございません。

以上

Kombu used for our product are Kombu from Hidaka, Hokkaido and Kushiro, Hokkaido. These varieties of Kombu are widely known to have high quality. We choose this Kombu because of its high quality. We have been responded by our Kombu suppier that no organic certified Kombu is currently available. (The document attached.) Therefore, we assume the difficulty of obtaining the equivalent of the same quality. We are not going to change the Kombu product

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COPY

(To: 御中) CBI-Deleted

（日 / 月 /西暦年号）

Date: 13 / 9 / 2006

(From:

（ご署名）

Signature:) CBI-Deleted

現在、米国ｵｰｶﾞﾆｯｸ基準（NOP-USA National Organic Program）において認定されているｵｰｶﾞﾆｯｸ昆布は、弊社の知る限りでは存在しておりません。

To the best of our knowledge, we hereby declare that there is no organic Kombu certified under NOP regulation.

Comments from the Yuzu Ponzu manufacture
Translation is attached.

Attachment 7

CBI-
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平成19年8月17日

株式会社　ミトク　御中
海外事業部　塩野　様

いつもお世話になっております。今回、お問い合わせの件ですが、下記の通り、ご報告申し上げます。

昆布は、コンブ科の海藻のことで古くから日本では食用やだし昆布として利用されてきました。だし昆布は日本の伝統的な食材のひとつです。それは、旨味成分である「グルタミン酸」が豊富に含まれているからです。ゆずぽん酢醤油はその旨味成分を活かした製品です。
オーガニック昆布は、弊社や仕入先の知る限りでは存在しません。

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COPY

Translated to English by Mitoku(Petitioner)

17 August 2007

To. Mitoku Co., Ltd.

We will report regarding your inquiry as below.

Kombu is a kind of seaweed belongs to Laminariaceae family. Kombu has been used as food and a stock for food from old times. The stock made with Kombu is one of traditional ingredient for Japanese cuisines, because it contains abundant glutamic acid, which produces flavor. Our Yuzu Ponzu product is characterized by the flavor.

To the best knowledge of us and our Kombu supplier, Organic Kombu does not exist.

( )

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