DEPARTMENT OF HEALTH & HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION

Public Health Service

Memorandum

Date JUL 29 1999

2785 '99 AUG -3 P3:27

From Senior Regulatory Scientist, Regulatory Branch, Division of Programs & Enforcement Policy (DPEP), Office of Special Nutritionals, HFS-456

Subject 75-day Premarket Notification for New Dietary Ingredient

To Dockets Management Branch, HFA-305

New Dietary Ingredient:	*Agaricus blazei* Murrill
Firm:	Iwade Research Institute of Mycology Co., Ltd.
Date Received by FDA:	May 24, 1999
90-day Date:	August 21, 1999

In accordance with the requirements of section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification for the aforementioned new dietary ingredient should be placed on public display in docket number 95S-0316 after August 21, 1999.

Robert J. Moore, Ph.D.

95S-0316

RPT 49

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington, DC 20204

JUL 29 1999

Kristi O. Smedley, Ph.D.
Consultant
Center for Regulatory Services
2347 Paddock Lane
Reston, Virginia 20191

Dear Dr. Smedley:

This letter is in response to your letter to the Food and Drug Administration (FDA) dated May 18, 1999 on behalf of Iwade Research Institute of Mycology Co., Ltd. of Japan, making a submission for a new dietary ingredient pursuant to 21 U.S.C. 350b(a)(2) (section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act). Your letter notified FDA of the intent of the Iwade Research Institute of Mycology Co., Ltd. to market a product containing a new dietary ingredient which consists of an extract of *Agaricus blazei* Murrill (Himematsutake extract).

21 U.S.C. 350b(a)(2) requires that a manufacturer or distributor of a dietary supplement that contains a new dietary ingredient submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under section 350b(a)(2), there must be a history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If this requirement is not met, the dietary supplement is deemed to be adulterated under 21 U.S.C. 342(f)(1)(B) because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness or injury.

FDA has carefully considered the information in your submission, and the agency has concerns about the evidence on which you rely to support your conclusion that a dietary supplement containing Himematsutake extract will reasonably be expected to be safe. You state in your submission that Himematsutake extract is used as an ingredient in foods consumed by humans. However, your submission does not provide a quantitative estimate of the typical exposure to this extract in the human diet that would provide a basis to conclude that the amount of it in the typical diet is a valid basis for determining that the amount provided by the recommended consumption of it in dietary supplements is safe or that the additive exposure to it and that typically present in the diet is safe.

Your submission also contained the results of several human and animal studies that you assert are adequate to evaluate the safety of ingested Himematsutake extract. However, your submission provides inadequate information about the nature and composition of the extracts used in the various studies as compared with the extract intended to be used in the dietary supplement. Therefore, we cannot confidently compare the expected exposure to Himematsutake extract to the dosages of the extracts used in the animal and human studies

cited as evidence in the submission. The paucity of information on the nature of the *Agaricus blazei* Murrill extracts used in the studies results in significant uncertainty in making dose comparisons and assessing the safety or hazards associated with human consumption of this dietary ingredient. Notwithstanding the limitations of these studies, a tolerable daily intake (TDI) for *Agaricus blazei* Murrill can be estimated using a no observable adverse effect level (NOAEL) in rodents after 3 months of exposure of 500 mg/kg body weight. Using an uncertainty factor of 1000 (factors of 10 for inter- and intra-species differences and for subchronic to chronic extrapolation), the TDI for this ingredient would be 0.5 mg/kg. Even using a less conservative analysis with an uncertainty factor of 100 gives a TDI of 5 mg/kg body weight. Each of these TDI is less than the exposure to the *Agaricus blazei* Murrill that would result from the recommended use of the dietary supplement containing this ingredient (50 mg/kg bodyweight for a 60 kg adult consuming the recommended 3000 mg of the ingredient per day). Therefore, given the limited nature of the data in this submission, we do not agree that the data from the studies you submitted provide evidence that establishes that the dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe.

Finally, the human studies contained in the submission provide little support for concluding that chronic or long-term consumption of dietary supplements containing this ingredient will reasonably be expected to be safe in healthy people. The studies submitted were not designed nor intended to examine the adverse or toxicological effects of *Agaricus blazei* Murrill in healthy people; instead, the dietary ingredient was used as a therapy in studies of persons with serious diseases. Such studies have limited utility for determining whether the long-term use of a substance as an ingredient in dietary supplements is safe.

For the reasons discussed above, the information in your submission does not provide an adequate basis to conclude that Himematsutake extract, when used under the conditions recommended or suggested in the labeling of your product, will reasonably be expected to be safe. Therefore, your product may be adulterated under 21 U.S.C. 342(f)(1)(B) as a dietary supplement that contains a new dietary ingredient for which there is inadequate information to provide reasonable assurance that such ingredient does not present a significant or unreasonable risk of illness or injury. Introduction of such a product into interstate commerce is prohibited under 21 U.S.C. 331(a) and (v).

Please contact us if you have questions concerning this matter.

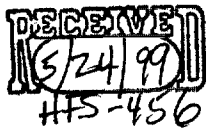
Sincerely,

Lynn A. Larsen, Ph.D.
Director
Division of Programs and Enforcement Policy
Office of Special Nutritionals
Center for Food Safety
and Applied Nutrition

ORIGINAL

65258

cfr center for regulatory services
2347 Paddock Lane • Reston, Virginia 20191 • 703-620-9175 • Fax 703-620-9476



May 18, 1999

Dr. Robert Moore
Director, Office of Special Nutritionals (HFS-450)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street SW
Washington, DC 20204

Dear Dr. Moore:

SUBJECT: Premarket Notification of a New Dietary Ingredient Extract of Agaricus blazei

On behalf of our client, Iwade Research Institute of Mycology Co., Ltd. (Iwade), notice is hereby given pursuant to the requirements of section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (21 USC §350b) of the intent of Iwade to introduce into interstate commerce in 75 days herefrom a new dietary ingredient, extract of Agaricus blazei. In accordance with 21 CFR §190.6, enclosed is one original plus two copies of the following information.

1. Manufacture
   Iwade Research Institute of Mycology Co., Ltd
   1-9, Suehiro-cho, Tsu, Mie
   514-0012, JAPAN

2. New Dietary Ingredient
   Extract of Agaricus blazei Murrill (Himematsutake extract)

3. Description Dietary Supplement
   Concentration of the hydrolysis of the culture of Agaricus blazei (30%) mixed with enzymatically hydrolyzed guar gum (70%).

   > It will be marketed in 5 gram packages (1.5 gram of Himematsutake) with directions to take orally after dissolving in tepid water.

   > Directions will suggest to use one or two packages each day on an empty stomach.

4. Iwade has concluded that the dietary supplement containing Himematsutake extract will reasonably be expected to be safe under the recommended conditions of use based on numerous studies and other information, including copies of the following attached documents.

I. List of Existing Food Additives, Japanese Government (excerpt listing himematsutake extract and enzymatically hydrolyzed guar gum, English translation and original Japanese)

II. Summary of Acute and Subacute Toxicological Studies of ABME from Cultured Agaricus blazei Murrill (Iwade Strain 101). Hitoshi Ito, M.D. Ph.D., Department of Pharmacology, MIE University School of Medicine, JAPAN (full reports available to FDA).

III. History of Himematsutake (Agaricus blazei Murrill). Iwade Research Institute of Mycology

IV. AGARICUS in North America: Type Studies. Alice E.H. Freeman. 1979. Mycotaxon 8:1.

V. Clinical studies conducted with Agaricus blazei indicating no safety problems with the extract:

a. Observation on the Treatment of Agaricus blazei for Chronic Hepatitis B. Wang Li Rong et al. Journal of Lanzhou Medical College. Vol. 20. 1994 (English translation and original Japanese)

b. Observation on Treatment Effect of Agaricus blazei against Alimentary Tract Tumor. Wang Jing, Mao Xin Min, Cheng Ru Zheng, Wang Jun Zhi, Hitoshi Ito, and Keishiro Shimaru. Gansu Medical Journal. 1994. (English translation and original Japanese)

c. Antitumor Activity and Some Properties of Water-soluble Polysaccharides from "Himematsutake," the Fruiting Body of Agaricus blazei Murill. Takaishi Mizuno, Toshihiko Hagiwara, et al. Agricultural and Biological Chemistry, 54:2889. 1990.

d. Antitumor Activity and Some Properties of Water-insoluble Hetero-glycans from "Himematsutake," the Fruiting Body of Agaricus blazei Murill. Takashi Mizuno, Ryuichi Inagaki, et al. Agriculture and Biological Chemistry, 54:2897-2905. 1990.

VI. Manufacturing Scheme **(CONFIDENTIAL)**

VII. Product specifications of Himematsutake Powder and Himematsutake Extract **(CONFIDENTIAL)**

Should you have any questions or comments on this request, please contact the undersigned.

Sincerely,

Kristi O. Smedley, Ph.D.
Consultant

Enclosures
Listed Above and
on Attachment Page

cc: I. Iwai

504:\043.fda

ATTACHMENTS

I. List of Existing Food Additives, Japanese Government (excerpt listing himematsutake extract and enzymatically hydrolyzed guar gum, English translation and original Japanese)

II. Summary of Acute and Subacute Toxicological Studies of ABME from Cultured Agaricus blazei Murrill (Iwade Strain 101). Hitoshi Ito, M.D. Ph.D., Department of Pharmacology, MIE University School of Medicine, JAPAN (full reports available to FDA).

III. History of Himematsutake (Agaricus blazei Murrill). Iwade Research Institute of Mycology

IV. AGARICUS in North America: Type Studies. Alice E.H. Freeman. 1979. Mycotaxon 8:1.

V. Clinical studies conducted with Agaricus blazei indicating no safety problems with the extract:

a. Observation on the Treatment of Agaricus blazei for Chronic Hepatitis B. Wang Li Rong et al. Journal of Lanzhou Medical College. Vol. 20. 1994 (English translation and original Japanese)

b. Observation on Treatment Effect of Agaricus blazei against Alimentary Tract Tumor. Wang Jing, Mao Xin Min, Cheng Ru Zheng, Wang Jun Zhi, Hitoshi Ito, and Keishiro Shimaru. Gansu Medical Journal. 1994. (English translation and original Japanese)

c. Antitumor Activity and Some Properties of Water-soluble Polysaccharides from "Himematsutake," the Fruiting Body of Agaricus blazei Murill. Takaishi Mizuno, Toshihiko Hagiwara, et al. Agricultural and Biological Chemistry. 54:2889. 1990.

d. Antitumor Activity and Some Properties of Water-insoluble Hetero-glycans from "Himematsutake," the Fruiting Body of Agaricus blazei Murill. Takashi Mizuno, Ryuichi Inagaki, et al. Agriculture and Biological Chemistry, 54:2897-2905. 1990.

VI. Manufacturing Scheme **(CONFIDENTIAL)**

VII. Product specifications of Himematsutake Powder and Himematsutake Extract **(CONFIDENTIAL)**

Provisional translation

List of Existing Food Additives

Note: This English version of the *List of Existing Food Additives* is published to meet the needs of the non- Japanese speaking people. In the case of any discrepancy between the Japanese origin and the English translation, the former will take priority.

Note: In this List, the names enclosed with brackets do not appear in the Japanese origin but are given just as reference.

121. Redbark cinchona extract

A substance composed mainly of quinidine, quinine and cinchonineobtained from the bark of redbark cinchona trees.

122. Phellodendron bark extract

A substance composed mainly of berberine obtained from the bark of phellodendron trees (*Phellodendron amurense* RUPR.).

123. Fish scale foil

A substance obtained by extraction from the epithelium of fish.

124. Quillaja extract

A substance composed mainly of saponins obtained from the bark of quillaia trees.

125. Gold

126. Silver

127. Guar gum

A substance composed mainly of polysaccharides obtained from guar seeds, excluding No. 128 Enzymatically hydrolyzed guar gum.

128. Enzymatically hydrolyzed guar gum

A substance composed mainly of polysaccharides obtained by grinding and hydrolyzing guar seeds.

129. Guaiac resin

A substance composed mainly of guaiaconic acid, guaiaretic acid, and β-resin obtained from the trunks/branches of guaiacum trees.

130. Guaiac resin (extract)

A substance composed mainly of α- and β-guaiaconic acids obtained from the secretion of guaiacum trees.

341. Microfibrillated cellulose

A substance composed mainly of cellulose obtained by microfibrillating pulp or cotton.

342. L-Histidine

343. Beet saponin

A substance composed mainly of saponins obtained from beets.

344. Beet red

A substance composed mainly of betanin and isobetanin obtained from beet roots.

345. L-Hydroxyproline

346. Peanut colour

A substance obtained from peanut astringent skins.

347. Sunflower seed extract

A substance composed mainly of isochlorogenic acid and chlorogenic acid obtained from sunflower seeds.

348. Himematsutake extract

A substance obtained from the mycelium or fruit body of HIME-MATSUTAKE (*Agricus blazei* MURR.) or its cultured solution.

349. Pimento extract

A substance composed mainly of eugenol and thymol obtained from pimento fruits.

350. Xanthomonas campestris protein [Ice nucleation protain, Ice nucleating protain]

A substance composed mainly of proteins obtained from the cytoplasm of bacteria belonging to Xanthomonas campestris.

351. Vermiculite

厚生省生活衛生局食品化学課　監修

食品衛生法の改正と食品添加物の規制

平成８年８月

日本食品添加物協会

百十一　カンゾウ油性抽出物（ウラルカンゾウ、チョウカカンゾウ又はヨウカンゾウの根又は根茎から得られた、フラボノイドを主成分とするものをいう。）

百十二　カンデリラロウ（カンデリラの茎から得られた、ヘントリアコンタンを主成分とするものをいう。）

百十三　キサンタンガム（キサントモナスの培養液から得られた、多糖類を主成分とするものをいう。）

百十四　キシラナーゼ

百十五　D－キシロース

百十六　キダチアロエ抽出物（キダチアロエの葉から得られた、多糖類を主成分とするものをいう。）

百十七　キチナーゼ

百十八　キチン

百十九　キトサナーゼ

百二十　キトサン

百二十一　キナ抽出物（アカキナの樹皮から得られた、キニジン、キニーネ及びシンコニンを主成分とするものをいう。）

百二十二　キハダ抽出物（キハダの樹皮から得られた、ベルベリンを主成分とするものをいう。）

百二十三　魚鱗箔（魚類の上皮部から抽出して得られたものをいう。）

百二十四　キラヤ抽出物（キラヤの樹皮から得られた、サポニンを主成分とするものをいう。）

百二十五　金

百二十六　銀

百二十七　グァーガム（グァーの種子から得られた、多糖類を主成分とするものをいう。ただし、次号のグァーガム酵素分解物を除く。）

百二十八　グァーガム酵素分解物（グァーの種子を粉砕し、分解して得られた、多糖類を主成分とするものをいう。）

百二十九　グアヤク脂（ユソウボクの幹枝から得られた、グアヤコン酸、グアヤレチック酸及びβ－レジンを主成分とするものをいう。）

百三十　グアヤク樹脂（ユソウボクの分泌液から得られた、α－グアヤコン酸及びβ－グアヤコン酸を主成分とするものをいう。）

百三十一　グアユーレ（グアユーレの幹枝から得られた、ポリイソプレンを主成分とするものをいう。）

百三十二　クエルセチン

百三十三　クサギ色素（クサギの果実から得られた、トリコトミンを主成分とするものをいう。）

百三十四　クチナシ青色素（クチナシの果実から得られたイリドイド配糖体とタンパク質分解物の混合物にβ－グルコシダーゼを添加して得られたものをいう。）

百三十五　クチナシ赤色素（クチナシの果実から得られたイリドイド配糖体のエステル加水分解物とタンパク質分解物の混合物にβ－グルコシダーゼを添加して得られたものをいう。）

百三十六　クチナシ黄色素（クチナシの果実から得られた、クロシン及びクロセチンを主成分とするものをいう。）

百三十七　グッタカチュウ（グッタカチュウの分泌液から得られた、アミリンアセタート及びポリイソプレンを主成分とするものをいう。）

を主成分とするものをいう。）

三百四十一　微小繊維状セルロース（パルプ又は綿を微小繊維状にして得られた、セルロースを主成分とするものをいう。）

三百四十二　Ｌ－ヒスチジン

三百四十三　ビートサポニン（サトウダイコンから得られた、サポニンを主成分とするものをいう。）

三百四十四　ビートレッド（ビートの根から得られた、イソベタニン及びベタニンを主成分とするものをいう。）

三百四十五　Ｌ－ヒドロキシプロリン

三百四十六　ピーナッツ色素（ピーナッツの渋皮から抽出して得られたものをいう。）

三百四十七　ヒマワリ種子抽出物（ヒマワリの種子から得られた、イソクロロゲン酸及びクロロゲン酸を主成分とするものをいう。）

三百四十八　ヒメマツタケ抽出物（ヒメマツタケの菌糸体若しくは子実体又はその培養液から抽出して得られたものをいう。）

三百四十九　ピメンタ抽出物（ピメンタの果実から得られた、オイゲノール及びチモールを主成分とするものをいう。）

三百五十　氷核菌細胞質液（キサントモナスの細胞質液から得られた、タンパク質を主成分とするものをいう。）

三百五十一　ひる石

三百五十二　ビンロウジュ抽出物（ビンロウの種子から抽出して得られたものをいう。）

三百五十三　ファーセレラン（フルセラリアの全藻から得られた、多糖類を主成分とするものをいう。）

三百五十四　ファーバルサム（ファーバルサムの分泌液から得られた、α－カナジノール酸及びβ－カナジノール酸を主成分とするものをいう。）

三百五十五　ファフィア色素（ファフィアの培養液から得られた、アスタキサンチンを主成分とするものをいう。）

三百五十六　フィシン

三百五十七　フィターゼ

三百五十八　フィチン酸（米ぬか又はトウモロコシの種子から得られた、イノシトールヘキサリン酸を主成分とするものをいう。）

三百五十九　フィチン（抽出物）（米ぬか又はトウモロコシの種子から得られた、イノシトールヘキサリン酸マグネシウムを主成分とするものをいう。）

三百六十　フェリチン

三百六十一　フェルラ酸

三百六十二　フクロノリ抽出物（フクロノリの全藻から得られた、多糖類を主成分とするものをいう。）

三百六十三　Ｌ－フコース

三百六十四　ブタン

三百六十五　ブドウ果皮色素（アメリカブドウ又はブドウの果皮から得られた、アントシアニンを主成分とするものをいう。）

三百六十六　ブドウ果皮抽出物（アメリカブドウ又はブドウの果皮から得られた、ポリフェノールを主成分とするものをいう。）

三百六十七　ブドウ種子抽出物（アメリカブドウ又はブドウの種子から得られた、プロアントシアニジンを主成分とするものをいう。）

II

CONFIDENTIAL

ACUTE AND SUBACUTE TOXICOLOGICAL STUDIES OF ABME

FROM CULTURED AGARICUS BLAZEI MURRILL

(IWADE STRAIN 101)

Hitoshi Ito, M.D.,PH.D.
Department of Phamacology,
Mie University School of Medicine.
2-174, Edobashi, Tsu-city,
Mie-pref. 514-0001
Japan
Tel: +81 59 232 1111 (Int.6342)

Agaricus blazei Murrill (Iwade Strain 101) - Himematsutake

Agaricus blazei Murrill (Iwade Strain 101), "Himematsutake" (Japanese official nomenclature), "Cogmelo de Deus" in Brazil, is an edible mushroom belonging to the genus Agaricus. Strains of this species were imported into Japan from Brazil in 1965, and an artificial cultivation process has been established at the Iwade Mushroom Institute in Mie Prefecture (1978). This mushroom is now being cultivated on a contract basis in various part of Japan, as well as in Indonesia since 1988.

This mushroom resembles a champignon ("Tsukuritake"), but has a thicker and longer stalk. It has been regarded as a suitable material for Japanese, Western and Chinese dishes because of its strong fragrance, sweet flesh and excellent texture. This mushroom has recently been attracting attention as a health-oriented food (physiologically functional food) and as a material for the development of drugs.

Table 1:

Acute toxicity (LD_{50}) in mice and rats treated with oral administration and intraperitoneal injection of ABME from cultured Agaricua blazei Murrill.

Routes	No. of animals	Mouse	LD_{50} (mg/kg) Rat
p.o.	10 male	>3000	>16000
	10 female	>3000	>16000
i.p.	10 male	>3000	>16000
	10 female	>3000	>16000

ABME dissolved in saline. Animal; Swiss albino mice (weight about 25g) and Sprague-Dawley-JCL rats (weight about 150g) were used.

General physical appearances and behaviors of animals and toxic symptoms of each group (10 animals) were observed for the period of 7 days following the administration; orally by means of a stomach tube (p.o.) and intraperitoneally into the cavity of the abdomen (i.p.). The LD_{50} value of each administration route was calculated by Behrens-karbers method.

Acute Toxicity in Mice by W.H.O. Standardarization and J.P.

Japanese Pharmacopoeia W.H.O.	Median Lethal I.P. (I.V.)	Dose (LD50) S.C.	(mg/kg) P.O.
Deadly Poison	< 10	< 20	< 30
Poison	< 100	< 200	< 300
Common Drug	> 100	> 200	> 300

These experiments concluded that ABME has highly LD_{50} value and is devoid of any specific acute toxicity of ABME. In the each group of ABME, no toxic signs were observed except the slight inflammatory change at the injection site.

Thus, acute toxicity of ABME in maximum dose was not found. ABME was considered as the highly safe substance. Male and female animals (rats and mice) were administered orally by means of a stomach tube (p.o.) and intraperitoneally into the cavity of the abdomen (i.p.) of ABME (500mg/kg) for 3 months.

Significant changes were not observed in this preliminary experiments; urinary test, organ wet weight, hematological test (hemoglobin, red blood cell, white blood cell, hematocrit) and histopathological test.

Therefore, the toxicity test of 1000 and 3000mg/kg for 1 month with P.O. or I.P administration were carried out under the same condition to confirm the safety of ABME.

Table 2:

Urinary findings of Spregue-Dawley rats treated with ABME orally for 30 days.

Sex	Dose mg/kg/day	No. of rats	Glucose		pH					Protein				Bilirubin	
			-	+	5	6	7	8	9	±	+	++	+++	-	+
M	3000x30	5	5	0	0	4	1	0	0	0	3	2	0	5	0
M	1000x30	5	5	0	0	3	2	0	0	0	2	3	0	5	0
M	Control	5	5	0	0	3	2	0	0	0	1	4	0	5	0
F	3000x30	5	5	0	0	4	0	1	0	0	3	2	0	5	0
F	1000x30	5	5	0	0	3	1	1	0	0	3	2	0	5	0
F	Control	5	5	0	0	4	1	0	0	0	2	2	1	5	0

Remarks: M=Male, F=Female

Urinarysis was performed at the end of an administration period on 5 animals in every groups. Each analysis included determination of pH, glucose, protein and bilirubin.

The glucose and bilirubin were not detected in urine of all male and female rats. However, protein was detected in urine of rats treated with ABME and untreated control. As above mentioned, there was no abnormal change in the urinalysis.

Table 3:

Average organ wet weight of Swiss albino mice treated with ABME orally for 30 days.

Sex	Group	No. of mice	Heart (g)	Liver (g)	Kidney (g)	Spleen (g)
M	3000x30	10	0.87 ± 0.04	5.37 ± 0.25	2.40 ± 0.05	0.54 ± 0.03
M	1000x30	10	0.79 ± 0.24	6.71 ± 0.69	2.44 ± 0.05	0.57 ± 0.03
M	Control	10	0.85 ± 0.03	5.45 ± 0.43	2.42 ± 0.02	0.56 ± 0.04
F	3000x30	10	0.51 ± 0.04	5.14 ± 0.12	1.69 ± 0.06	0.49 ± 0.04
F	1000x30	10	0.49 ± 0.03	5.12 ± 0.29	1.77 ± 0.08	0.48 ± 0.04
F	Control	10	0.52 ± 0.04	5.40 ± 0.34	1.99 ± 0.23	0.47 ± 0.05

Remarks: M=Male, F=Female
The values represent means ± standard deviations

The animals of both sexes from each group including the control were sacrificed by exsanguination at 30 days after the start of administration of ABME. After gross observations, the wet weight of the heart, liver, kidney and spleen were measured.

From these above results, it is concluded that the organ weights of mice has no significant change between the treated group and untreated control. No organ weight changes were observed in the stomach, small intestine, large intestine, lungs, adrenals or thymus.

Table 4:

The wet weight of the organs per 100g of body weight in Sprague-Dawley rats treated with ABME orally for 30 days.

Sex	Group	No. of rats	Weight of the organs 100g of body weight (Mean ± S.E.)			
			Heart (mg)	Liver (mg)	Kidney (mg)	Spleen (mg)
M	3000x30	5	383 ± 31	3994 ± 240	840 ± 49	277 ± 29
M	1000x30	5	376 ± 29	4014 ± 249	893 ± 63	279 ± 52
M	Control	5	385 ± 29	4079 ± 199	872 ± 59	260 ± 39
F	3000x30	5	367 ± 36	4157 ± 249	809 ± 74	251 ± 28
F	1000x30	5	370 ± 29	4341 ± 281	794 ± 80	249 ± 79
F	Control	5	359 ± 32	4069 ± 233	820 ± 65	229 ± 46

Remarks: M=Male, F=Female

Table 5:

The hematological findings in Swiss albino mice treated with ABME orally for 30 days

Sex	Dose mg/kg/day	R.B.C. (x 10^4/mm^3)	Hb. (g/dl)	Ht. (%)	W.B.C. (x 10^3/mm^3)
M	3000x30	757 ± 29	15.2 ± 0.9	47.0 ± 2.5	59.2 ± 8.9
M	1000x30	723 ± 21	13.4 ± 0.6	45.2 ± 1.3	52.6 ± 4.9
M	Control	725 ± 34	14.6 ± 0.7	44.6 ± 1.4	60.2 ± 6.4
F	3000x30	789 ± 29	14.0 ± 0.4	49.0 ± 0.9	53.2 ± 3.9
F	1000x30	769 ± 30	14.9 ± 1.4	46.0 ± 0.7	57.2 ± 8.1
F	Control	756 ± 31	14.9 ± 1.7	49.2 ± 1.2	50.4 ± 6.3

a) Average in 10 animals b) Mean ± S.E.

Hematological findings:

Determination of hemoglobin, red blood cell counts, white blood cell counts and hematocrit were performed on each sample taken from 10 animals in every groups. In all points, there was no abnormal change in the hematological findings.

Table 6:

The hematological findings in Sprague-Dawley rats treated with ABME orally for 30 days

Sex	Dose mg/kg/day	R.B.C. (x 10^4/mm^3)	Hb. (g/dl)	Ht. (%)	W.B.C. (x 10^2/mm^3)
M	3000x30	567 ± 21	14.0 ± 1.4	46.8 ± 0.8	99.5 ± 2.2
M	1000x30	557 ± 27	14.9 ± 0.9	42.3 ± 0.5	109.0 ± 2.8
M	Control	600 ± 34	14.7 ± 0.5	44.3 ± 0.9	110.0 ± 6.6
F	3000x30	552 ± 34	14.9 ± 0.9	41.2 ± 1.7	98.1 ± 4.2
F	1000x30	549 ± 61	15.0 ± 0.5	41.0 ± 4.0	99.3 ± 4.0
F	Control	541 ± 39	14.6 ± 0.4	40.9 ± 4.2	93.9 ± 6.9

a) Average in 5 animals b) Mean ± S.E.

A slight decrease of W.B.C. in male rats of 3000 mg/kg/day groups was noted. In other points, all value within normal range.

Body Weight:

- The body weight of each animal in every group was checked every 3 days.

Food Consumption:

- Individual consumptions of food was measured every 3 days in all groups. The conditions of health and toxic symptoms were checked every day.

- The animals were kept in air-conditioned rooms at the temperature of between 22 to 25 Celsius degree and relative humidity of between 50 to 60 percent. They were caged individually and fed on a synthetic diet (Oriental NMF for rats and CMF for mice, Oriental Kobo Co., Tokyo) as solid basal diet. The foods and water were available ad libitum.

- No significant differences were found in the mean growth curves of mice and rats treated with ABME. There seems no sex difference in clinical symptoms and the change of body weight.

- The food consumption in male rats of 3000mg/kg daily for 30 days was lesser than that of control group. However, female rats were not observed the tendency of the food consumption change as mentioned above.

- The experiments were carried out in the Institute of Laboratory Animals, Mie University of School of Medicine, Tsu, Mie, Japan, under the condition of Good Laboratory Practice (GLP).

Pathological Studies:

- For microscopical examinations, following tissues from each sacrificed animal were fixed in 10% buffered formalin, and paraffin sections of the tissues were stained with hematoxylin and eosin: the heart, liver, kidney, lung, pancreas, spleen, thymus, adrenal gland, stomach, small and large intestines.

- No histopathological changes were observed in the above organs.

Conclusion:

- The acute toxicological studies of ABME from cultured Agaricus blazei Murrill were performed in mice and rats in 2 different routes of administration, orally, and intraperitoneally.

- The present studies prove no particular acute toxicity of ABME in mice and rats. The estimate i.p. and p.o. of LD_{50} values of ABME were not definitely because of lack toxicity.

- The subacute toxicity studies of ABME were performed in mice and rats, ABME was administered orally at doses of 1000 and 3000 mg/kg/day x 30 days. Throughout the administration periods, ABME induced no toxic symptoms to any of the experimental animals.

- The changes of food consumption, body weight, urinarysis and pathological studies were within normal range except hematologically, the number of white blood cells was decreased at the dose of 1000 and 3000 mg/kg of ABME in male rats. In other point, all results were within normal range.

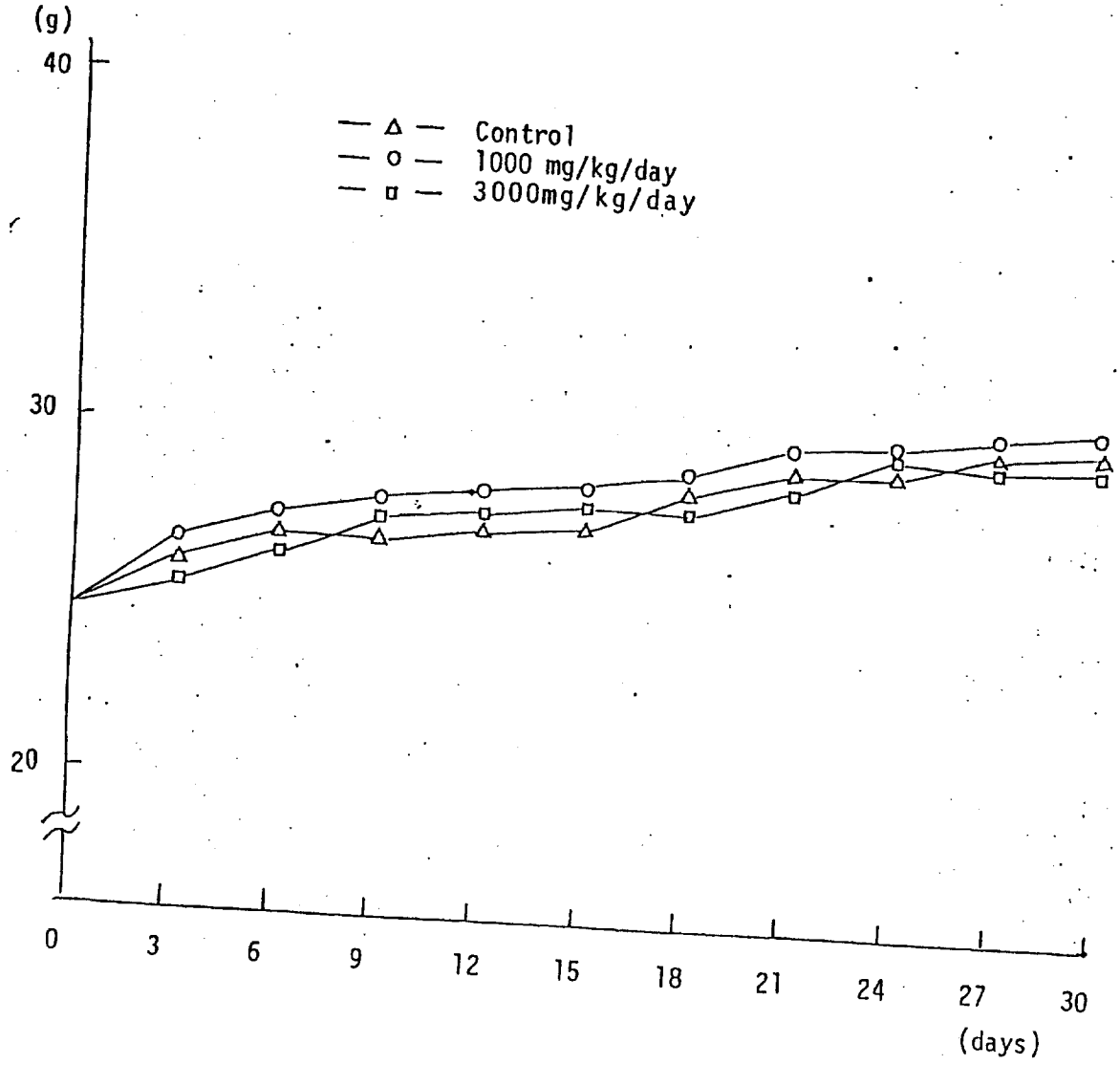


Fig. 1 Changes body weight in female mice orally administered with ABME for 30 days

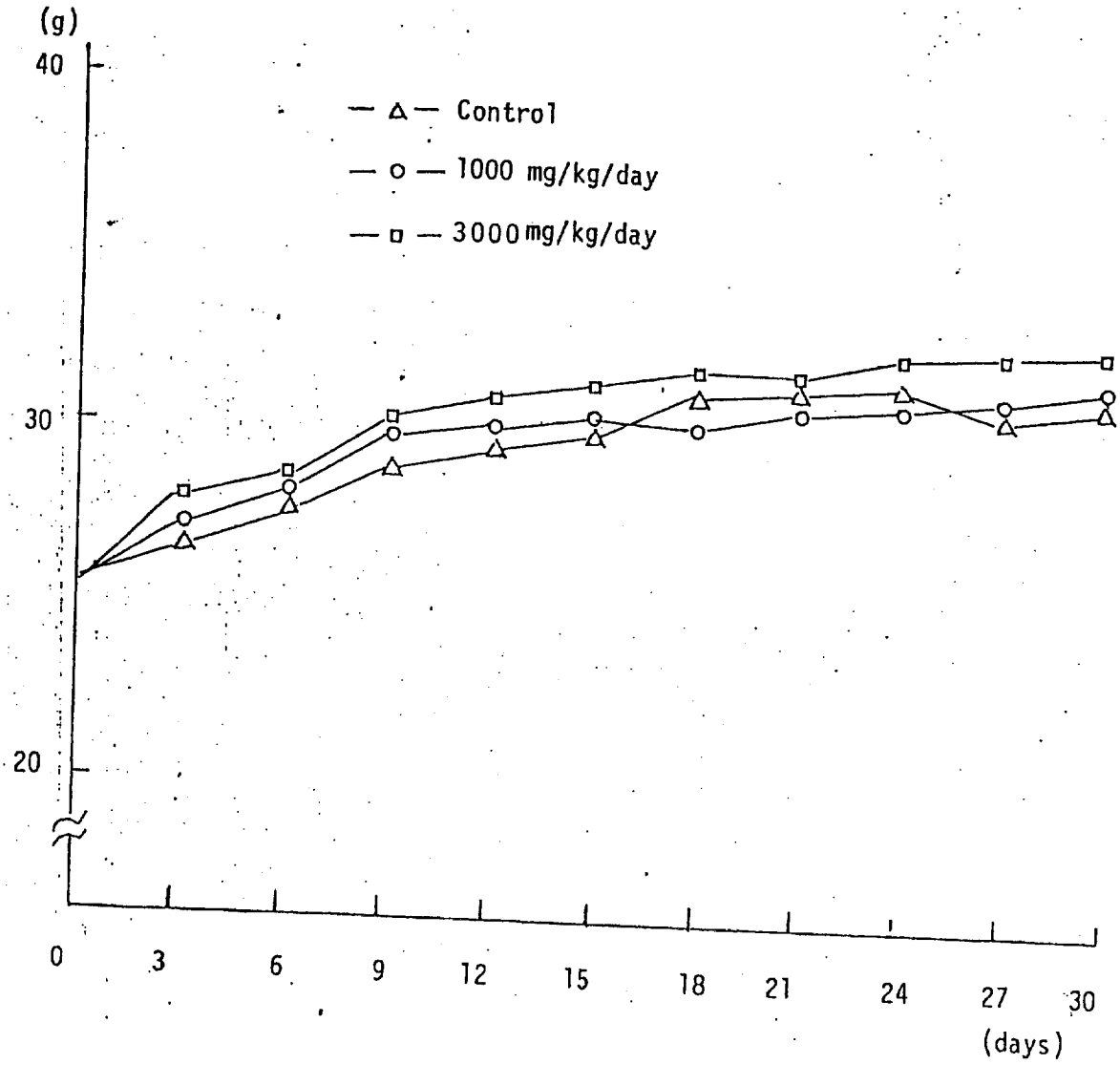


Fig. 2 Changes body weight in male mice orally administered with ABME for 30 days

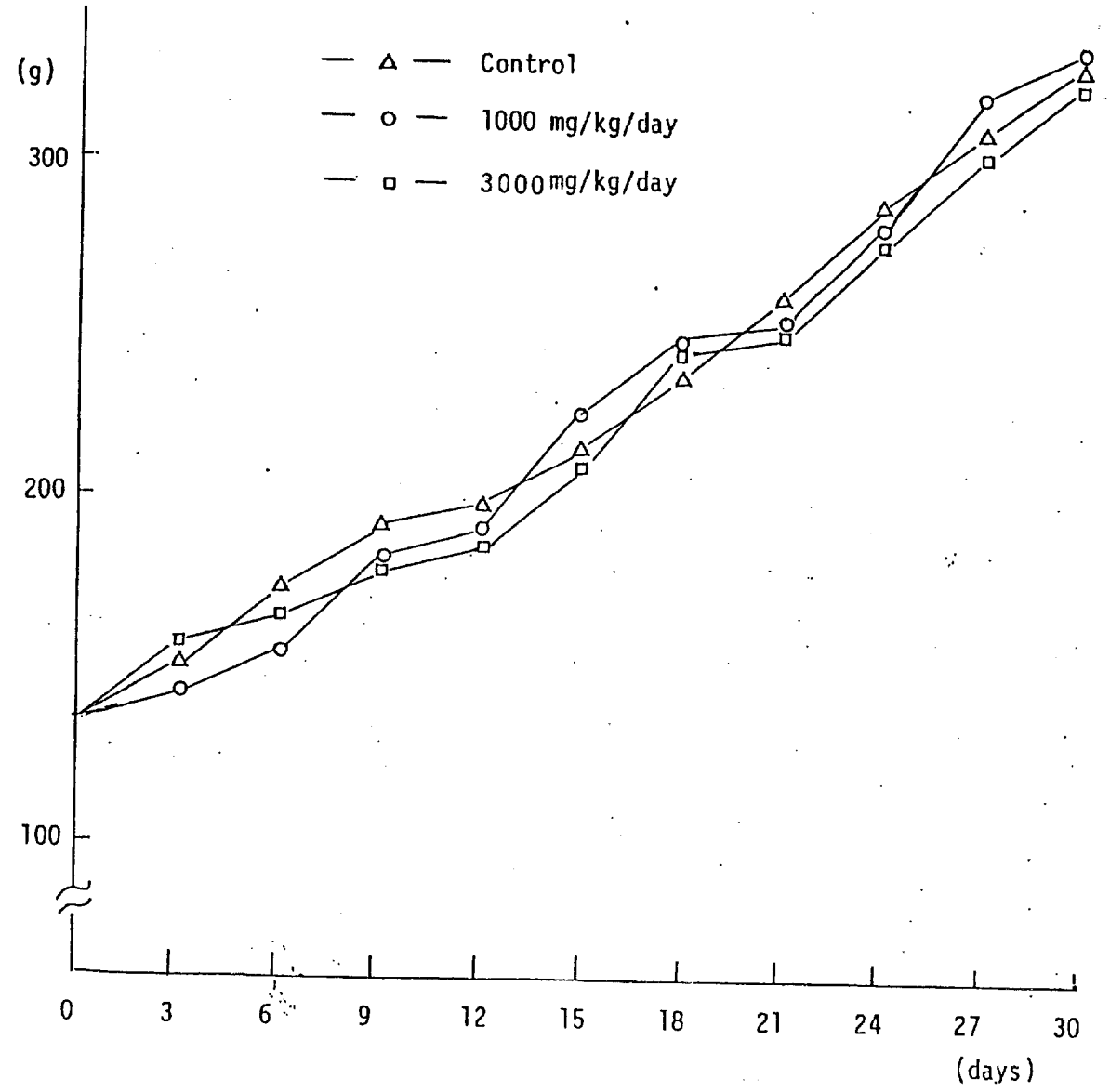


Fig. 3 Changes body weight in male rats treated orally with ABME for 30 days.

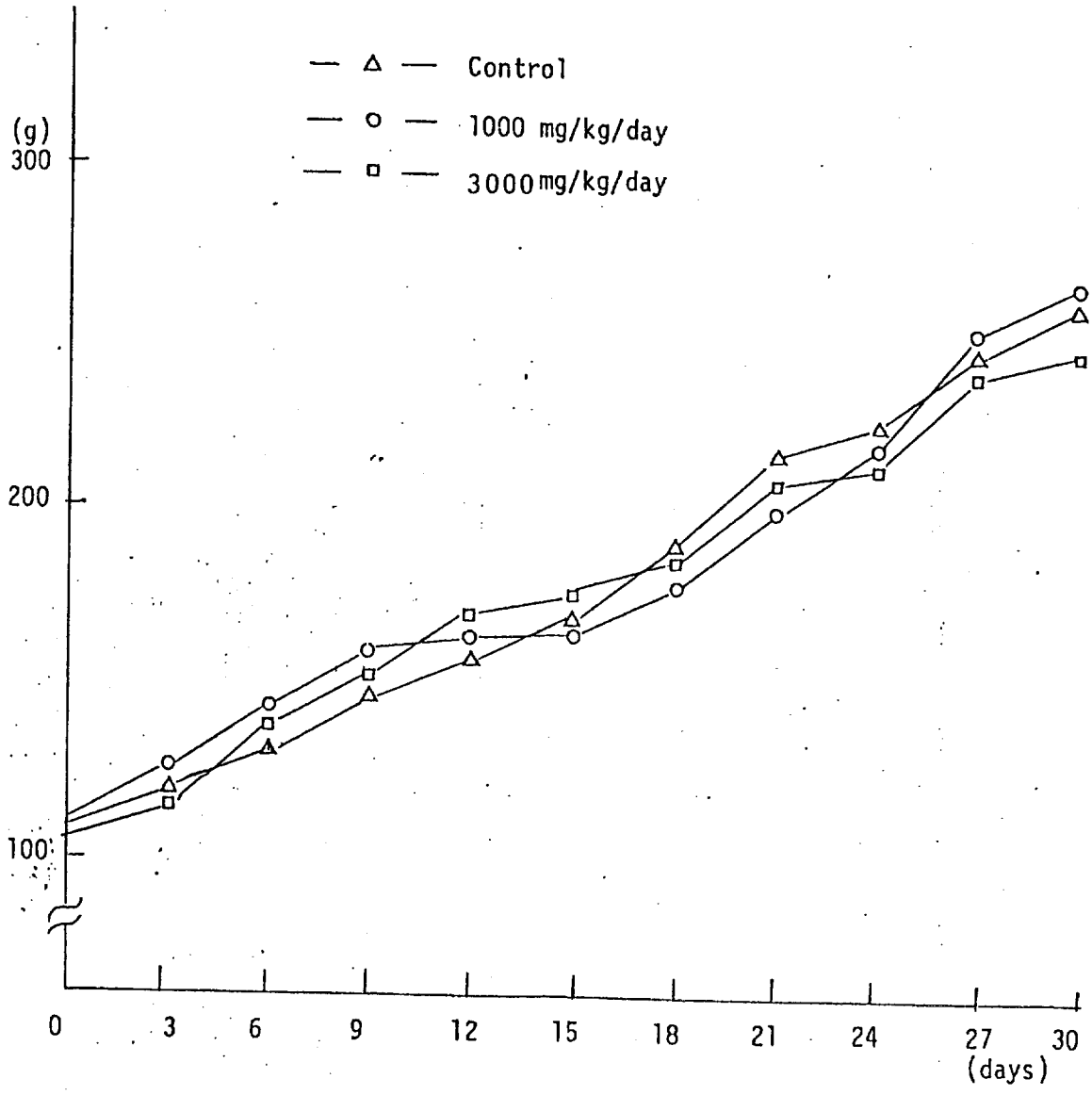


Fig. 4 Changes body weight in female rats treated orally with ABME for 30 days.

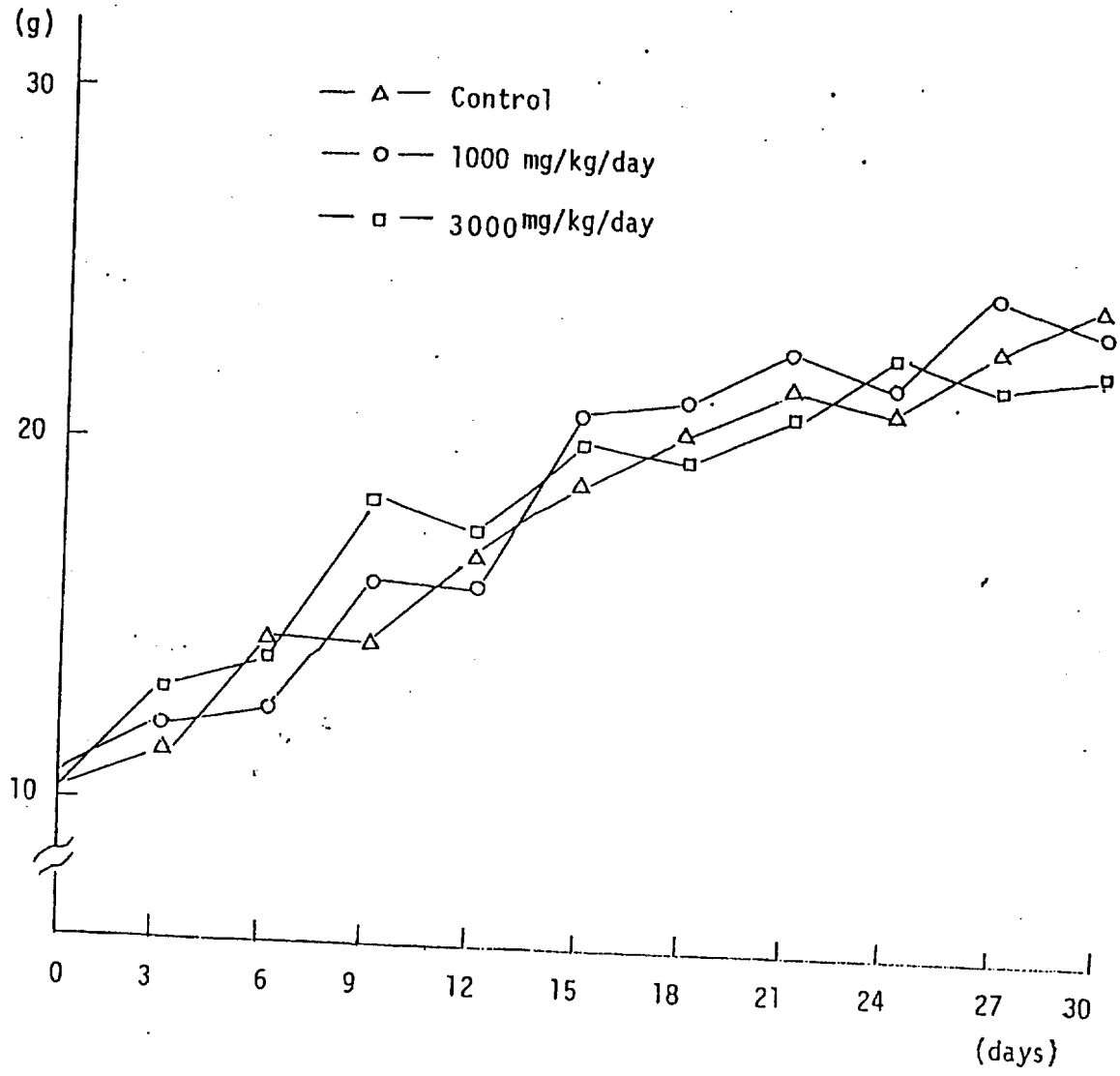


Fig. 5 Changes of food intake in female rats treated orally with ABME for 30 days.

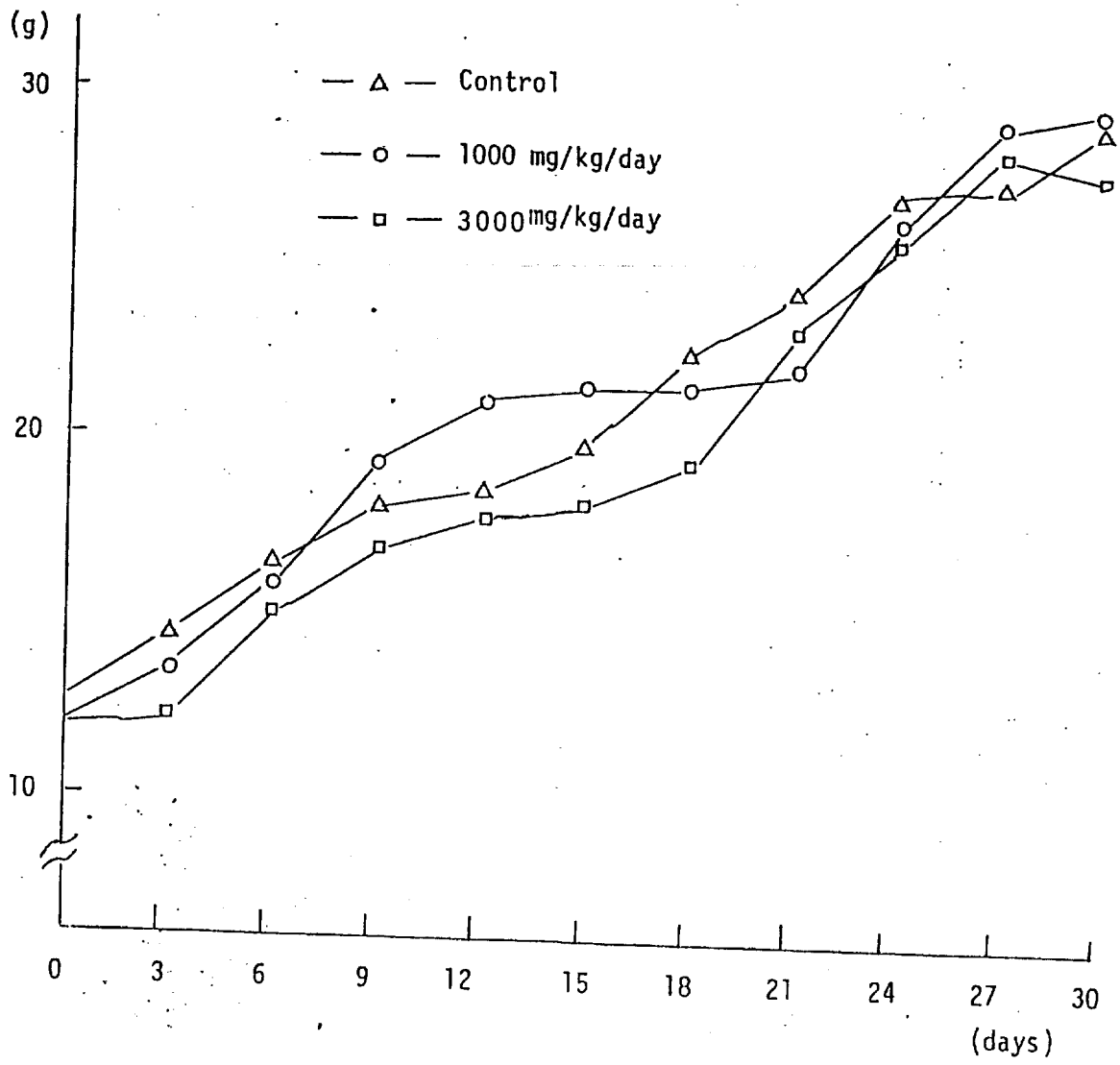


Fig. 6 Changes of food intake in male rats treated orally with ABME for 30 days.

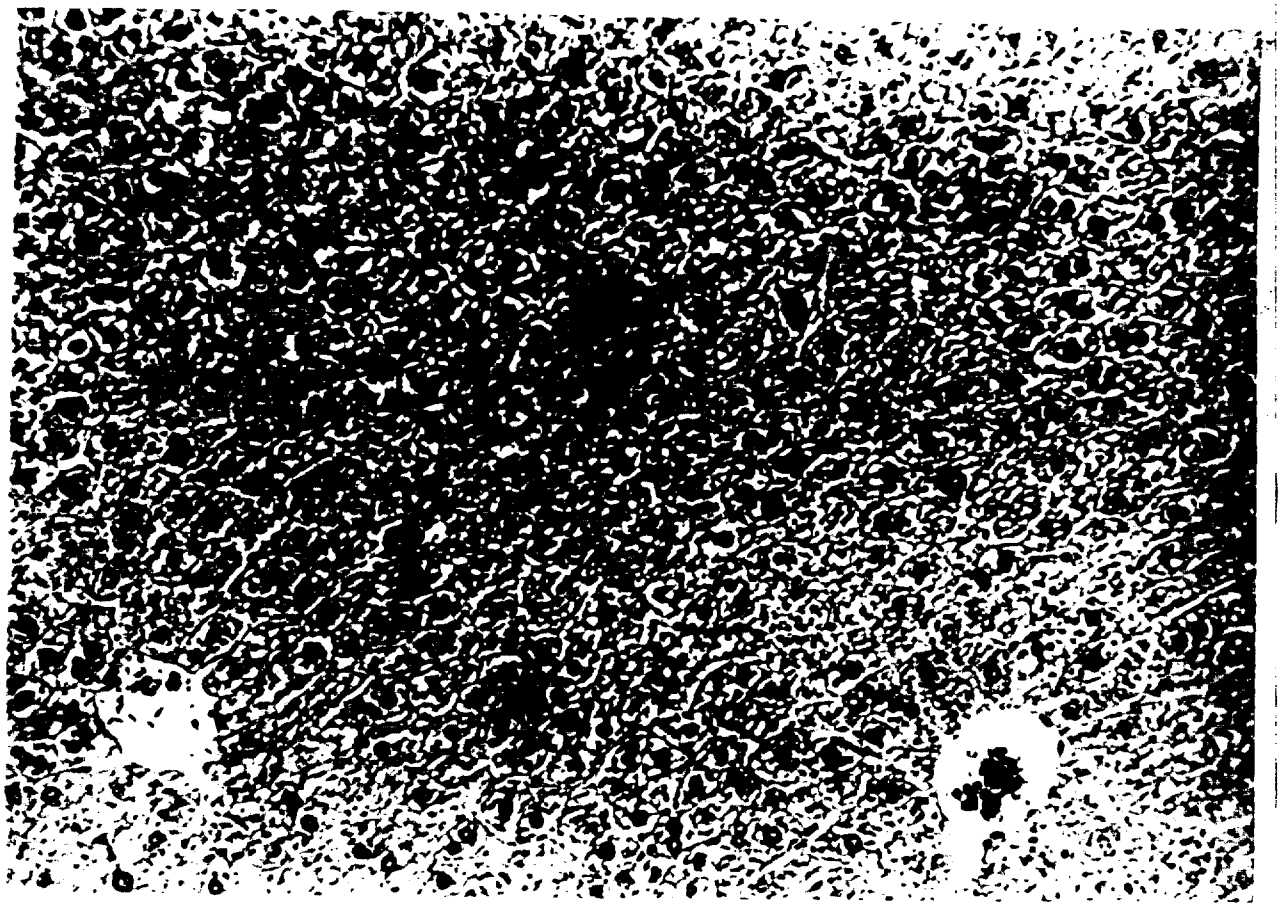


Photo 1. Liver (treated with ABME)
No significant change.

Photo 2. Kidney (treated with ABME)
No significant change.

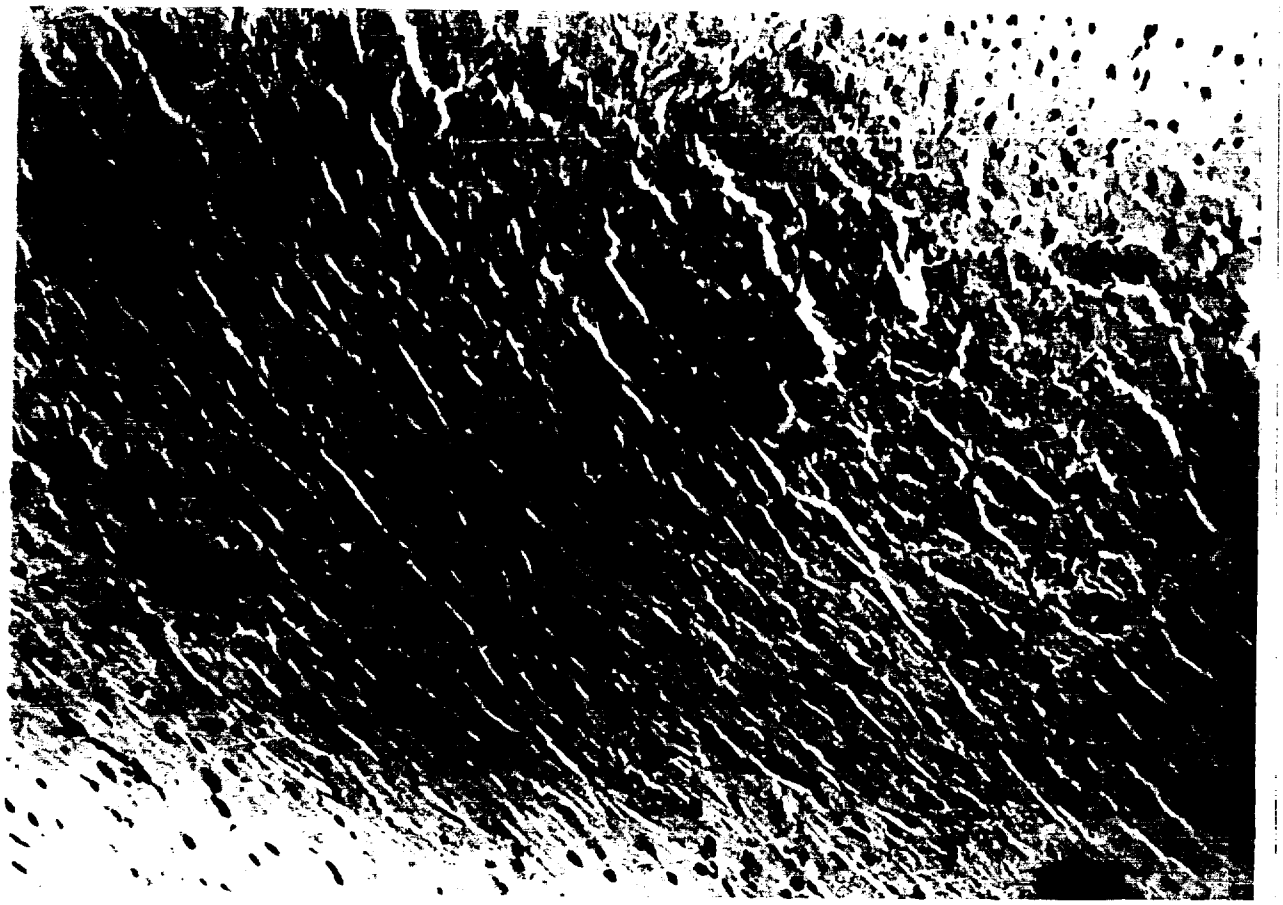


Photo 3. Heart (treated with ABME)
No significant change.

Photo 4. Spleen (treated with ABME)
No significant change.

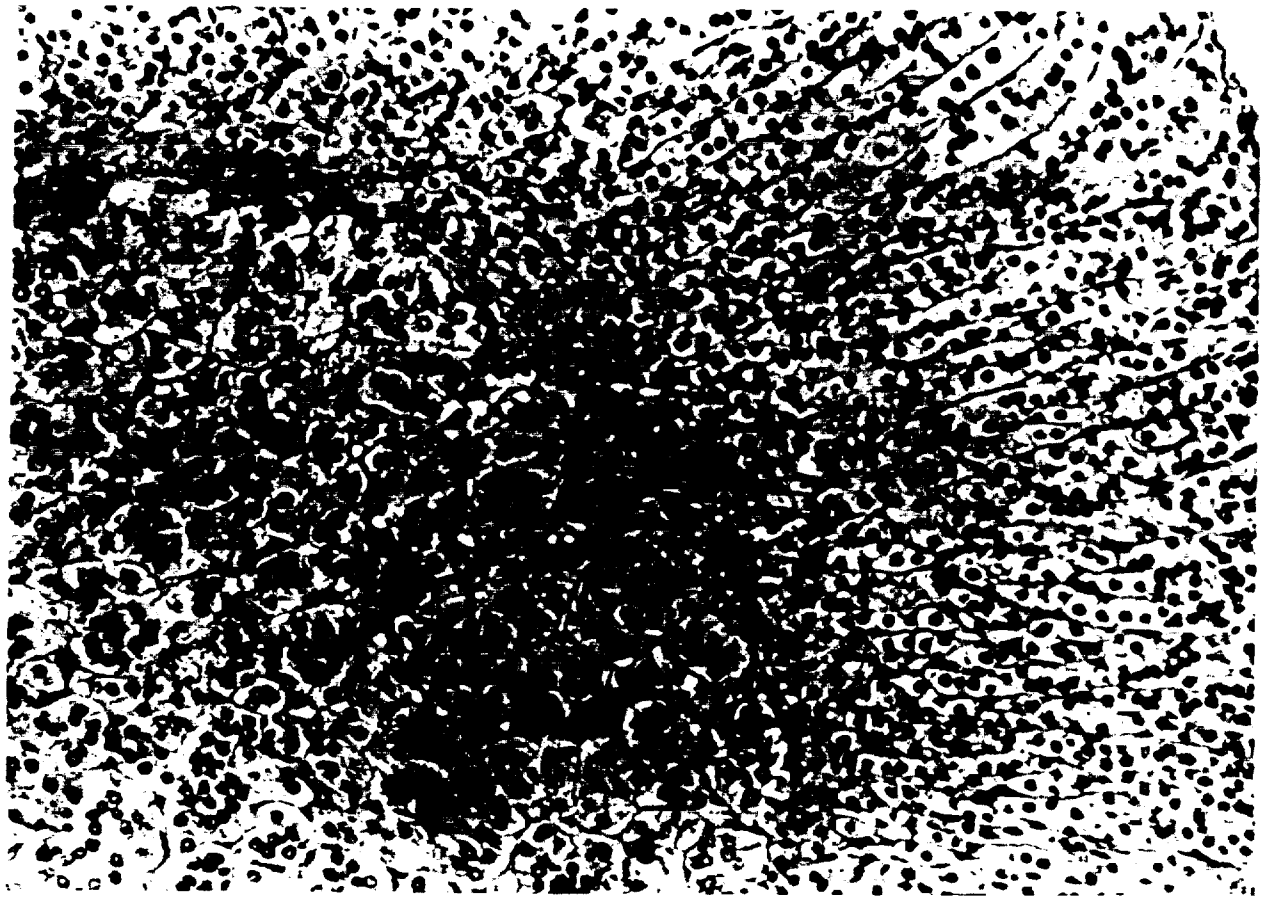


Photo 5. Adrenal (treated with ABME)
No significant change.

Photo 6. Small intestine (treated with ABME)
No significant change.

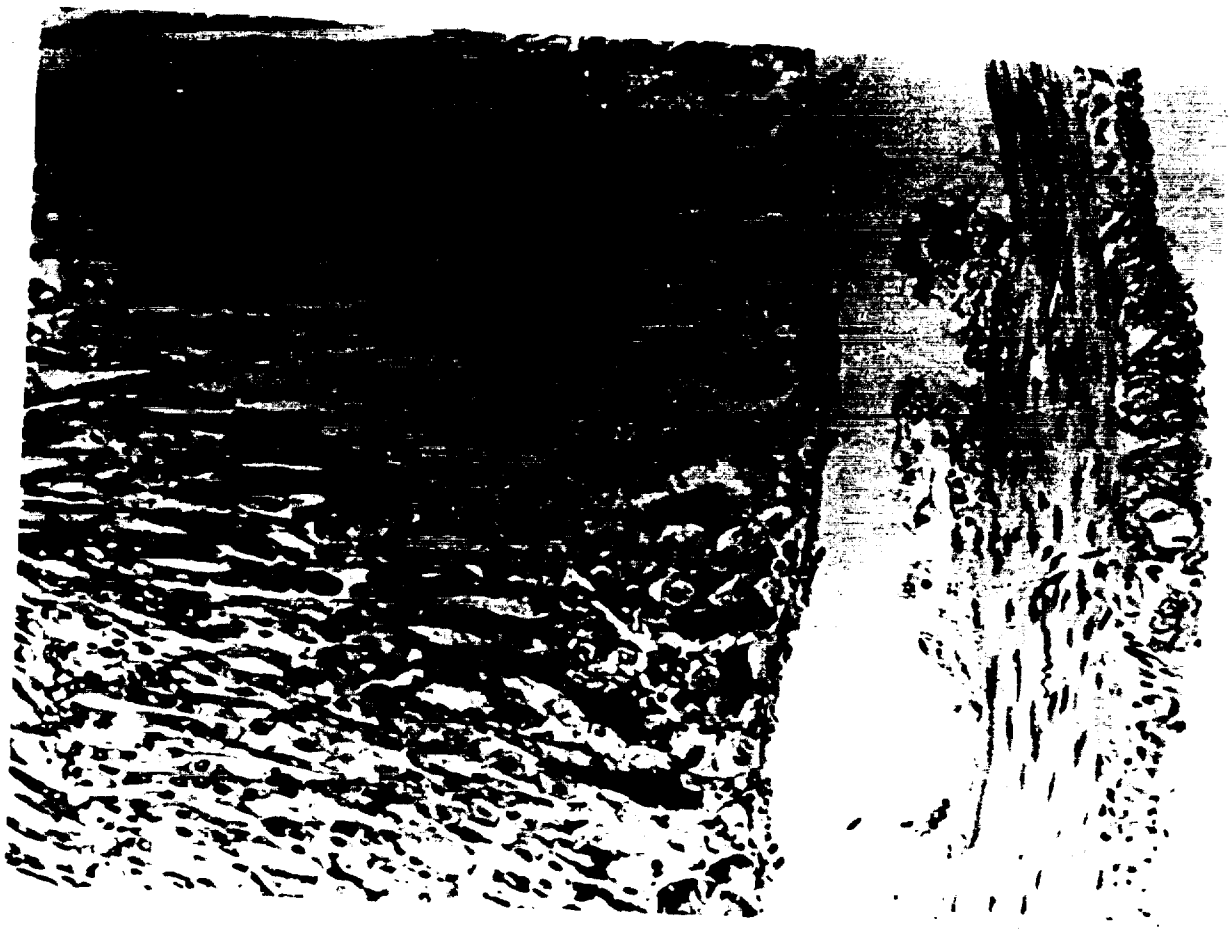


Photo 7. Stomach (treated with ABME)
No significant change.

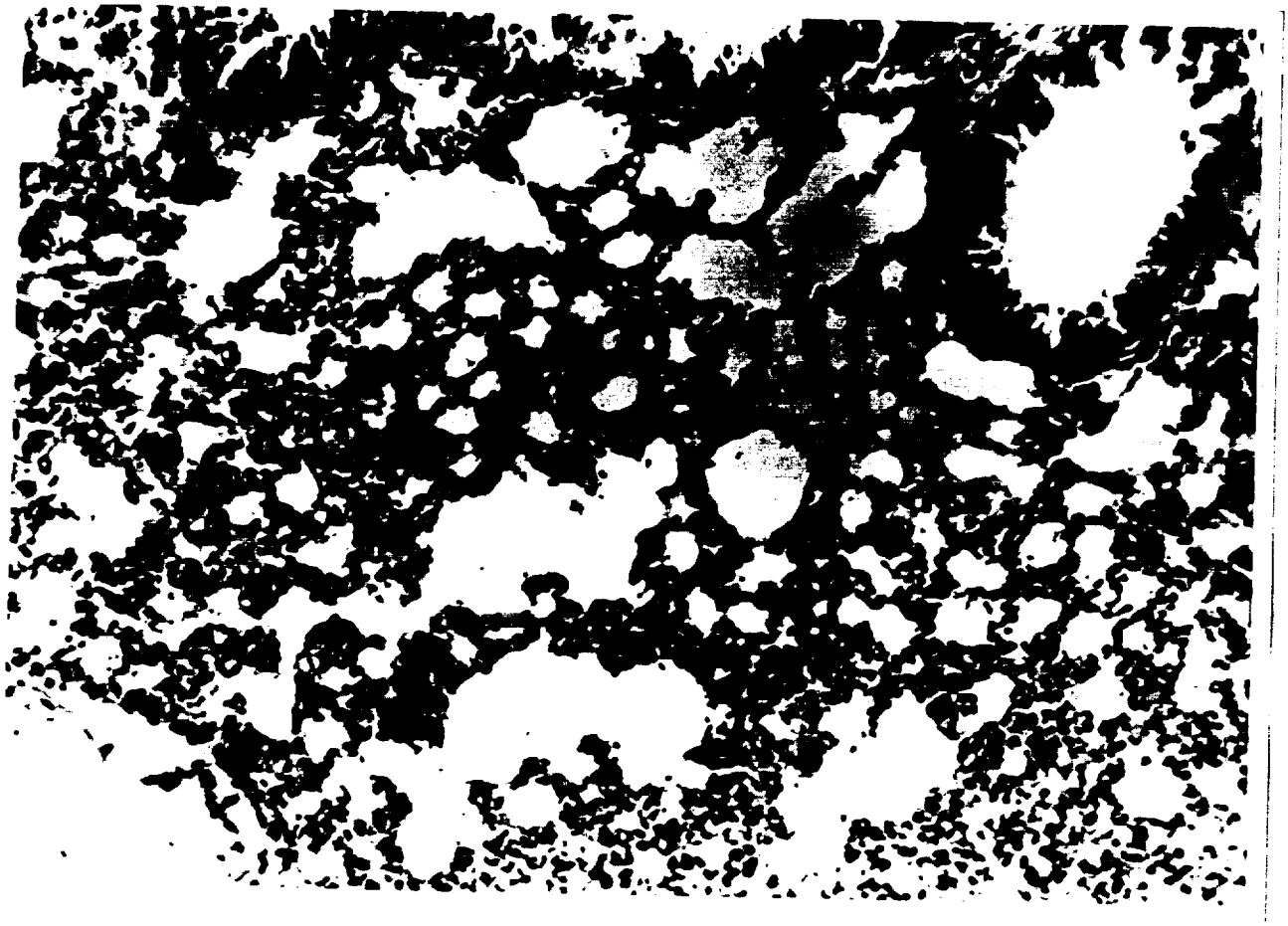


Photo 8. Lung (treated with ABME)
No significant change.

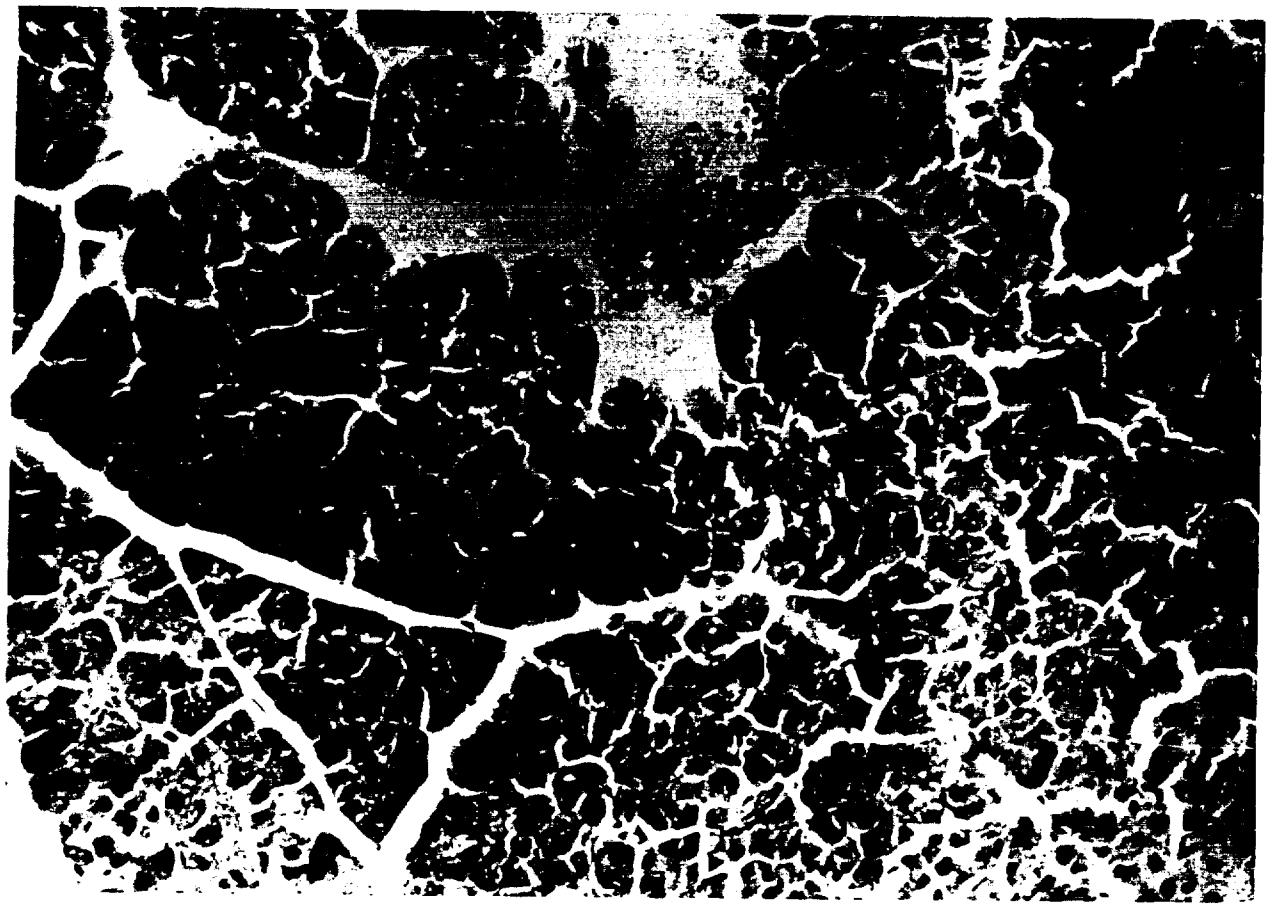


Photo 9. Pancreas (treated with ABME)
No significant change.

History of Himematsutake (*Agaricus blazei* Murrill)

Iwade Research Institute of Mycology

Contents

Dr. Inosuke Iwade who succeeded in cultivating Himematsutake for the first time in Japan mentioned in the article of "Himematsutake" in "Transactions of the Mycological Society of Japan" in 1982 as follows:

Himematsutake
Written by Inosuke Iwade

This mushroom was found by Mr.Takatoshi Furumoto who was my old friend and resident of Brazil. He noticed wild mushroom coming out around a Japanese Brazilian farmer's house located on the mountains of Piedade of Sanpaulo Brazil in the summer of 1965. Since this mushroom was edible and tasty, he made the seeds fungi and brought them to me. So, I spent a few years and completed cultivation method of this mushroom to be adaptable to Japanese climate. This mushroom was one of *Agaricus genus*. However, this scientific name was not known, I sent the mushroom twice to Dr. Heineman, Belgian scholar of mushroom classification for the judgement through the introduction of Dr.Hongo. Finally, this mushroom was recently proved to be *Agaricus blazei* Murrill. Compared to other mushrooms' forms in the same genus, its stem part is thick and long. And the spore part is slow to be changed into black. As for its characteristics, its aroma is high and the stem part is tasty and sweet.

Considering of its classification, I first named it Kawariharatake as its Japanese name. However, after due consideration thinking of its characteristics and practical use, I changed it and named it Himematsutake.

The rest omitted.

Therefore, before the scientific society held in 1982, the scientific name (nomenclature)of Himematsutake was provisionally *Agaricus heterosistes* Heinem et Gooss. However, after Dr. Iwade's publication, its Japanese name was confirmed to be Himematsutake and its scientific name was confirmed to be *Agaricus blazei* Murrill.

Scientific Report of Antitumor Effects of Polysaccharide from Himematsutake (Iwade Strain 101) and Its Active Mechanism

1980/4 The 53rd Japanese Society for Bacteriology (Niigata)
Studies on Antitumor Activities of Basidiomycetes (27): The relationship between C3 conversion and macrophages by antitumor polysaccharides.
Keishiro Shimura et al. Institute of Laboratory Animals, Dept. of Bacteriology, Mie University School of Medicine, Mie Japan

1980/11 Proceeding of the Japanese Cancer Association (Tokyo). The 39th Annual Meeting
Studies on Antitumor Activities of Polysaccharide (28):
Antitumor activities of prepared from *Agaricus heterosistes* Heinem et Gooss.
Keishiro Shimura et al. Institute of Laboratory Animals, Dept. of Pharmacology, Mie University School of Medicine

1981/3 Proceeding 54 th General Meeting Japanese Pharmacological Society (Fukuoka)
Studies on Antitumor Activities of Polysaccharides (29):
Antitumor effects and biological activities mannan fraction prepared from *Agaricus heterosistes* Heinem et Gooss.
Hitoshi Ito et al. Dept. of Pharmacology, Institute of Laboratory Animals, Mie University School of Medicine

1982 Transactions of the Mycological Society of Japan 23 543-561
Himematsutake
Inosuke Iwade, Iwade Research Institute of Mycology

1982/11 The 35th Japanese Society for Bacteriology Kansai Area (Shiga)
Studies on Antitumor Activities of Basidiomycetes: Antitumor Activities of Polysaccharides Prepared from *Agaricus blazei.*

Keishiro Shimura et al. Institute of Laboratory Animals, Dept. of Bacteriology , Mie University School of Medicine

1983 Japan Journal of Pharmacology 33 403-408
Screening of Host-mediated Antitumor Polysaccharides by Crossed Immunoelectrophoresis Using Fresh Human Serum
Keishiro Shimura et al. Institute of Laboratory Animals, Dept. of Pharmacology, Mie University School of Medicine

1983/3 Proceeding 56th General Meeting Japanese Pharmacological Society (Osaka)
Studies on Antitumor Activities of Polysaccharides (32): Antitumor mechanism of polysaccharides "ATOM" prepared from *Agaricus blazei* (Himematsutake).
Hitoshi Ito et al. Dept. of Pharmacology, Institute of Laboratory Animals, Mie University School of Medicine

1984/3 Proceeding 57th General Meeting Japanese Pharmacological Society (Kyoto)
Studies on Antitumor Activities of Polysaccharide (33): Antitumor mechanism of polysaccharides "ATOM" prepared from *Agaricus blazei* (Himematsutake).
Hitoshi Ito et al. Dept. of Pharmacology, Institute of Laboratory Animals, Mie University School of Medicine

1984/10 Proceeding of the Japanese Cancer Association (Fukuoka).
The 43rd Annual Meeting
Studies on Antitumor Activities of Basidiomycetes Polysaccharide (34): Antitumor activity of ATOM prepared from Himematsutake (*Agaricus blazei*).
Keishiro Shimura et al. Institute of Laboratory Animals, Dept. of Pharmacology, Mie University School of Medicine

1984/11 Medicine and Biology 109 (5) 299-302
Activation of the Reticuloendothelial System by Antitumor

Polysaccharide from *Agaricus blazei* Iwade.
Mitsuo Kawade et al. Iwade Research Institute of Mycology,
Dept. of Pharmacology, Institute of Laboratory Animals,
Mie University School of Medicine

1985/10 Proceeding of the Japanese Cancer Association (Tokyo).
The 44th Annual Meeting
Effect of Oral Administration of Antitumor Polysaccharides, ATSO and ATOM, on Mouse Peritoneal Macrophages.
Keishiro Shimura et al. Institute of Laboratory Animals,
Dept. of Pharmacology, Mie University School of Medicine

1985/10 Medicine and Biology 111 (4) 201-204
The Preventive Effect of Himematsutake on Experimental Liver Damage Induced by Carbon Tetrachloride.
Mitsuo Kawade et al. Iwade Research Institute of Mycology,
Dept. of Pharmacology, Institute of Laboratory Animals,
Mie University School of Medicine

1986/1 Medicine and Biology 112 (1) 29-32 (1986)
Antitumor Activity and Macrophage Activation by Lipid Fraction from *Agaricus blazei* Iwade
Hitoshi Ito et al. Dept. of Pharmacology, Institute of Laboratory Animals, Mie University School of Medicine, Iwade Research Institute of Mycology, Nichirei Laboratory

1986/10 Proceeding of the Japanese Cancer Association (Sapporo).
The 45th Annual Meeting
Studies on Antitumor Activity of Basidiomycete Polysaccharide (Report 37): Tumor cell binding activity of ATOM prepared from Himematsutake (*Agaricus blazei*).
Keishiro Shimura et al. Institute of Laboratory Animals,
Dept. of Pharmacology, Mie University School of Medicine

1987/3 The 34th Nippon Shokuhin Kougyou Daikai (in Japanese)
Studies on the Lipid of Himematsutake.
Haruo Sekiguchi et al. Department of Veterinary Medicine, Faculty of Agriculture, Nippon University

1987/4 Medicine and Biology 114 (4) 259-261
Antitumor Effect of Pretreatment with Polysaccharide from *Agaricus blazei* on the Solid sarcoma-180 in mice.
Hitoshi Ito et al. Dept. of Pharmacology, Institute of Laboratory Animals, Mie University School of Medicine, Iwade Research Institute of Mycology,

1987/6 New Fungi of Japan in Color, Hoikusha Publishing Co., Ltd. by
Edited Rokuya Imazeki and Tsuguo Hongo
Himematsutake (Kawariharatake) *Agaricus blazei* Murrill.

1987/9 Proceeding of the Japanese Cancer Association (Tokyo).
The 46th Annual Meeting
Studies on Antitumor Activity of Basidiomycete Polysaccharide(38)
: Antitumor activity of polysaccharide, ribonucleotide- complex and Lipid fraction prepared from Himematsutake (*Agaricus blazei*).
Hitoshi Ito et al. Dept. of Pharmacology, Institute of Laboratory Animals, Mie University School of Medicine,

1987 Proceeding of the 10th Symposium of the Sugar (Japan)
Antitumor Activity and Structural Features of Some Glycans Obtained from "Himematsutake", Fruiting Body of *Agaricus blazei* Murrill.
Takashi Mizuno et al. Dept. of Agriculture, Shizuoka Univ.
Dept. of Pharmacology, Institute of Laboratory Animals,
Mie University School of Medicine, Nichirei Laboratory,
Iwade Research Institute of Mycology

1988/9 Proceeding of the Japanese Cancer Association (Tokyo).
The 47th Annual Meeting

Studies on Antitumor Activity of Basidiomycete Polysaccharide(39) : Antitumor and biological activities of purified polysaccharide (FIII-2-b) prepared from Himematsutake (*Agaricus blazei*).
Hitoshi Ito et al. Dept. of Pharmacology, Institute of Laboratory Animals, Mie University School of Medicine

1988 Bull. Fac. Agri. Shizuoka Univ. 38 29-35 (1988)
Immunostimulative Antitumor Effects of β-D-Glucans and Chitin Substances Isolated from Medicinal Mushrooms.
Takashi Mizuno et al. Dept. of Agriculture, Shizuoka Univ., Dept. of Pharmacology, Institute of Laboratory Animals, Mie University School of Medicine

1989 Carbohydrate Research
Fractionation and Antitumor Activity of the Water-soluble Residue of *Agaricus blazei* Fruiting Bodies
Takashi Mizuno et al. Dept. of Agriculture, Shizuoka Univ., Dept. of Pharmacology, Institute of Laboratory Animals, Mie University School of Medicine, Iwade Research Institute of Mycology

1990 Agricultural and Biological Chemistry 54 (11), 2897-2905
Antitumor Activity and Some Properties of Water- insoluble Heteroglycans from "Himematsutake" the Fruiting Body of *Agaricus blazei* Murrill
Takashi Mizuno et al. Dept. of Agriculture, Shizuoka Univ., Dept. of Pharmacology, Institute of Laboratory Animals, Mie University School of Medicine

1990 Agricultural and Biological Chemistry 54 (11)
Antitumor Activity and Some Properties of Water-soluble Polysaccharides from "Himematsutake" the Fruiting Body of *Agaricus blazei* Murrill
Takashi Mizuno et al. Dept. of Agriculture, Shizuoka Univ., Dept. of Pharmacology, Institute of Laboratory Animals, Mie University School of Medicine, Iwade Research Institute of Mycology

1990 Carbohydrate Polymers 12 303-403

Formolysis of Antitumor $(1 \rightarrow 6)$-β-D-Glucan-Protein Complex from *Agaricus blazei* Fruiting Bodies and Antitumor Activity of the Resulting Products

Hirokazu Kawagishi et al. Dept. of Applied Biological Chemistry Faculty of Agriculture, Shizuoka Univ., Institute of Laboratory Animals, Dept. of Pharmacology, Mie University School of Medicine

1991/11 Laboratory Animal Technology and Science 2 (6) 341-348

Application of Biological Response Modifiers in Cancer Field.

Hitoshi Ito et al. Dept. of Pharmacology, Mie University School of Medicine

1991/5 The 38th Proceedings of the Japanese Association for Laboratory Experimental Animal Science (Sapporo)

Studies on Antitumor Activities of Basidiomycetes (42): Relation between antitumor activities and rapidly C3 release by macrophages from polysaccharides.

Keishiro Shimura et al. Institute of Laboratory Animals, Mie University School of Medicine

1992 Chemical and Biochemical Effect of Mushroom Fungi, Gakkai Publishing Center "Complement and Macrophages Stimulator".

Keishiro Shimura et al. Institute of Laboratory Animals, Dept. of Pharmacology, Mie University School of Medicine

1992 Chemical and Biochemical Effect of Mushroom Fungi, Gakkai Publishing Center "Himematsutake".

Takashi Mizuno, Dept. of Agriculture, Shizuoka Univ.

1992 Center for Cooperative Research and Development Kobe University.

Findings on the Activator of Competent Cells with Immuno-Response System and Its Chemical Characterization, and Separation.

Masashi Mizuno et al. Agricultural, Medical Dept., Kobe Univ.

1993/10 Medicine and Biology 127 (4) 239-242

Activation of Reticuloendothelial System after Oral Administration of Himematsutake (*Agaricus blazei*) with Special Reference to Analysis of Administration Schedule.

Hiroko Ito et el. Faculty of Bioresources, Mie University, Iwade Research Institute of Mycology, Dept. of Pharmacology, Mie University School of Medicine

1994 Kansensho (Infections). Fujisawa Pharmacy Co., Ltd. 14 (1) 18-22

Antitumor Polysaccharides.

Hitoshi Ito, Dept. of Pharmacology, Institute of Laboratory Animals, Mie University School of Medicine

1994/5 The 2nd International Symposium on the Efficacy of Fungi (Korea)

Immunopharmacological Studies of Antitumor Polysaccharides Isolated from Basidiomycetes.

Hitoshi Ito et al. Dept. of Pharmacology, Mie University School of Medicine

The 2nd International Symposium on the Efficacy of Fungi (Korea)

A Clinical Studies of ATSO from Coriolus versicolor Iwade and ABWS from *Agaricus blazei* (Himematsutake) on Patients with Tumor and Chronic Hepatitis B.

Wang Jun Zhi et al. Dept. of Pharmacology, Institute of Laboratory Animals, Mie University School of Medicine

1994 Journal of Lanzhou Medical College 20 (3) 169-171 1994

Clinical Observation on Treatment of Acute Nonlymphocytic Leukemia with *Agaricus blazei*.

Tian Xiaohui et al. Lanzhou Medical College, Dept. of Pharmacology, Institute of Laboratory Animals, Mie University School of Medicine

1994 Gansu Medical Journal 13 (1) 5-7 1994

Clinical Observation on Treatment of Gastro-Intestinal Cancer with *Agaricus blazei.*

Wang Jing et al. Lanzhou Medical College, Dept. of Pharmacology, Institute of Laboratory Animals, Mie University School of Medicine

1994 Journal of Lanzhou Medical College 20 (1) 24-26 1994

Observation on Treatment of Agaricus blazei Chronic Hepatitis B.

Wang Lirong et al. Lanzhou Medical College, Dept. of Pharmacology, Institute of Laboratory Animals, Mie University School of Medicin

1994 Japan Journal of Pharmacology 6 6 265-271

Inhibitory Action of a (1→6) β-D-Gulcan-Protein Complex (FIII-2-b) Isolated from *Agaricus blazei* Murrill ("Himematsutake") on Meth A Fibrosaroma-Bearing Mice and Its Antitumor Mechanism

Hitoshi Ito et al. Dept. of Pharmacology, Mie University School of Medicine

1995 Medicine and Biology 131 (1) 11-15 1995

Antiallergic Action of *Agaricus blazei* (Iwade Strain 101) "Himematsutake".

Hiroko Ito et al. Faculty of Bioresources, Mie University, Iwade Research Institute of Mycology,

1995/11 Proceedings of the Third International Symposium of the Mycological Society of Japan (Chiba) New Initiative in Mycological Research

Immunopharmacological Studies of Antitumor Polysaccharides Prepared from *Agaricus blazei* (Iwade Strain 101) "Himematsutake" on Tumor-Bearing Mice.

Hitoshi Ito, Dept. of Pharmacology, Mie University School of Medicine

1996 The 43rd Proceedings of the Japanese Association for Laboratory Experimental Animal Science (Niigata)
Studies on the Binding Ability to Ehrlich Ascites Carcinoma Cells by Polysaccharide "ATOM" Prepared from Cultured Himematsutake.
Keishiro Shimura et al. Institute of Laboratory Animals, Mie University School of Medicine

1996/10 Proceeding of the Japanese Cancer Association(Yokohama).
The 55th Annual Meeting
Antitumor Effects of a New Polysaccharide-Protein Complex (ATOM) Prepared from *Agaricus blazei* (Iwade Strain 101) "Himematsutake" and its Mechanisms in Tumor-Bearing Mice.
Hitoshi Ito et al. Dept. of Pharmacology, Institute of Laboratory Animals, Mie University School of Medicine, Iwade Research Institute of Mycology

1996/11 Medicine and Biology 133 (5) 179-181
Availability of *Agaricus blazei* (Iwade Strain 101) "Himematsutake" in Human Advanced Cancer as a Biological Response Modifier.
Koichi Yokono et al. The 2nd Dept. of Internal Medicine, Kobe University School of Medicine, Iwade Research Institute of Mycology

1997/3 The 44th Nippon Shokuhin Kagaku Kougaku Taikai (in Japanese)
Separation, Purification and Chemical Structure of Antitumor Active Substances in Mycelia Prepared from Cultured Himematsutake.
Yukitaka Taniguchi et al. Faculty of Agriculture of Kobe University Dept. of Pharmacology, Mie University School of Medicine, Iwade Research Institute of Mycology

1997 Anticancer Research (International Journal of Cancer Research and Treatment) 17 277-284
Antitumor Effects of a New Polysaccharide-Protein Complex (ATOM) Prepared from *Agaricus blazei* (Iwade strain 101) "Himematsutake" and its Mechanisms in Tumor-Bearing Mice

Hitoshi Ito et al. Dept. of Pharmacology, Institute of Laboratory Animals, Mie University School of Medicine, Iwade Research Institute of Mycology

1997/9 Proceeding of the Japanese Cancer Association (Kyoto). The 56th Annual Meeting
Antitumor Effect and Its Mechanism of Preparation Prepared from Himematsutake (*Agaricus blazei*)
Kohichi Ohshima et al. Sumitomo Ringyo (Sumitomo Forestry Co., Ltd. Tsukuba Research Institute), Miyagi Cancer Center

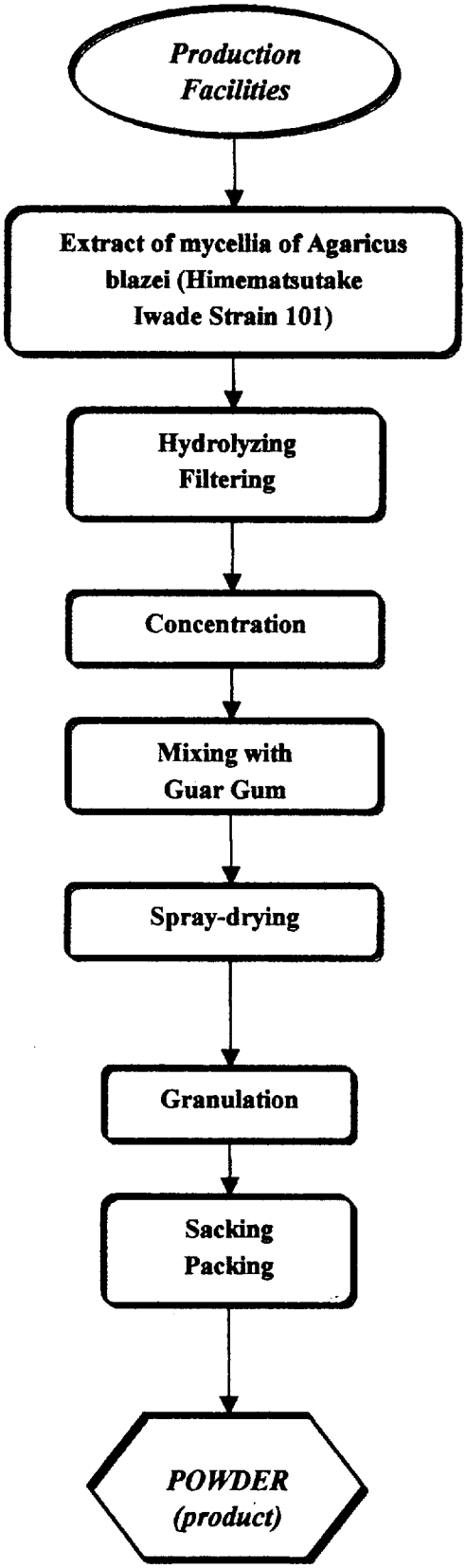
1997/10 Food Style 21 1 (5) 25-30
Studies of Medicinal Effect of Mushroom Fungi: A New Findings In Mycological Research.
Hitoshi Ito et al. Dept. of Pharmacology, Mie University School of Medicine

The above mentioned are scientific reports about antitumor effects of Himematsutake. However, any scientific reports about Agaricus was not published at all. Therefore, there's no record that the group including Dr. Shoji Shibata, former member of Pharmaceutical Dept. of Tokyo University and Dr. Tetsuro Ikekawa, former member of National Cancer of Japan made research about Agaricus. The name of Agaricus means "Genus of Agaricus". According to Singer, world famous mushroom scholar, reports that Agaricus can be devided into 37 kinds of mushrooms, which means Agaricus is a genus name of mushrooms and not an individual name.

VI

CONFIDENTIAL

SCHEME



VII

CONFIDENTIAL

Iwade Research Institute of Mycology Co., Ltd.

CONFIDENTIAL

Product Specification

HIMEMATSUTAKE POWDER

MATERIAL

* Himematsutake extract
* Enzymatically hydrolyzed guar gum

DESCRIPTION

* Himematsutake Powder is obtained by mixing Himematsutake Extract and Guar Gum and then spray drying it.

CHEMICAL SPECIFICATIONS

Energy		kcal/100g	350
Water content	*1	g/100g	1.2
Crude ash	*2	g/100g	1.2
Crude protein	*3	g/100g	7.0
Crude fat	*4	g/100g	0.6
Crude fiber	*5	g/100g	0.9
Total sugar	*6	g/100g	19.1
GS fiber	*7	g/100g	70.0

*1 Heat-drying method, 105℃ 3hr
*2 Ashnized method, 550℃ (Carbonizing)
*3 Lowry method
*4 Ether extracting method
*5 Henneberg-Stohmann modified method
*6 Phenol-Sulfuric acid method
*7 Diluent

CARBOHYDRATES PROFILE

Glucose	g/100g	4.9
Galactose	g/100g	2.2
Mannose	g/100g	10.0
Xylose	g/100g	0.2
Arabinose	g/100g	0.06
Ribose	g/100g	1.5
Fucose	g/100g	trace
Unknown	g/100g	0.27
GS fiber*	g/100g	70.0

GLC: gas liquid chromatography
* Diluent

POLYSACCHARIDE PROFILE

â-Glucan	p/100g	7.5
á-Glucan	p/100g	2.2
â-Glucomannan	p/100g	8.4
â-Galactoglucan	p/100g	2.2
Ribonucleotide	p/100g	2.2
Protein bound·â-Glucan	p/100g	8.6
Xyloglucan	p/100g	1.1

^{13}C-NMRanalysis
Two-dimensional COSY analysis

AMINO ACID PROFILE

Aspartic acid	mg/100g	236
Threonine	mg/100g	136
Serine	mg/100g	129
Glutamic acid	mg/100g	230

Glycine	mg/100g	194
Alanine	mg/100g	208
Valine	mg/100g	135
Methionine	mg/100g	36
Leucine	mg/100g	216
Tyrosine	mg/100g	60
Phenylalanine	mg/100g	107
Histidine	mg/100g	57
Lysine	mg/100g	143
Arginine	mg/100g	291
Isoleucine	mg/100g	59
Proline	mg/100g	62

Amino acid analyser

SENSORIC

Texture/consistency	Powder
Appearance/color	Brown
Taste/odor	Bitter and odorless

SHELF LIFE: 18 months

CONFIDENTIAL

Iwade Research Institute of Mycology Co., Ltd.

Formula / Recipe

HIMEMATSUTAKE POWDER

INGREDIENTS	P/100g
Himematsutake extract	30
Enzymatically hydrolyzed guar gum	70
Total	100

CONFIDENTIAL

Iwade Research Institute of Mycology Co., Ltd.

Ingredient Specification

Himematsutake Extract

MATERIAL *Himematsutake Extract

DESCRIPTION *Himamatsutake is an extract obtained by first hydrolising the mushrooms (*Agaricus blazei* Murrill, Iwade Strain 101)and then concentrating its Liquid.

CHEMICAL SPECIFICATIONS

Energy		kcal/100g	350
Water content	*1	g/100g	1.2
Crude ash	*2	g/100g	1.2
Crude protein	*3	g/100g	7.0
Crude fat	*4	g/100g	0.6
Crude fiber	*5	g/100g	0.9
Total sugar	*6	g/100g	19.1

*1 Heat-drying method, 105□ 3hr
*2 Ashnized method, 550□ (Carbonizing)
*3 Lowry method
*4 Ether extracting method
*5 Henneberg-Stohmann modified method
*6 Phenol-Sulfuric acid method

CONFIDENTIAL

Iwade Research Institute of Mycology Co., Ltd.

Ingredient Specification

Enzymatically Hydrolyzed Guar Gum

MATERIAL * Enzymatically Hydrolyzed Guar Gum

DESCRIPTION * Guar gum is obtained from Cyamopsis seeds by grinding and Hydrolyses

CHEMICAL SPECIFICATIONS

Moisture	under 7.0 %
Ash	under 2.0 %
Protein	under 1.0 % (kendall test)
Viscosity	under 10 cps (5 % solution)
pH	4-7 (20 % solution)
Arsenic	under 4 ppm
Heavy metal	under 10 ppm
Mycology	under 1000 ps/g
Melt	melt about 40 % for water