DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date AUG 4 2000

From (Acting) Division Director, Division of Standards and Labeling Regulations,
Office of Nutritional Products, Labeling and Dietary Supplements, HFS-820

Subject 75-Day Premarket Notification of New Dietary Ingredients

To Dockets Management Branch, HFA-305

New Dietary Ingredient:	extract of *Agaricus blazei* Merrill
Firm:	Iwade Research Institute of Mycology Co., Inc.
Date Received by FDA:	May 23, 2000
90-Day Date:	August 20, 2000

In accordance with the requirements of section 413(a) 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 No. 95S-0316 after August 20, 2000.

Felicia B. Satchell

Felicia B. Satchell

95S-0316

RPT76

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington DC 20204

AUG 4 2000

Kristi O. Smedley, Ph.D.
Consultant
Center for Regulatory Services
5200 Wolf Run Shoals Road
Woodbridge, Virginia 22192

Dear Dr. Smedley:

This is in response to your letter submitted on behalf of Iwade Research Institute of Mycology Company, Inc. of Suehiro-cho, Tsu, Mie, Japan (client) to the Food and Drug Administration (FDA) dated May 22, 2000, 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 (the Act)). Your letter notified FDA of your client's intent to market a dietary supplement product containing A new dietary ingredient, namely, an extract of *Agaricus blazei* Murrill. This new dietary ingredient notification contains information that supplements that contained in a previous submission dated May 18, 1999. We concluded in our letter dated July 29, 1999, that the information in the previous submission did not provide a basis to conclude that a dietary supplement containing this new dietary ingredient will reasonably be expected to be safe.

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 ingredients do not present a significant or unreasonable risk of illness or injury.

FDA has carefully considered the information in your submission, and the agency has significant concerns about the evidence on which you rely to support your conclusion that the new dietary ingredients stated above will reasonably be expected to be safe. In our letter of July 29, 1999, we stated that there was a lack of quantitative estimates of dietary exposure to *Agaricus blazei* Murrill extract (ABME) that would provide a basis to support the history of use of this substance in Japan to conclude that its use in a dietary supplement is safe. The current submission states that your client "is not requesting that

FDA make a determination of safety based on historical use." Since the history of use will not be used as a basis to conclude that this new dietary ingredient will reasonably be expected to be safe, we have not considered prior human food use of the ingredient in our review of your notification.

Your client's submission contained data from two animal studies and three human studies that your client asserts support a determination that the dietary supplement ABME will reasonably be expected to be safe. All animal studies were performed using adult rodents.

Your client's submission included, in Attachment A, a derived tolerable daily intake (TDI) for "Himematsutake powder" based on the findings of animal studies. Several issues need to be addressed to clarify the basis of your calculations in deriving the TDI. First, no references are noted for the studies cited in Attachment A as the basis for the calculations. Second, it appears the animals used in the studies that were the basis of the TDI (section 4.B.I and 4.B.II) were administered ABME. Thus, a TDI derived from these animal studies represents a TDI for ABME, not Himematsukate powder. In turn, the TDI for ABME cannot be directly compared in a meaningful way to the doses of exposure to Himematsukate powder in human studies as is done in Attachment A. Himematsukate powder is indicated in the submission to contain ABME extract and guar gum. These differences need to be considered in the estimates. This section also indicated that the result of chronic toxicity studies (6-month rat and mouse studies) demonstrated a no adverse effect level (NOAEL) of 3000 mg/kg body weight (bw) and your client derived a TDI from this NOAEL of 30 mg/kg bw/day using an uncertainty factor (UF) of 100 (10 X 10 for intra- and inter-species differences). However, the rat study that was indicated as having a NOAEL of 3000 mg/kg body weight revealed small increases in liver weight expressed as g per 100g body weight in males at 3000 mg ABME/kg bw/day dose level at week 13 and 26, and in females at the 3000 mg/kg bw/day dose level at 13 weeks. The authors of the study suggest that the effect on liver weight is not due to the test material, but the pattern and consistency of this effect suggests that it is ABME-treatment induced. Clarification of the statistical analyses of these changes is warranted. Other changes such as increase in food intake and decrease in cholesterol were seen at 1000 and 3000 mg ABME /kg bw/day in the rat study submitted. It appears that your client concluded that it is reasonable not to consider these changes as adverse effects. If the alteration in liver weight represents an adverse effect, then the lowest adverse effect level (LOAEL) would be 3000 mg/kg bw/day and the NOAEL would instead be 1000 mg/kg bw/day (or possibly 50 mg/kg bw/day based on the mouse study). Then the TDI for ABME based on animal studies would be lower than suggested (e.g., 10 mg/kg bw/day). Finally, in the TDI derivation, your client notes that "the formulated product" administered to healthy humans was 3X-6X the recommended dosage (4500 – 8000 mg/person/day). It is not clear to which study this refers.

Three studies performed in adult humans was also provided in the notification. In the first study (Section 4.B.III), the AMBE used was confirmed as identical to the AMBE

dietary supplement product that Iwade intends to market in the United States (U.S.) (see letter in Section 4.B. III). However, interpretation of the information presented in the letter is difficult. It is not clear if it indicates that the ABME used in the study is identical to ABME used in the Iwade dietary supplement or the ABME used in the study actually represents the Himematsutake powder which contains a diluent and/or is identical to the Iwade dietary supplement product. In addition, the volume (ml) of ABME fluid administered is indicated in the study but the concentration of ABME in this fluid is not noted. Without information on the dose of exposure, it is difficult to draw conclusions about the significance of the paucity of substantial ABME-induced changes indicated for a range of measures in this experiment. Also with respect to this study, the results on these various measures were presented for each individual. However, no summary data were provided nor were statistical analyses performed. Some individual changes or trends were noted. However, the significance of these changes associated with ABME exposure were not clearly delineated or addressed. Considerations of the response of the subset of individuals with pre-existing medical conditions (hypertension, diabetes and high triglycerides, hyper-triglycerides and lipidemia) with respect to the findings from the healthy subjects may also be of concern.

Another human study (Section 4.B.V) involved 10 female patients with cancers of the reproductive system (malignant tumors of the uterus, cervix, ovaries). Some of the subjects underwent a surgical operation (8/10), chemotherapy (1/10) and/or radiotherapy (7/10) prior to the administration of Himematsutake powder (indication that the Himematsutake powder is identical in nature to the Iwade proposed dietary supplement product is not noted). Studies in seriously ill patients that are confounded with different medical conditions, different degrees and types of cancer, and different treatments are of limited utility in evaluating safety of a substance in healthy people. Changes in the immune system along with blood and liver measures were seen with Himematsutake powder intake. The nature and significance of these potential effects being elicited chronically in normal, healthy individuals consuming Himematsutake-based products have not been addressed. Therefore, this human study provides little support for concluding that chronic or long-term consumption of dietary supplements containing ABME will reasonable be expected to be safe in healthy people.

In the third human study (Section 4.B.IV), 20 healthy male and 15 healthy female university student volunteers (19-23 years old , no body weight provided) consumed 30 and 15 g Himematsutake powder per day, respectively, for 6 months. It is reported by the investigator of this study that no significant side effects were observed in this study. In contrast to this statement, examination of Table 3 and 4 in Section 4.B.II suggests some side effects emerged with exposure to Himematsutake powder such as changes in appetite, digestion, general condition, etc. However, exact interpretation of these tables is difficult because many table elements are not clearly labeled or explained. Clarification on the nature of the changes would be useful.

If adequate human data are available, a toxicological–based safety/risk assessment approach should utilize these data to derive a human TDI with estimates from animal work to support it. Some of the human studies presented in this notification could potentially be addressed in this manner. However, deficiencies and uncertainties exist in the information provided in the human studies and in the notification on the Himematsutake powder utilized in the various experiments, i.e., the Iwade dietary supplement (i.e., 1.5g ABME / 3.5 g guar gum), the powder described in Section 4.B.VI (no % ABME to diluent information provided), and how they compare. This information is vital for determining the merits of the arguments made by your client on the safe use of the Himematsutake dietary supplement product. Furthermore, the information you submitted does not address the safety of use of ABME in children or developing animals.

For the reasons discussed above, the information in your submission does not provide an adequate basis to conclude that extract of *Agaricus blazei* Murrill, when used under the conditions recommended or suggested in the labeling of your client's products, will reasonably be expected to be safe in adults or children. Therefore, the products may be adulterated under 21 U.S.C. 342(f)(1)(B) as dietary supplements that contain the new dietary ingredient specified 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 products into interstate commerce is prohibited under 21 U.S.C. 331(a) and (v).

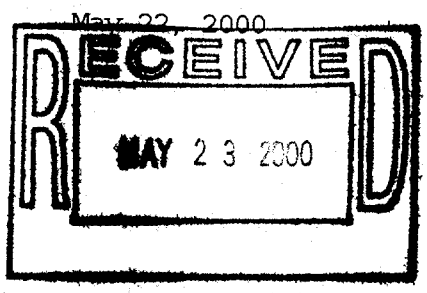
Please contact us if you have any questions concerning this matter.

Sincerely yours,

Felicia B. Satchell

Felicia B. Satchell
(Acting) Division Director
Division of Standards
and Labeling Regulation
Office of Nutritional Products, Labeling
and Dietary Supplement

center for regulatory services
5200 Wolf Run Shoals Road * Woodbridge, VA 22192 * 703 590 7337 * Fax 703 580 8637 * cfrsrv@aol.com



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*-- **SUPPLEMENTAL** Information

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*. This information is provided in addition to the information submitted on May 18, 1999, and responded to by the agency on July 29, 1999. In accordance with 21 CFR §190.6, enclosed is one original plus two copies of the following information.

We understood that the agency had four concerns regarding the notification submitted on behalf of Iwade: 1) lack of historical quantitative data on consumption; 2) inadequate information about the nature and composition of the extract used in the studies to demonstrate safety; 3) safety information did not support the requested level of supplementation; and 4) the agency requested additional human studies using healthy subjects.

Iwade is not requesting FDA make a determination of safety based on historical use; therefore, we have not addressed that concern. The other concerns are addressed below and in the attached studies.

Iwade has modified the labeling on the product to be used at a level of one package per day (a total of 1.5 grams of Himematsutake extract). We have calculated an NOAEL of 1800 mg; therefore the suggested dose is below the NOAEL (Attachment A).

Information cited under Section 4.B. includes the new information submitted by Iwade to support their determination of safety. You will note that toxicity data referred to by 4.B. I. (Chronic Study of Cultured Agaricus blazei Murrill (Iwade Strain 101) (Japanese name; Himematsutake) Preparation, "ABME" Administered Orally in Rats for 26 weeks) and 4.B.II. (Chronic Study of Cultured Agaricus blazei Murrill (Iwade Strain 101) (Japanese name; Himematsutake) Preparation, "ABME"

Administered Orally in Mice for 26 weeks) were completed by Mie University School of Medicine, as was the complete analysis of the Himematsutake. Also included for your review are two studies completed with healthy volunteers (a 12-week and a 6-month study). Iwade Research has provided a letter of confirmation that the 12-week study was completed with the identical material covered by the notification. The chemical analysis of the product used in the 6 month studies are provided (Attachment 4.B.I.).

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*

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

   > Directions will suggest using one package 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.

   A. Previously Iwade provided the following documents and they are not included again in this filing.

      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 Murrill. 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 Murrill. 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)**

B. In this filing Iwade is providing additional information in support of their determination that the dietary supplement containing Himematsutake extract will reasonably be expected to be safe under the recommended conditions of use.

I. Chronic Study of Cultured Agaricus blazei Murrill (Iwade Strain 101) (Japanese name; Himematsutake) Preparation, "ABME" Administered Orally in Rats for 26 weeks.

II. Chronic Study of Cultured Agaricus blazei Murrill (Iwade Strain 101) (Japanese name; Himematsutake) Preparation, "ABME" Administered Orally in Mice for 26 weeks.

III. Safety of Cultured Agaricus blazei Murrill (Iwade Strain 101) (Japanese name; Himematsutake) Preparation, ABME, for Humans in Relatively Long Term Oral Administration.

IV Safety Test for Long-term Administration of Himematsutake (Iwade Strain 101) Powder in Healthy Volunteers.

V. Clinical Trail with Himematsutake (Iwade Strain 101) Powder on Patients with Malignant Tumor (Study on Long-Term Administration and Side Effect.

VI. Revised Product specifications of Himematsutake Powder. **(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

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ATTACHMENTS

A. Tolerable Daily Intake Estaimte – Himematsutake Powder

4.B. I. Chronic Study of Cultured Agaricus blazei Murrill (Iwade Strain 101) (Japanese name; Himematsutake) Preparation, "ABME" Administered Orally in Rats for 26 weeks.

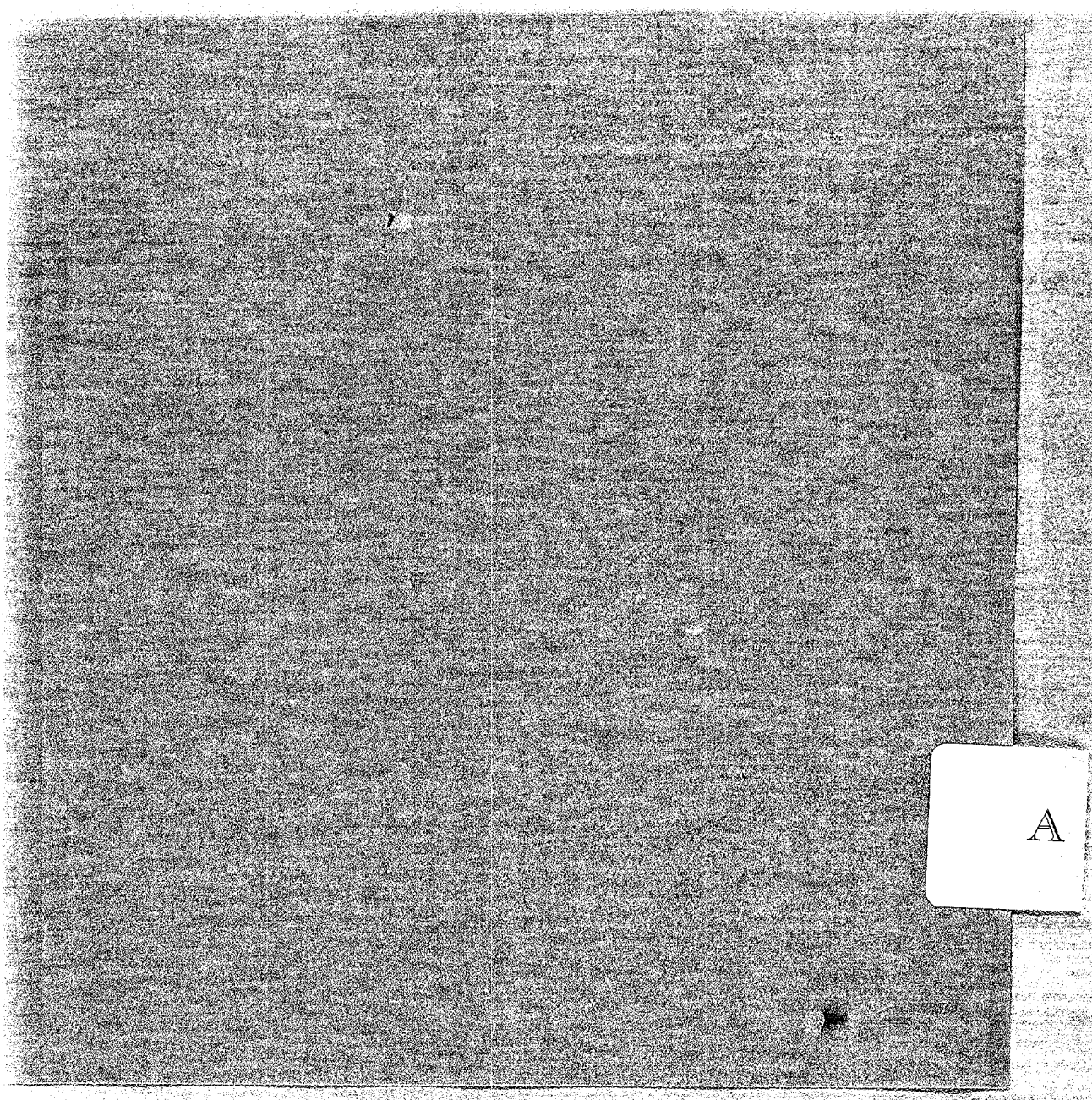
4.B. II. Chronic Study of Cultured Agaricus blazei Murrill (Iwade Strain 101) (Japanese name; Himematsutake) Preparation, "ABME" Administered Orally in Mice for 26 weeks.

4.B.III. Safety of Cultured Agaricus blazei Murrill (Iwade Strain 101) (Japanese name; Himematsutake) Preparation, ABME, for Humans in Relatively Long Term Oral Administration.

4.B. IV. Safety Test for Long-term Administration of Himematsutake (Iwade Strain 101) Powder in Healthy Volunteers.

4.B. V. Clinical Trial with Himematsutake (Iwade Strain 101) Powder on Patients with Malignant Tumor (Study on Long-Term Administration and Side Effect).

4.B. VI. Revised Product Specifications of Himematsutake Powder. **(CONFIDENTIAL)**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Attachı

TOLERABLE DAILY INTAKE ESTIMATE

Himematsutake Powder

The Chronic Toxicity Studies (6-month rat and mouse studies) determined a no adverse effe level (NOAEL) of 3000 mg/kg.

Also, studies of healthy volunteers were administered with the formulated product at levels o to 6x the recommended dosage (4500 mg/person/day - females and 9000 mg/person/day - ma

Consideration of both the chronic toxicity studies in laboratory animals and the lack of a toxic effect in healthy volunteers when administered Himematsutake Powder at 3 or 6x the recommended consumption, it would be appropriate to apply an uncertainty factor of 100 (a factor of 10 for inter-species differences and a factor of 10 for intra-species differences, ie., extrapolation of animal data to human data).

The tolerable daily intake would be 30 mg/kg/day.

With a 60 kg person the tolerable daily intake would be 1800 mg/person/day.

The recommended use is 1500 mg/person/day, thus, the recommended use is under the tolerable daily intake.

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4.B. I

CHRONIC TOXICITY STUDY OF CULTURED *AGARICUS BLAZEI* MURRILL (IWADE STRAIN 101) [JAPANEASE NAME ; HIMEMATSUTAKE] PREPARATION, "ABME" ADMINISTERED ORALLY IN RATS AND MICE FOR 26 WEEKS.

Hitoshi Ito, M.D., Ph.D. and Keishiro Shimura, M.D.*

Department of Pharmacology Mie University School of Medicine
*Institute of Laboratory Animals, Mie University School of Medicine
2-174, Edobashi, Tsu, Mie 514-0001, Japan

DEPARTMENT OF PHARMACOLOGY
MIE UNIVERSITY SCHOOL OF MEDICINE
EDOBASHI, TSU, MIE 514, JAPAN

Analysis of Experimental Material

Requested by Iwade Research Institute of Mycology, Japan, toxicity studies on ABME with mice and rats were performed. The composition of ABME (cultured *Agaricus blazei* Murrill Extracts) analyzed are as follows;

Material: Himematsutake extract
[ABME : Cultured *Agaricus blazei* Murrill(Iwade strain 101) Extracts]

Description: Himematsutake extract (ABME) is obtained as follows:
Cultured *Agaricus blazei* Murrill(Iwade strain 101) " Himematsutake " washed with distilled water, disintegrated in a mixer, and extracted with boiling water for 5 hours. The suspension was filtered to remove the insoluble material. After concentrating the aqueous extract under reduced pressure, and then spray drying it.

Analytical results

Chemical Specifications

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

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EDOBASHI, TSU, MIE 514, JAPAN

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

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

GLC: gas liquid chromatography

Polysaccharide Profile

β-Glucan	p/100g	7.5
α-Glucan	p/100g	2.2
β-Glucomannan	p/100g	8.4
β-Galactogulucan	p/100g	2.2
Ribonucleotide	p/100g	2.2
Protein bound・β-Glucan	p/100g	8.6
Xyloglucan	p/100g	1.1

^{13}C-NMRanalysis

Two-dimensional COSY analysis

Chronic Toxicity Study of Cultured *Agaricus blazei* Murrill (Iwade Strain 101) (Japanease name ; Himematsutake) Preparation, "ABME" Administered Orally in Rats for 26 Weeks.

Hitoshi Ito, M.D., Ph.D. and Keishiro Shimura, M.D.*

Department of Pharmacology Mie University School of Medicine
*Institute of Laboratory Animals, Mie University School of Medicine
2-174, Edobashi, Tsu, Mie 514-0001, Japan

Introduction

A chronic toxicity study of the edible mushroom, *Agaricus blazei* Murrill (Japanese name; Himematsutake) preparation, "ABME" - *Agaricus blazei* Murrill Extract, Japanese name: Himematsutake, was carried out with Sprague-Dawley / SLC (SD) rats. The ABME was administered orally for 26 weeks in doses of 0, 1000 and 3000 mg/kg/day.

Based on the series of animal experiments studied for the antitumor effect of ABME, the usual dose for human is estimated 25mg/kg. The chronic toxicity study on rats in this report includes 1000mg/kg - 40 times and 3000mg/kg - 120 times more dose compared to the usual dose for human.

With the limitation of the capacity of a rat's stomach and the physical condition of ABME in mind, over 3000mg/kg dose to a rat would be impossible.

ABME was provided by Iwade Research Institute of Mycology, Japan.

Chronic toxicity studies

Animals employed were SD strain rats (Japan SLC, Inc.) The animals were housed and fed in an animal room of the temperature of 23±2℃ and the humidity of 55±5%. Each animal was given solid diet (CLEA Japan CE-2) and water ad libitum.

One group of animals was used of 10 males and 10 females. Doses of administration were determined by the results of subacute toxicity studies, and two grades were adopted; 1000 and 3000 mg/kg/day (The maximum dose are able to the oral administration).

Test materials are easily soluble in water but high concentration used the state of suspensions. Their water solutions were, therefore, prepared as to be at a level of 1000 and 3000 mg/kg of rats body weight. They were compulsorily administered with a gastric catheter of teflon orally. After the test materials were administered, general symptoms of animals were observed for every day.

Results

(1) Behavior

In rat administered orally with 1000 and 3000 mg/kg for 26 weeks, any abnormal findings that seemed to be caused by the administration of the test material were not observed.

(2) Body Weight Changes (Table 1 and Table 2)

The animals were weighed weekly. No inhibition of body weight gain was found during the periods of the experiments among the test animals, both male and female.

(3) Amount of Diet Ingested (Table 3 and Table 4)

The amount of diet ingested weekly per head in every group is as shown Table 3 and Table 4. Food consumption was slightly increased in the early period (at 2nd and 3rd week) and intermediate period (at 8th and 9th week) of administration in male rats. No significant change was found in female rats, so it was not considered that the testing material caused the diet efficacy.

(4) Findings in Hematological Examinations (Table 5, 6, 7 and Table 8)

Hematological examination was performed on 5 cases of each group. No variation of significance was found in red blood cell count, hematocrit value, hemoglobin content, platelet value and white blood cell count. Differential leukocyte was found by fixing blood smear and staining by May-Grünwald Giemsa method. In differential leukocyte count, no abnormal findings were found due to the administration of the test material.

(5) Biochemical Examination of Blood (Table 9, 10, 11 and Table 12)

Biochemical examinations of blood were performed on 5 cases each of the groups, and results obtained are shown in Table (Table 9 - 12). Total cholesterol values in the male animals of the 3000 mg/kg group at 13th and 26th week and in the female animals of the 1000 mg/kg and 3000 mg/kg groups at 26th week were found with significant decrease. With regard to glucose, urea, total protein, albumin, alkaline phosphatase, GOT, GPT, Na and K content, however, no change was observed.

(6) Findings in Urine (Table 13 and Table 14)

Urine protein was assayed in the concentration of trace to 100mg/dl in most of the groups, regardless of the administered or the control. Inspecting urine volume, pH, specific gravity, urobilinogen, bilirubin, ketone body and glucose, no abnormal data was found in all groups.

(7) Findings at Autopsy Organ Weight (Table 15, 16, 17 and Table 18)

A slight increasing tendency was observed in the liver of male groups with administered 3000mg/kg/day at 13 weeks and 26 weeks, and in the female group with administered 3000 mg/kg/day at 13 weeks. However, no remarkable change was found between the control group and the treated groups in either absolute organ weight or comparative organ weight. The changes found were not considered to be caused by the test material.

(8) Histopathological Findings (PHOTO 1—PHOTO 13)

After autopsy and gross observation of changes, the organs were fixed with 10% formalin, embedded in paraffin and cut in slices ca. 6 μ thick, then stained with hematoxylin and eosine. Bone marrows were decalcified by dipping them in 5% nitric acid (10% formalin) for 48 hours.

Microscopic examinations were performed on 5 samples each of the groups at the end of 13 weeks and 26weeks after the administration. Histopathological examination was conducted by Sensake Naruse, M.D., at Department of Pathology, Mie University School of Medicine, Tsu, Mie, 514-0001, Japan.

Lungs : Tuberous infiltrations of cells composed mainly of lymphocytes were seen around the blood vessels in almost all cases including those of the control group.

Liver : Almost no difference between the control and the administered groups; a slight degeneration of liver and enlargement at sinus were observed in 1 case of the control group.

Kidneys : Congestions of glomeruli and slight degenerations of the epithelium of tubules were observed both in the control and the administered groups.

Spleen : A slight hemosiderosis was observed in almost all cases including those of the control group.

* No remarkable changes were observed in brain, heart, testes, ovaries, thymus, pituitary, thyroids, adrenals, pancreas, digestive tracts and bone marrow.

Summary

A chronic toxicity of edible mushroom, *Agaricus blazei* Murrill (Japanese name: Himematsutake) preparation, "ABME" was studied with SD rats.

ABME was administered orally for 26 weeks in dose of 0 (control), 1000 and 3000 mg/kg/day. During the period of oral administration for 26 weeks, no general symptoms to be marked were observed in SD rats, and there was no death throughout the whole period.

With regard to the amount of diet ingested, no significant change was found in all the administered group.

No inhibition of body weight gain was found during the periods of the experiments

among the test animals, both male and female.

In hematological findings, any significant variation in red blood cell count, hematocrit value, hemoglobin content, platelet value and white blood cell count was not found. In differential count, too, no abnormal findings due to the administration of the test material was found.

In biochemical examination of blood, no change was found in glucose, urea, total protein, albumin, alkaline phosphatase, GOT, GPT, Na and K content. However, the significant decrease in total cholesterol values was observed in the male and female of 3000 mg/kg administered groups after 26 weeks.

No abnormality was found in the urine volume, pH, specific gravity, urobilinogen, bilirubin, ketone body, protein and glucose in the control and the administered groups.

In assaying organ weight, a slight tendency of increase of liver was observed in the male and female groups of 3000 mg/kg/day administered group. However, the effect was found to be very slight. With regard to the other organ weight, no change was found in either the administered group of male or female rats compared with the control group.

In the histopathogical examinations, any abnormal figures specific to the administered group compared with the control group was not observed in rats. Furthermore, any toxicity to be caused by ABME could not be found.

Therefore, the safety dose for rats was estimated to be over 3000 mg/kg/day, but the sure intoxication dose could not be determined.

End of report

Table 1 , Body weight changes in male rats given ABME orally for 26 weeks

Dosing periods (weeks)	Dose level (mg/kg/day)			
	Male (g)			
	Number of rats	Control	1000	3000
0	15	225	217	210
1	15	268	270	265
2	15	331	328	319
3	15	357	366	358
4	15	401	394	396
5	15	419	415	417
6	15	433	450	448
7	15	451	461	453
8	15	476	472	469
9	15	501	516	507
10	15	519	523	520
11	15	525	530	528
12	15	537	541	536
13	15	542	549	540
14	10	546	552	547
15	10	554	561	557
16	10	561	567	562
17	10	567	569	567
18	10	574	578	575
19	10	587	590	583
20	10	600	607	603
21	10	619	617	615
22	10	623	621	619
23	10	629	627	620
24	10	630	632	626
25	10	632	636	630
26	10	636	642	638

Table 2 Body weight changes in female rats given ABME orally for 26 weeks

Dosing periods (weeks)	Dose level (mg/kg/day)			
	Female (g)			
	Number of rats	Control	1000	3000
0	15	164	167	170
1	15	187	189	193
2	15	198	199	204
3	15	213	226	227
4	15	229	238	238
5	15	241	250	249
6	15	263	261	258
7	15	266	267	264
8	15	275	279	278
9	15	281	285	284
10	15	289	290	287
11	15	295	294	293
12	15	298	297	299
13	15	302	304	301
14	10	303	306	304
15	10	305	307	305
16	10	307	312	308
17	10	309	314	311
18	10	315	316	312
19	10	320	319	318
20	10	321	323	320
21	10	323	325	321
22	10	323	326	324
23	10	326	328	324
24	10	326	329	326
25	10	329	332	330
26	10	331	336	334

Table 3 Food consumption of male rats given ABME orally for 26 weeks

Dosing periods (weeks)	Dose level (mg/kg/day)			
	Male (g)			
	Number of rats	Control	1000	3000
1	15	26.6±0.5	28.1±0.6	27.8±0.6
2	15	27.9±0.4	29.9±0.5*	29.5±0.6*
3	15	28.4±0.6	31.0±0.8*	30.8±0.7*
4	15	29.9±0.5	31.7±0.7	31.2±0.8
5	15	30.6±0.5	31.4±0.5	31.4±0.9
6	15	30.4±0.5	30.4±0.6	31.3±1.1
7	15	29.5±0.7	30.1±0.7	31.3±1.0
8	15	30.6±1.0	33.2±0.8*	33.0±1.0*
9	15	30.3±0.5	32.0±0.5*	33.1±0.7*
10	15	30.2±0.5	32.1±0.9	31.9±0.8
11	15	30.7±0.4	31.7±0.5	31.5±0.6
12	15	30.5±0.5	33.0±0.5*	31.8±0.9
13	15	30.7±0.5	31.8±0.9	31.1±0.8
14	10	30.8±0.6	31.5±0.8	31.8±0.9
15	10	31.7±1.0	31.0±0.7	31.1±1.0
16	10	33.3±1.1	31.7±0.7	31.9±0.9
17	10	32.1±1.0	31.8±0.6	31.7±0.8
18	10	32.0±1.1	31.5±0.6	31.6±0.9
19	10	31.1±1.3	31.5±0.7	31.7±0.8
20	10	31.5±1.0	31.7±0.7	30.9±1.0
21	10	32.0±1.1	31.1±0.8	31.7±1.1
22	10	31.6±1.0	31.9±0.7	30.6±1.0
23	10	30.8±1.1	30.5±0.6	30.4±0.8
24	10	31.3±0.9	30.9±0.6	30.0±0.9
25	10	31.0±0.9	31.5±0.7	30.8±0.8
26	10	31.0±0.8	32.9±1.3	31.6±0.9

Values represent mean ± standard error (g/day/rat)
* Significantly different from control at $p < 0.05$

Table 4 Food consumption of female rats given ABME orally for 26 weeks

Dosing periods (weeks)	Dose level (mg/kg/day)			
	Female (g)			
	Number of rats	Control	1000	3000
1	15	20.5±0.5	19.2±0.5	19.6±0.5
2	15	19.6±0.4	20.1±0.4	20.8±0.5
3	15	19.5±0.5	20.2±0.6	20.9±0.4
4	15	21.2±0.6	20.1±0.7	21.2±0.5
5	15	21.0±0.4	20.2±0.5	21.2±0.6
6	15	21.2±0.5	20.2±0.5	21.0±0.5
7	15	21.0±0.7	20.3±0.4	21.5±0.6
8	15	22.8±0.6	21.7±0.7	22.5±0.5
9	15	22.3±0.4	21.2±0.5	22.6±0.5
10	15	22.5±0.4	21.4±0.8	22.1±0.4
11	15	22.0±0.5	20.7±0.5	19.9±0.9
12	15	22.6±0.5	21.5±0.8	22.3±0.5
13	15	21.8±0.6	21.6±0.3	21.5±0.5
14	10	22.0±0.6	21.2±0.5	21.8±0.4
15	10	22.4±0.6	22.2±0.5	22.4±0.5
16	10	23.8±1.0	22.9±0.9	23.6±0.8
17	10	20.6±0.7	21.2±0.6	21.3±0.6
18	10	20.9±0.6	21.8±0.7	22.0±1.1
19	10	20.2±0.7	21.9±0.9	21.7±0.7
20	10	20.6±0.6	21.0±0.8	21.2±0.6
21	10	20.9±0.5	21.6±0.6	21.4±0.5
22	10	20.5±0.6	20.6±0.7	20.7±0.5
23	10	20.9±0.6	21.2±0.8	20.7±0.7
24	10	21.4±0.8	21.8±0.7	21.1±0.7
25	10	20.5±0.5	21.1±0.7	20.9±0.5
26	10	20.9±0.9	21.0±0.7	20.4±0.7

Values represent mean ± standard error (g/day/rat)
* Significantly different from control at $p < 0.05$

Table 5 Hematological findings in male rats given ABME orally for 13 weeks

Male											
Dose level (mg/kg/day)	Number of rats	RBC ($\times 10^4/mm^3$)	Ht (%)	Hb (g/dl)	BP ($\times 10^4/mm^3$)	WBC ($\times 10^2/mm^3$)	Differential count (%) [a]				
							L	M	N	E	B
Control	5	857±40.3	44±0.9	15.2±0.3	107±9.3	91±15.2	80.6	4.1	14.7	0.6	0
							(71-89)	(2-5)	(9-23)	(0-1)	
1000	5	861±37.2	45±1.3	14.9±0.4	111±8.4	119±25.3	81.0	3.4	14.9	0.7	0
							(70-89)	(2-6)	(8-26)	(0-2)	
3000	5	860±42.1	46±1.7	15.1±0.2	109±8.1	107±21.1	79.4	3.7	15.9	1.0	0
							(72-84)	(1-5)	(9-30)	(0-2)	

Values represent mean ± standard error [a] Ranges are given parentheses.
RBC (Red blood cell) : TOA Microcell Counter CC-108
Ht (Hematocrit) : Microhematocrit method
Hb (Hemoglobin) : TOA Hemoglobin Counter Hb-100
BP (Blood platelet) : TOA Platelet Counter PL-100
WBC (White blood cell) : TOA Microcell Counter CC-108
L (Lymphocyte), M (Monocyte), N (Neutrophil), E (Eosinocyte) and B (Basocyte) :
Leucocyte ratio (May-Grunwald Giemsa stained method)

Table 6 Hematological findings in female rats given ABME orally for 13 weeks

Female											
Dose level (mg/kg/day)	Number of rats	RBC ($\times 10^4/mm^3$)	Ht (%)	Hb (g/dl)	BP ($\times 10^4/mm^3$)	WBC ($\times 10^2/mm^3$)	Differential count (%) [a]				
							L	M	N	E	B
Control	5	752±30.5	39±1.2	14.2±0.4	94±5.7	73±11.4	81.4	2.7	14.5	1.4	0
							(73-88)	(1-5)	(8-24)	(1-3)	
1000	5	750±29.3	41±1.4	14.8±0.6	97±8.1	85±10.0	82.3	2.6	14.1	1.0	0
							(70-87)	(1-4)	(9-22)	(1-2)	
3000	5	769±26.0	40±1.2	14.5±0.6	92±9.8	91±9.4	82.0	2.8	14.0	1.2	0
							(74-89)	(1-5)	(8-21)	(0-3)	

Values represent mean ± standard error [a] Ranges are given parentheses.
RBC (Red blood cell) : TOA Microcell Counter CC-108
Ht (Hematocrit) : Microhematocrit method
Hb (Hemoglobin) : TOA Hemoglobin Counter Hb-100
BP (Blood platelet) : TOA Platelet Counter PL-100
WBC (White blood cell) : TOA Microcell Counter CC-108
L (Lymphocyte), M (Monocyte), N (Neutrophil), E (Eosinocyte) and B (Basocyte) :
Leucocyte ratio (May-Grunwald Giemsa stained method)

Table 7 Hematological findings in male rats given ABME orally for 26 weeks

Male											
Dose level (mg/kg/day)	Number of rats	RBC ($\times 10^4/mm^3$)	Ht (%)	Hb (g/dl)	BP ($\times 10^4/mm^3$)	WBC ($\times 10^2/mm^3$)	Differential count (%) [a]				
							L	M	N	E	B
Control	10	865±43.1	43±1.0	15.0±0.3	106±9.6	89±11.2	77.9	2.8	17.0	2.3	0
							(62-89)	(0-4)	(6-33)	(1-3)	
1000	10	868±39.7	44±0.9	15.6±0.2	107±8.5	97±13.1	78.8	2.8	15.9	2.5	0
							(64-87)	(1-5)	(7-32)	(1-4)	
3000	10	870±44.2	43±1.2	15.5±0.4	104±9.0	101±15.8	73.9	2.6	21.0	2.5	0
							(60-84)	(0-5)	(8-37)	(1-3)	

Values represent mean ± standard error [a] Ranges are given parentheses.
RBC (Red blood cell) : TOA Microcell Counter CC-108
Ht (Hematocrit) : Microhematocrit method
Hb (Hemoglobin) : TOA Hemoglobin Counter Hb-100
BP (Blood platelet) : TOA Platelet Counter PL-100
WBC (White blood cell) : TOA Microcell Counter CC-108
L (Lymphocyte), M (Monocyte), N (Neutrophil), E (Eosinocyte) and B (Basocyte) :
Leucocyte ratio (May-Grunwald Giemsa stained method)

Table 8 Hematological findings in female rats given ABME orally for 26 weeks

Female											
Dose level (mg/kg/day)	Number of rats	RBC ($\times 10^4/mm^3$)	Ht (%)	Hb (g/dl)	BP ($\times 10^4/mm^3$)	WBC ($\times 10^2/mm^3$)	Differential count (%) [a]				
							L	M	N	E	B
Control	10	769±40.1	41±0.7	15.2±0.3	96±9.9	75±11.0	71.8	1.9	24.9	1.4	0
							(60-83)	(0-4)	(14-39)	(0-2)	
1000	10	771±39.3	44±1.3	14.9±0.2	101±9.1	79±9.5	70.8	2.0	25.2	2.0	0
							(59-81)	(1-4)	(13-41)	(0-3)	
3000	10	760±36.0	43±1.5	15.3±0.4	103±8.7	83±12.1	72.3	2	23.8	1.9	0
							(61-85)	(0-5)	(11-35)	(0-4)	

Values represent mean ± standard error [a] Ranges are given parentheses.
RBC (Red blood cell) : TOA Microcell Counter CC-108
Ht (Hematocrit) : Microhematocrit method
Hb (Hemoglobin) : TOA Hemoglobin Counter Hb-100
BP (Blood platelet) : TOA Platelet Counter PL-100
WBC (White blood cell) : TOA Microcell Counter CC-108
L (Lymphocyte), M (Monocyte), N (Neutrophil), E (Eosinocyte) and B (Basocyte) :
Leucocyte ratio (May-Grunwald Giemsa stained method)

Table 9 Biochemical findings in male rats given ABME orally for 13 weeks

Male											
Dose level (mg/kg/day)	Number of rats	Glucose (mg/dl)	Urea nitrogen (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Alkaline phoshatase (IU/l)	GOT (IU/l)	GPT (IU/l)	Total cholesterol (mg/dl)	Na (mEq/l)	K (mEq/l)
Control	5	192±36	18±2	7.6±0.2	2.6±0.2	262±46.3	61±6.4	30±4.9	80±8.9	141±2	-
1000	5	184±21	19±2	7.9±0.4	2.7±0.1	259±43.9	60±6.0	29±3.7	72±7.3	143±1	-
3000	5	180±22	20±3	8.0±0.4	2.9±0.2	244±52.0	62±8.6	31±5.2	62±5.7*	144±2	-

Values represent mean ± standard error
* Significantly different from control at $p < 0.05$
Glucose : GLK/G6PDH method
Urea nitrogen : Urease/GLDH method
Albumin : BCG method
Alkaline phosphatase : King-Armstrong method
GOT : NADH-UV (IFCC method)
GPT : NADH-UV (IFCC method)
Total cholesterol : CE/CO/POD method
Na and K : Flame reaction

Table 10 Biochemical findings in female rats given ABME orally for 13 weeks

Female											
Dose level (mg/kg/day)	Number of rats	Glucose (mg/dl)	Urea nitrogen (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Alkaline phoshatase (IU/l)	GOT (IU/l)	GPT (IU/l)	Total cholesterol (mg/dl)	Na (mEq/l)	K (mEq/l)
Control	5	142±19	20±1	7.9±0.2	3.0±0.5	181±32.6	59±7.8	27±5.5	64±7.2	-	5.4±0.4
1000	5	128±17	19±1	8.1±0.4	3.2±0.3	201±39.3	63±9.3	26±6.1	58±5.8	-	5.9±0.7
3000	5	123±10	18±1	7.8±0.3	2.9±0.2	190±40.2	67±6.5	24±6.8	59±3.2	-	5.2±0.6

Values represent mean ± standard error
* Significantly different from control at $p < 0.05$
Glucose : GLK/G6PDH method
Urea nitrogen : Urease/GLDH method
Albumin : BCG method
Alkaline phosphatase : King-Armstrong method
GOT : NADH-UV (IFCC method)
GPT : NADH-UV (IFCC method)
Total cholesterol : CE/CO/POD method
Na and K : Flame reaction

Table 11 Biochemical findings in male rats given ABME orally for 26 weeks

Male											
Dose level (mg/kg/day)	Number of rats	Glucose (mg/dl)	Urea nitrogen (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Alkaline phoshatase (IU/l)	GOT (IU/l)	GPT (IU/l)	Total cholesterol (mg/dl)	Na (mEq/l)	K (mEq/l)
Control	10	170±12	19±3	7.5±0.2	2.7±0.1	293±46.6	69±5.6	46±3.1	92±8.0	145±2	5.1±0.5
1000	10	165±27	18±2	8.0±0.3	2.5±0.2	263±40.3	72±7.3	45±3.0	84±8.1	143±2	4.9±0.4
3000	10	159±16	19±1	7.7±0.2	2.7±0.2	287±24.9	66±9.1	46±6.2	76±5.3*	142±1	4.7±0.5

Values represent mean ± standard error
* Significantly different from control at $p < 0.05$
Glucose : GLK/G6PDH method
Urea nitrogen : Urease/GLDH method
Albumin : BCG method
Alkaline phosphatase : King-Armstrong method
GOT : NADH-UV (IFCC method)
GPT : NADH-UV (IFCC method)
Total cholesterol : CE/CO/POD method
Na and K : Flame reaction

Table 12 Biochemical findings in female rats given ABME orally for 26 weeks

Female											
Dose level (mg/kg/day)	Number of rats	Glucose (mg/dl)	Urea nitrogen (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Alkaline phoshatase (IU/l)	GOT (IU/l)	GPT (IU/l)	Total cholesterol (mg/dl)	Na (mEq/l)	K (mEq/l)
Control	10	132±21	20±3	8.2±0.3	3.1±0.4	169±38.5	86±10.1	49±6.2	82±9.1	143±2	4.9±0.5
1000	10	125±13	19±1	8.7±0.3	3.6±0.3	227±29.6	92±9.8	47±7.0	64±5.3*	142±1	5.0±0.4
3000	10	133±9	19±0	7.9±0.2	3.6±0.2	230±32.7	84±5.6	43±7.4	63±6.0*	143±0	4.8±0.3

Values represent mean ± standard error
* Significantly different from control at $p < 0.05$
Glucose : GLK/G6PDH method
Urea nitrogen : Urease/GLDH method
Albumin : BCG method
Alkaline phosphatase : King-Armstrong method
GOT : NADH-UV (IFCC method)
GPT : NADH-UV (IFCC method)
Total cholesterol : CE/CO/POD method
Na and K : Flame reaction

Table 13 Urinalysis of male rats given ABME orally for 26 weeks

Male											
Dosing period (week)	Dose level (mg/kg/day)	Number of rats	Appearance	Volume (ml)	pH	Specific gravity	Urobilinogen (Ehrlich unit/dl)	Bilirubin	Ketone body	Protein	Glucose
13	Control	10	Normal	12.9±1.4	7.2 (6.8-7.8)[a)]	1.048 (1.004-1.065)	0.1-1	−	−	±~+	−
	1000	10	Normal	11.6±1.0	7.0 (6.7-7.5)	1.063 (1.051-1.074)	0.1-1	−	−	±~+	−
	3000	10	Normal	13.2±1.2	7.4 (6.6-7.7)	1.044 (1.029-1.053)	0.1-1	−	−	±~+	−
26	Control	10	Normal	13.0±1.0	6.7 (6.3-7.0)	1.056 (1.053-1.073)	0.1-1	−	−	±~+	−
	1000	10	Normal	14.1±1.9	6.6 (6.2-7.1)	1.050 (1.039-1.076)	0.1-1	−	−	±~+	−
	3000	10	Normal	12.9±0.9	6.7 (6.1-7.2)	1.051 (1.039-1.060)	0.1-1	−	−	±~+	−

[a)] Ranges are given parentheses.
pH : pH meter,
Specific grarity : Weight determination,
Urobilinogen, Bilirubin, Ketone body,Protein and Glucose : Uro-Labstix (Ames reagent strips for urinalysis)

Table 14 Urinalysis of female rats given ABME orally for 26 weeks

Female											
Dosing period (week)	Dose level (mg/kg/day)	Number of rats	Appearance	Volume (ml)	pH	Specific gravity	Urobilinogen (Ehrlich unit/dl)	Bilirubin	Ketone body	Protein	Glucose
13	Control	10	Normal	10.7±1.5	7.1 (6.9-8.2)[a]	1.044 (1.023-1.063)	0.1-1	−	−	±～+	−
	1000	10	Normal	10.4±1.3	7.0 (6.5-8.7)	1.047 (1.032-1.060)	0.1	−	−	±～+	−
	3000	10	Normal	9.8±1.0	7.4 (6.9-9.0)	1.048 (1.037-1.065)	0.1-1	−	−	±～+	−
26	Control	10	Normal	12.3±2.0	7.0 (6.3-7.1)	1.048 (1.035-1.067)	0.1	−	−	−～+	−
	1000	10	Normal	13.0±1.7	7.3 (6.9-7.7)	1.050 (1.040-1.071)	0.1	−	−	−～+	−
	3000	10	Normal	13.4±1.9	7.1 (6.2-7.6)	1.046 (1.029-1.063)	0.1	−	−	−～+	−

[a] Ranges are given parentheses.
pH : pH meter,
Specific grarity : Weight determination,
Urobilinogen, Bilirubin, Ketone body,Protein and Glucose : Uro-Labstix (Ames reagent strips for urinalysis)

Table 15 Organ weights in male rats given ABME orally for 13 weeks

Male													
Dose level (mg/kg/day)	Number of rats	Final body wt. (g)	Brain (g)	Heart (g)	Lung (g)	Liver (g)	Kidneys (g)	Spleen (g)	Testes (g)	Thymus (g)	Pituitary (mg)	Thyroids (mg)	Adrenals (mg)
Control	5	542±30	1.99±0.02	1.43±0.07	1.83±0.05	14.80±1.21	3.59±0.20	0.78±0.02	3.52±0.10	0.35±0.03	15±1	27±2	62±2
			(0.37±0.01)	(0.26±0.02)	(0.34±0.01)	(2.73±0.06)	(0.66±0.01)	(0.14±0.01)	(0.65±0.04)	(0.065±0.004)	(2.8±0.3)	(4.9±0.7)	(11±1)
1000	5	549±27	1.94±0.04	1.41±0.09	1.85±0.06	15.10±1.07	3.73±0.15	0.80±0.04	3.26±0.19	0.45±0.08	16±2	28±1	64±3
			(0.35±0.03)	(0.26±0.02)	(0.34±0.02)	(2.75±0.07)	(0.68±0.03)	(0.15±0.01)	(0.59±0.06)	(0.082±0.012)	(2.9±0.4)	(5.1±0.3)	(12±1)
3000	5	540±35	2.12±0.04	1.43±0.08	1.86±0.12	15.91±0.79	3.62±0.09	0.82±0.05	3.50±0.09	0.41±0.06	15±2	28±1	61±3
			(0.39±0.02)	(0.26±0.01)	(0.34±0.02)	(2.95±0.06)	(0.67±0.02)	(0.15±0.01)	(0.65±0.04)	(0.076±0.011)	(2.8±0.5)	(5.2±0.2)	(11±1)

Values represent mean ± standard error.
Values in parentheses represent organ weights in grams or milligrames per 100g body weight.

Table 16 Organ weights in female rats given ABME orally for 13 weeks

Female													
Dose level (mg/kg/day)	Number of rats	Final body wt. (g)	Brain (g)	Heart (g)	Lung (g)	Liver (g)	Kidneys (g)	Spleen (g)	Ovaries (mg)	Thymus (g)	Pituitary (mg)	Thyroids (mg)	Adrenals (mg)
Control	5	302±18	1.91±0.03	0.87±0.03	1.37±0.06	8.25±0.40	2.04±0.06	0.52±0.03	72±4	0.31±0.03	16±1	22±2	72±2
			(0.63±0.03)	(0.29±0.01)	(0.45±0.02)	(2.73±0.08)	(0.68±0.02)	(0.17±0.01)	(24±2)	(0.102±0.009)	(5.3±0.3)	(7.3±0.6)	(24±1)
1000	5	304±19	1.88±0.03	0.85±0.04	1.38±0.09	8.40±0.51	1.97±0.06	0.50±0.04	73±4	0.32±0.04	16±2	23±1	69±2
			(0.62±0.04)	(0.28±0.01)	(0.45±0.03)	(2.76±0.07)	(0.65±0.02)	(0.16±0.01)	(24±2)	(0.105±0.015)	(5.3±0.3)	(7.6±0.4)	(23±1)
3000	5	301±16	1.89±0.04	0.90±0.05	1.30±0.07	8.67±0.43	2.01±0.09	0.53±0.04	70±6	0.33±0.02	18±3	23±2	71±3
			(0.63±0.02)	(0.30±0.02)	(0.43±0.04)	(2.88±0.07)	(0.67±0.03)	(0.18±0.01)	(23±3)	(0.110±0.010)	(6.0±0.4)	(7.6±0.4)	(24±1)

Values represent mean ± standard error.
Values in parentheses represent organ weights in grams or milligrames per 100g body weight.

Table 17 Organ weights in male rats given ABME orally for 26 weeks

Male													
Dose level (mg/kg/day)	Number of rats	Final body wt. (g)	Brain (g)	Heart (g)	Lung (g)	Liver (g)	Kidneys (g)	Spleen (g)	Testes (g)	Thymus (g)	Pituitary (mg)	Thyroids (mg)	Adrenals (mg)
Control	10	636±24	2.09±0.04	1.52±0.06	2.16±0.05	16.85±0.90	3.69±0.11	0.93±0.05	3.55±0.26	0.27±0.03	17±1	29±1	60±2
			(0.33±0.01)	(0.24±0.01)	(0.34±0.01)	(2.65±0.07)	(0.58±0.01)	(0.15±0.01)	(0.56±0.03)	(0.043±0.004)	(2.6±0.2)	(4.6±0.2)	(9±0)
1000	10	642±20	2.05±0.03	1.60±0.07	2.25±0.10	17.79±1.11	3.78±0.15	1.05±0.04	3.34±0.15	0.25±0.03	16±0	28±1	61±2
			(0.32±0.01)	(0.25±0.02)	(0.35±0.02)	(2.77±0.07)	(0.59±0.03)	(0.16±0.01)	(0.52±0.03)	(0.039±0.002)	(2.5±0.1)	(4.4±0.2)	(10±1)
3000	10	638±19	2.16±0.04	1.65±0.05	2.25±0.09	18.29±0.81	3.85±0.16	1.00±0.05	3.62±0.21	0.28±0.03	17±1	30±1	60±3
			(0.34±0.01)	(0.26±0.02)	(0.35±0.02)	(2.87±0.08)	(0.60±0.02)	(0.16±0.02)	(0.57±0.03)	(0.044±0.003)	(2.7±0.1)	(4.6±0.2)	(9±0)

Values represent mean ± standard error.
Values in parentheses represent organ weights in grams or milligrames per 100g body weight.

Table 18 Organ weights in female rats given ABME orally for 26 weeks

Female													
Dose level (mg/kg/day)	Number of rats	Final body wt. (g)	Brain (g)	Heart (g)	Lung (g)	Liver (g)	Kidneys (g)	Spleen (g)	Ovaries (mg)	Thymus (g)	Pituitary (mg)	Thyroids (mg)	Adrenals (mg)
Control	10	331±12	1.88±0.03	0.97±0.03	1.53±0.04	8.49±0.30	2.10±0.07	0.57±0.03	57±6	0.17±0.01	24±1	25±1	79±4
			(0.57±0.01)	(0.29±0.01)	(0.46±0.01)	(2.56±0.03)	(0.63±0.02)	(0.17±0.01)	(17±3)	(0.053±0.003)	(7.3±0.5)	(7.6±0.5)	(23±1)
1000	10	336±16	1.86±0.03	1.04±0.05	1.58±0.08	8.91±0.39	2.14±0.06	0.61±0.02	58±5	0.18±0.02	25±2	25±1	82±5
			(0.55±0.02)	(0.31±0.01)	(0.47±0.01)	(2.65±0.07)	(0.64±0.02)	(0.18±0.01)	(17±1)	(0.054±0.004)	(7.4±0.3)	(7.4±0.6)	(24±2)
3000	10	334±13	1.87±0.06	0.95±0.03	1.51±0.07	8.35±0.50	1.99±0.05	0.60±0.03	56±6	0.17±0.02	23±1	25±1	80±3
			(0.56±0.03)	(0.28±0.01)	(0.45±0.02)	(2.50±0.06)	(0.60±0.02)	(0.18±0.01)	(17±1)	(0.051±0.008)	(6.9±0.4)	(7.5±0.3)	(24±1)

Values represent mean ± standard error.
Values in parentheses represent organ weights in grams or milligrames per 100g body weight.

PHOTO 1 **Lungs**: 3000mg/kg/day×26 weeks, male, ×100,
No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

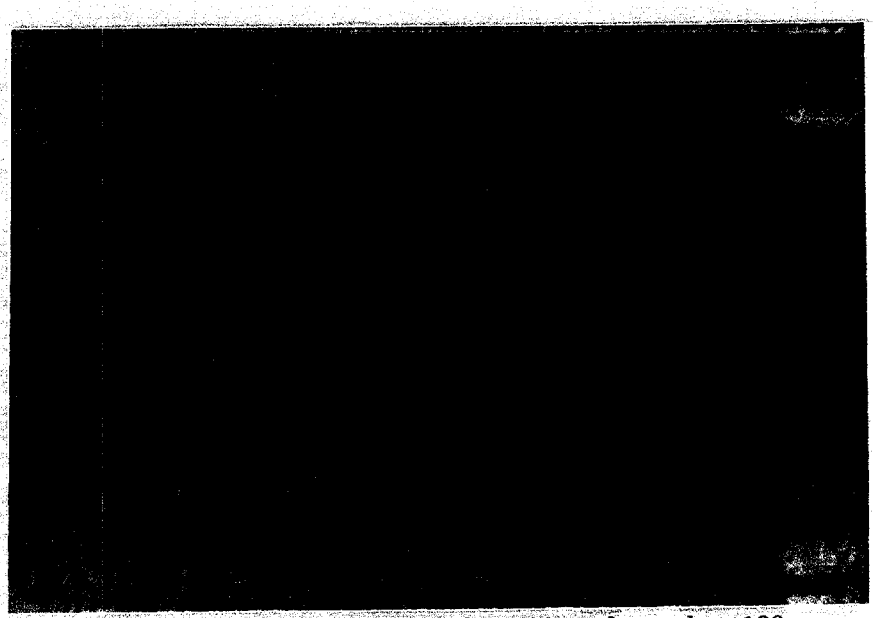


PHOTO 2 **Spleen**: 3000mg/kg/day×26 weeks, male, ×100
No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

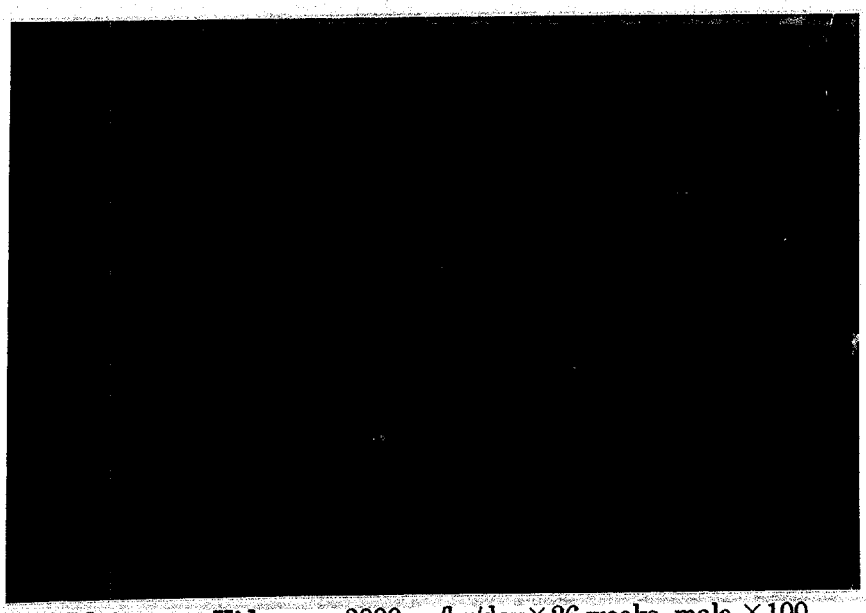


PHOTO 3 **Kidneys**: 3000mg/kg/day×26 weeks, male, ×100,
No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

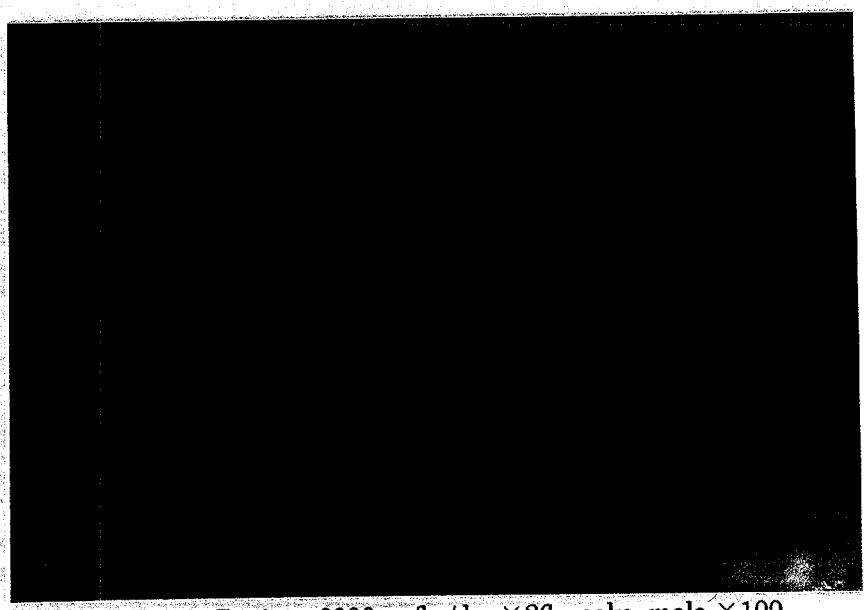


PHOTO 4 **Brain**: 3000mg/kg/day×26 weeks, male, ×100.
No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

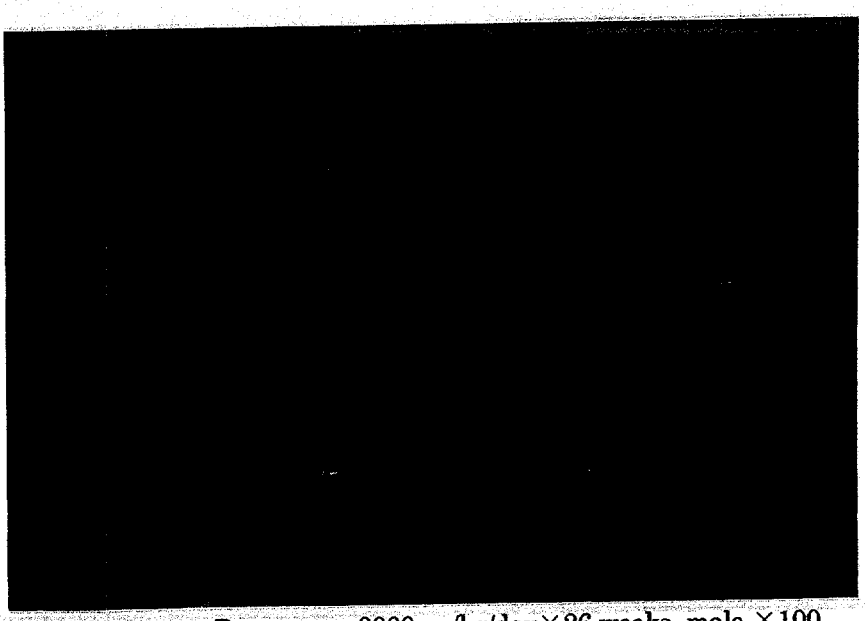


PHOTO 5 **Pancreas:** 3000mg/kg/day×26 weeks, male, ×100, No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

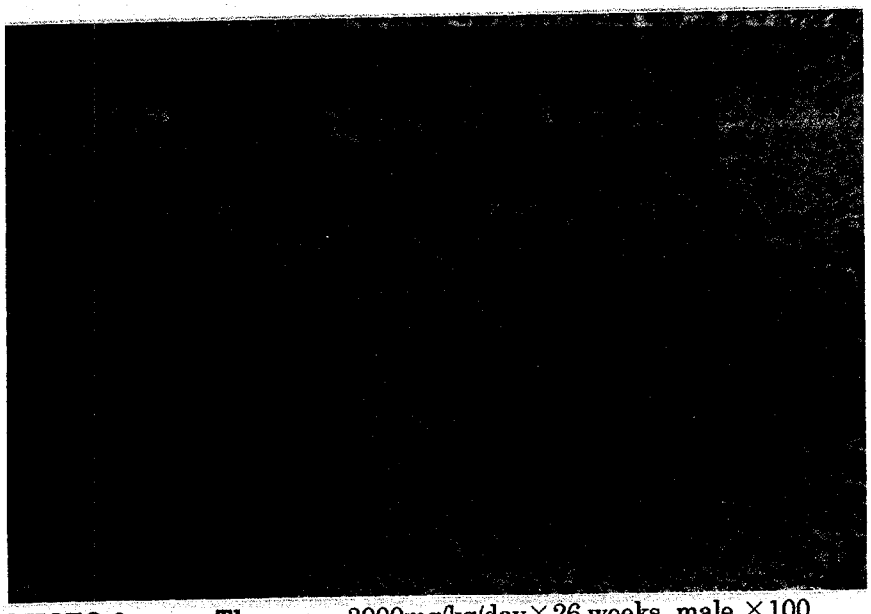


PHOTO 6 **Thymus:** 3000mg/kg/day×26 weeks, male, ×100, No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

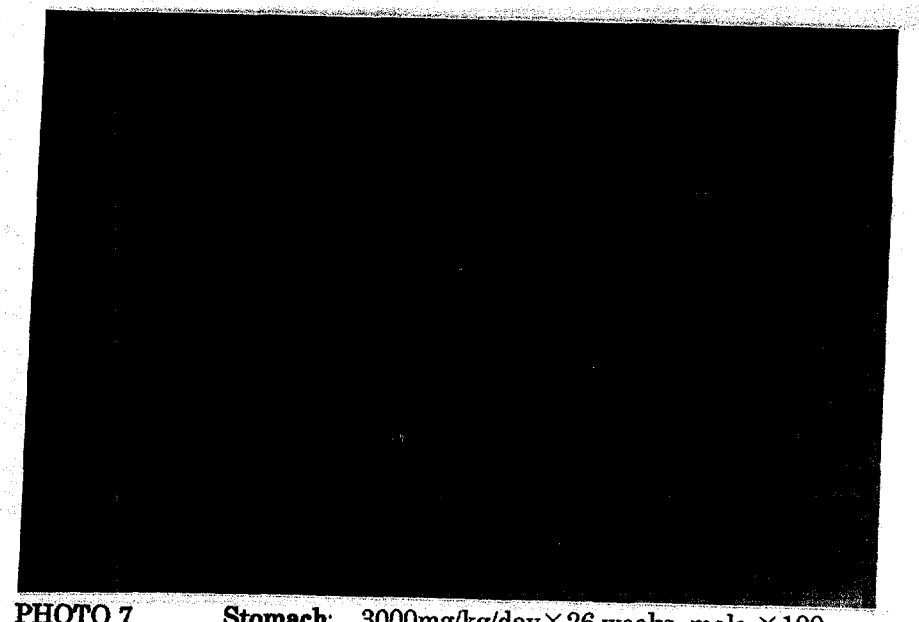


PHOTO 7 **Stomach**: 3000mg/kg/day×26 weeks, male, ×100, No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

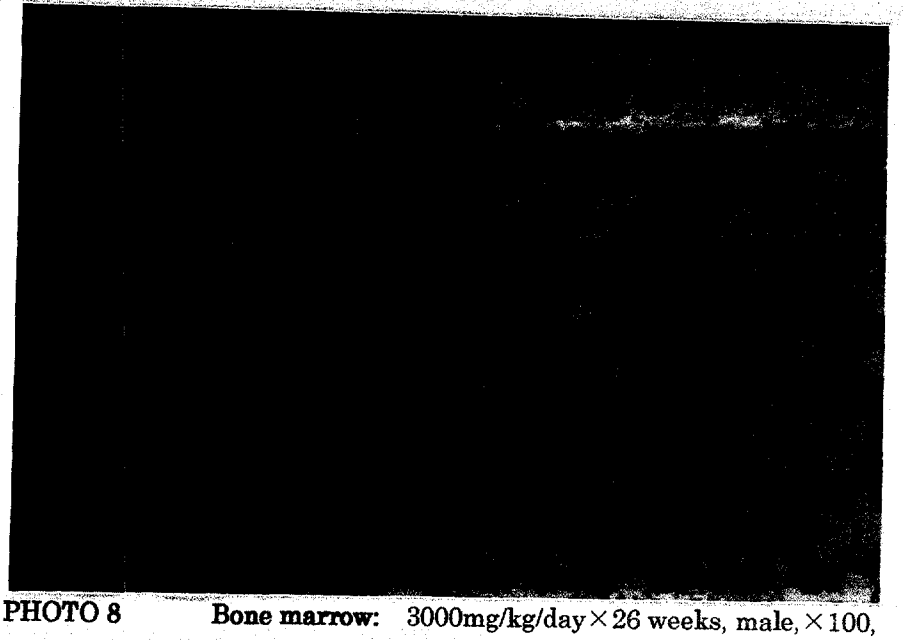


PHOTO 8 **Bone marrow:** 3000mg/kg/day×26 weeks, male, ×100, No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

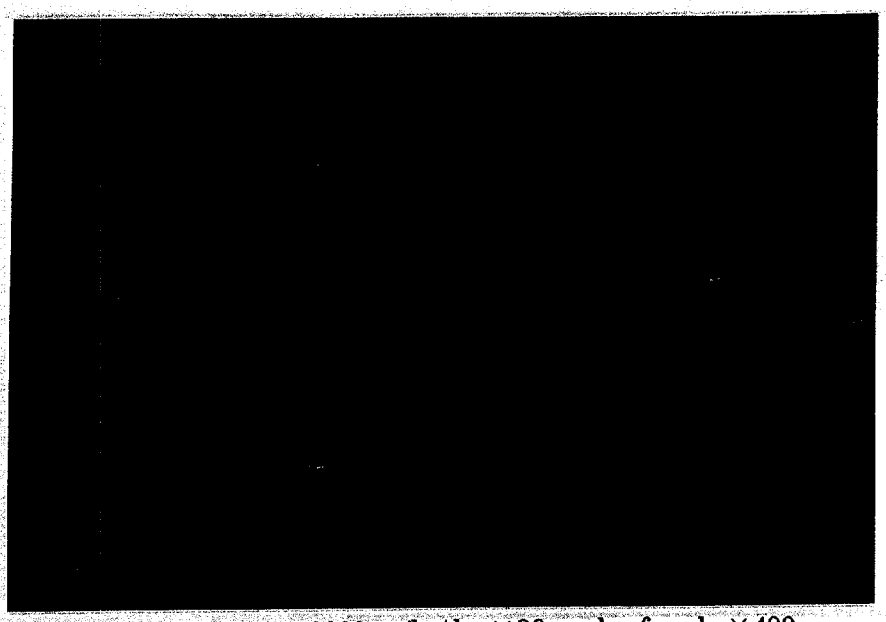


PHOTO 9 **Liver:** 3000mg/kg/day×26 weeks, female, ×400, No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

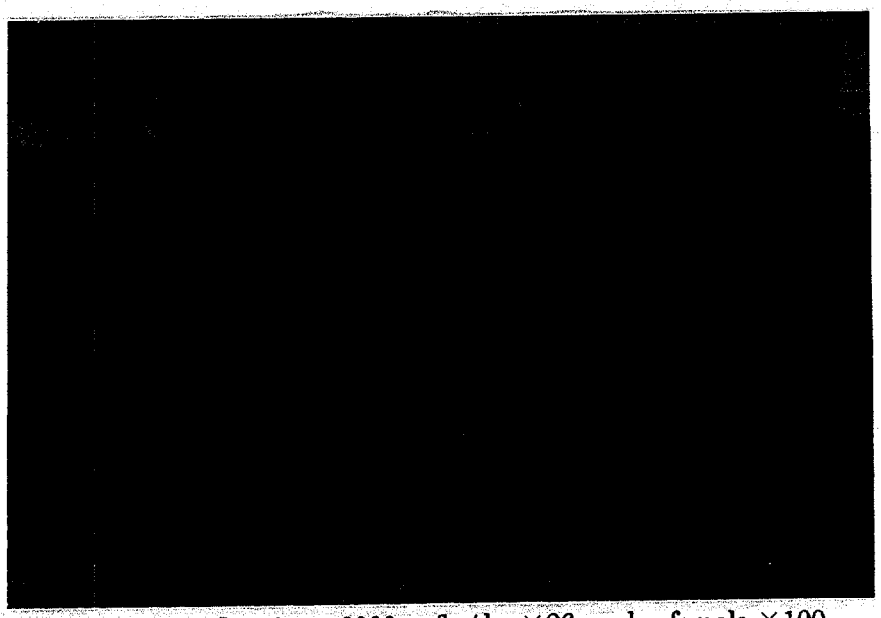


PHOTO 10 **Ovaries:** 3000mg/kg/day×26 weeks, female, ×100, No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

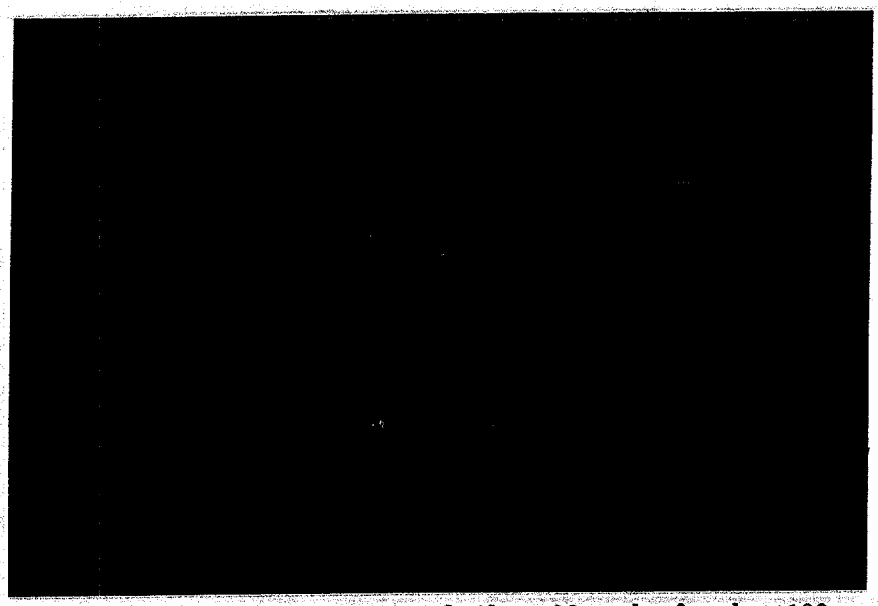


PHOTO 11 **Thyroid**: 3000mg/kg/day×26 weeks, female, ×100, No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

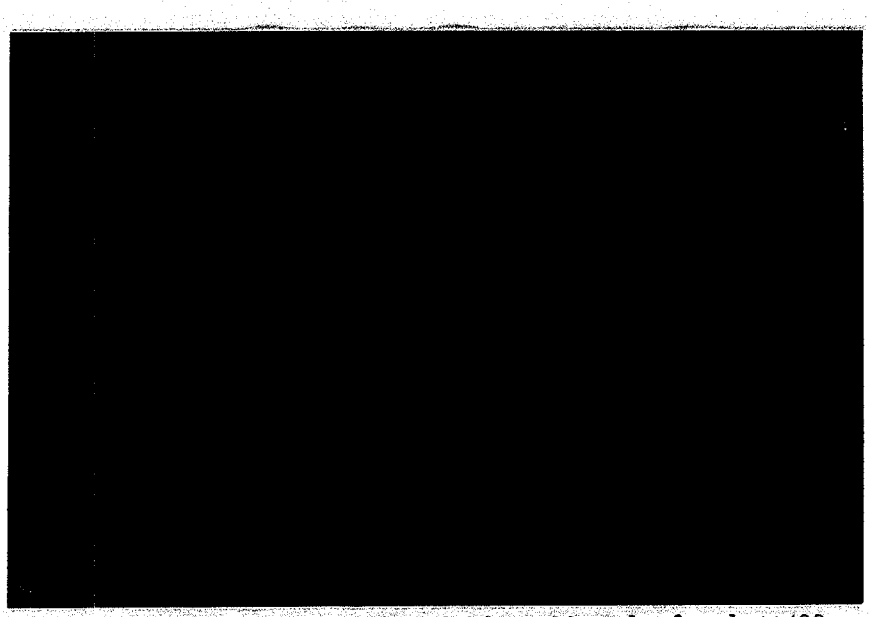


PHOTO 12 **Thyroid**: 3000mg/kg/day×26 weeks, female, ×400, No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

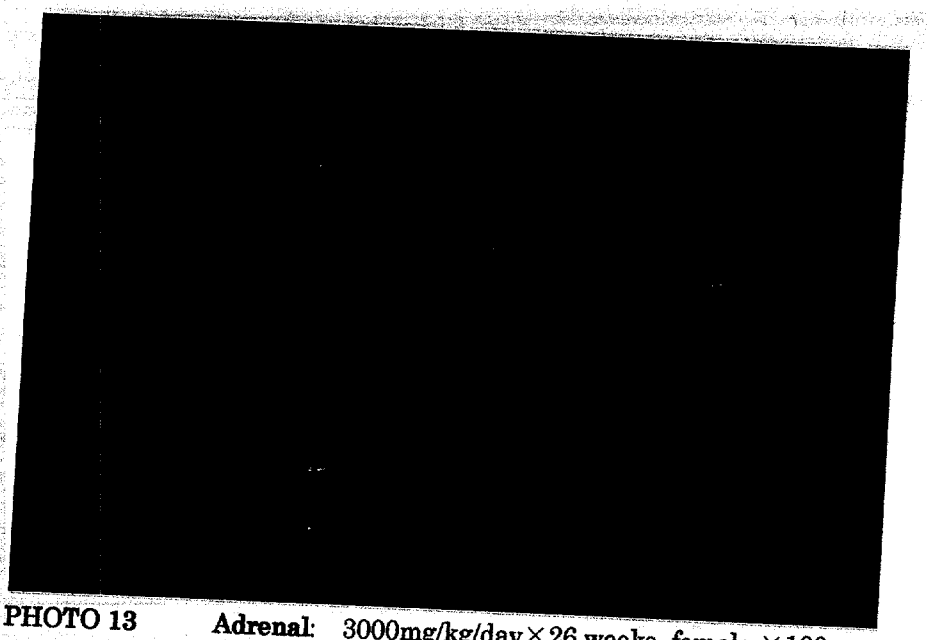


PHOTO 13 **Adrenal**: 3000mg/kg/day×26 weeks, female, ×100, No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

4.B. II.

Chronic Toxicity Study of Cultured *Agaricus blazei* Murrill (Iwade Strain 101) (Japanease name ; Himematsutake) Preparation, "ABME" Administered Orally in Mice for 26 Weeks.

Hitoshi Ito, M.D., Ph.D. and Keishiro Shimura, M.D.*

Department of Pharmacology Mie University School of Medicine
*Institute of Laboratory Animals, Mie University School of Medicine
2-174, Edobashi, Tsu, Mie 514-0001, Japan

Introduction

A chronic toxicity study of the edible mushroom, *Agaricus blazei* Murrill (Japanese name; Himematsutake) preparation, "ABME" - *Agaricus blazei* Murrill Extract, Japanese name: Himematsutake, was carried out with ICR-Slc strain mice (specific pathogen free animals). The ABME was administered orally for 26 weeks in doses of 0, 500 and 3000 mg/kg/day.

Based on the series of animal experiments studied for the antitumor effect of ABME, the usual dose for human is estimated 25mg/kg. The chronic toxicity study on mice in this report includes 500mg/kg - 20 times and 3000mg/kg - 120 times more dose compared to the usual dose for human.

With the limitation of the capacity of mice stomach and the physical condition of ABME in mind, over 3000mg/kg dose to a mouse would be impossible.

ABME was provided by Iwade Research Institute of Mycology, Japan.

Chronic toxicity studies

Animals were employed 5 weeks old ICR-Slc strain mice, both male and female (Japan SLC, Inc.). The animals were housed and fed in an animal room of the temperature of 23±2℃ and the humidity of 55±5%. Each animal was given solid diet (CLEA Japan CE-2) and water ad libitum.

One group of animals was used of 10 males and 10 females. Doses of administration were determined by the results of subacute toxicity studies, and two grades were adopted; 500 and 3000 mg/kg/day (The maximum dose are able to the oral administration).

Test materials are easily soluble in water but high concentration used the state of suspensions. Their water solutions were, therefore, prepared as to be at a level of 0.1 to 0.15ml per 10g of mice body weight. They were abstained from food for several hours before administration. They were compulsorily administered with a gastric catheter of teflon into the stomach. After the test materials were administered, general symptoms of animals were observed for every day.

Results

(1) Behavior

In mouse administered orally with 500 and 3000 mg/kg for 26 weeks, any abnormal findings that seemed to be caused by the administration of the test material were not observed.

(2) Body Weight Changes (Table 1 and Table 2)

The animals were weighed weekly. No inhibition of body weight gain was found during the periods of the experiments among the test animals, both male and female.

(3) Amount of Diet Ingested (Table 3 and Table 4)

As shown in Table 3 and Table 4, differences between the two administered groups were not observed. As the correct amount of ingested diet was not found, it was impossible to calculate the diet efficacy.

(4) Findings in Hematological Examinations (Table 5, 6, 7 and Table 8)

Hematological examination after 13 weeks of administration was performed on 5 cases of each group, and other items of examinations after 26 weeks of administration were done on 10 cases. No variation of significance was found in red blood cell count, hematocrit value, hemoglobin content, platelet value and white blood cell count. Differential leukocyte was found by fixing blood smear and staining by May-Grünwald Giemsa method. In differential leukocyte count, no abnormal findings were found due to the administration of the test material.

(5) Biochemical Examination of Blood (Table 9, 10, 11 and Table 12)

Biochemical examinations of blood after 13 weeks of administration were performed on 5 cases each of the groups. With regard to glucose, urea, total protein, albumin, alkaline phosphatase, GOT, GPT and total cholesterol value, no abnormal data was found in the female animals of the 500 mg/kg and 3000 mg/kg groups at 13^{th} and 26^{th} week.

(6) Findings in Urine (Table 13 and Table 14)

Urine protein was assayed in the concentration of trace to 100mg/dl in most of the groups, regardless of the administered or the control. Inspecting , pH, urobilinogen, bilirubin, ketone body and glucose, no abnormal data was found in all groups.

(7) Findings at Autopsy Organ Weight (Table 15, 16, 17 and Table 18)

At the end of administration, blood was sampled under anesthesia of ether. Immediately after the mice were sacrificed by blood-letting, autopsy was performed, organs were excised, principal organs were weighted wet, fixed with 10% formalin, and put to the histopathological examinations. As shown in Table 15, 16, 17 and Table 18, no remarkable change was found between the control group and the treated groups in either absolute organ weight or mean comparative organ weight. The changes found were not considered to be caused by the test material.

(8) Histopathological Findings (Table 19, 20, 21 and Table 22, PHOTO 1—PHOTO 14)

After autopsy and gross observation of changes, the organs were fixed with 10% formalin, embedded in paraffin and cut in slices ca. 6 μ thick, then stained with hematoxylin and eosine.

Microscopic examinations were performed on 5 samples each of the groups at the end of 13 weeks and 26weeks after the administration. Histopathological examination was conducted by Sensake Naruse, M.D., at Department of Pathology, Mie University School of Medicine, Tsu, Mie, 514-0001, Japan.

Findings in the survived mice are as follows:

Lungs : Two cases including the control group at 26 weeks showed a slight chronic bronchitis and inflammation of interstitial cells.

Liver : Almost no difference between the control and the administered groups; a slight cell infiltration and degeneration of liver were observed in 1 case of the control group.

Kidneys : In one case of the 3000mg/kg group, kidney tubules were found enlarged like cyst, and may have been caused by pyelitis and nephritis. However, a slight pyelitis and nephritis were found in the control group, too.

Spleen : A slight hemosiderosis was observed in a few case including those of the control and the administered groups.

* No marked changes were observed in brain, heart, testes, ovaries, thymus, pituitary, adrenals, pancreas and digestive tracts.

Summary

A chronic toxicity of edible mushroom, *Agaricus blazei* Murrill (Japanese name: Himematsutake) preparation, "ABME" was studied with ICR strain mice.

ABME was administered orally for 26 weeks in dose of 0 (control), 500 and 3000 mg/kg/day. During the period of oral administration for 26 weeks, no general symptoms to be marked were observed in ICR strain mice, and there was no specific change of male and female mice.

With regard to the amount of diet ingested, no significant change was found in all the administered group.

No inhibition of body weight gain was found during the periods of the experiments among the test animals, both male and female.

In hematological findings, any significant variation in red blood cell count, hematocrit value, hemoglobin content, platelet value and white blood cell count was not found. In differential count, too, no abnormal findings due to the administration of the test material was found.

In biochemical examination of blood, no change was found in glucose, urea, total protein, albumin, alkaline phosphatase, GOT, GPT and total cholesterol value.

No abnormality was found in the pH, urobilinogen, bilirubin, ketone body, protein and glucose in the control and the administered groups.

In assaying organ weight, no change was found in either the administered group of male or female mice compared with the control group.

In the histopathogical examinations, any abnormal figures specific to the administered group compared with the control group was not observed in mice. Furthermore, any toxicity to be caused by ABME could not be found.

As a conclusion of chronic toxicity studies in mice, the safety dose for mice was estimated to be over 3000 mg/kg/day, but the sure intoxication dose could not be determined.

End of report

Table 1 Body weight changes in male mice given ABME orally for 26 weeks

Dosing periods (weeks)	Dose level (mg/kg/day)			
	Male (g)			
	Number of mice	Control	500	3000
0	15	28.4	28.1	28.7
1	15	32.7	31.8	33.4
2	15	34.5	35.3	36.2
3	15	36.2	37.0	36.8
4	15	38.0	39.2	37.8
5	15	39.9	40.8	39.4
6	15	41.3	42.0	41.0
7	15	42.4	43.5	41.9
8	15	43.9	45.0	42.7
9	15	44.5	45.5	43.2
10	15	45.5	46.7	44.1
11	15	45.3	47.2	45.4
12	15	45.9	47.0	45.2
13	15	46.4	48.3	46.7
14	10	46.3	49.0	47.2
15	10	46.4	49.4	47.7
16	10	47.0	50.6	47.9
17	10	47.8	51.3	47.6
18	10	48.3	51.7	48.5
19	10	48.9	52.0	48.7
20	10	49.8	52.7	49.2
21	10	51.0	53.4	51.6
22	10	51.9	53.7	52.1
23	10	52.2	53.5	53.0
24	10	52.7	54.1	53.4
25	10	53.0	54.2	53.7
26	10	53.1	54.5	53.9

Table 2 , Body weight changes in female mice given ABME orally for 26 weeks

Dosing periods (weeks)	Dose level (mg/kg/day)			
	Female (g)			
	Number of mice	Control	500	3000
0	15	22.8	23.4	23.4
1	15	25.5	26.0	26.6
2	15	26.3	26.9	27.9
3	15	28.2	29.0	28.9
4	15	29.7	30.5	30.5
5	15	30.3	32.0	31.9
6	15	31.0	32.2	32.3
7	15	32.2	33.2	33.5
8	15	34.0	34.4	34.7
9	15	34.4	35.2	35.3
10	15	35.6	35.5	36.3
11	15	35.5	36.6	37.7
12	15	35.7	37.5	39.3
13	15	36.6	38.0	39.4
14	10	36.5	38.7	41.3
15	10	37.0	39.0	41.5
16	10	37.8	39.8	42.4
17	10	38.4	41.0	43.0
18	10	39.9	41.7	43.9
19	10	41.6	42.5	44.5
20	10	41.8	43.6	45.6
21	10	42.7	44.0	45.6
22	10	43.4	44.8	46.0
23	10	43.4	44.7	45.9
24	10	44.0	45.1	46.1
25	10	44.5	45.3	46.2
26	10	44.8	45.9	46.3

Table 3' Food consumption of male mice given ABME orally for 26 weeks

Dosing periods (weeks)	Dose level (mg/kg/day)			
	Male (g)			
	Number of mice	Control	500	3000
1	15	4.9±0.3	5.2±0.4	5.2±0.4
2	15	5.6±0.3	5.5±0.3	5.9±0.5
3	15	7.5±0.8	7.8±0.7	6.5±0.6
4	15	8.2±0.9	9.3±0.9	8.1±0.8
5	15	8.7±0.7	9.0±0.6	8.1±0.5
6	15	8.8±0.4	10.1±0.5	9.0±0.6
7	15	9.3±0.5	10.1±0.4	9.4±0.7
8	15	8.9±0.5	9.6±0.6	8.5±0.6
9	15	8.1±0.7	9.7±0.5	8.3±0.3
10	15	8.6±0.5	9.8±0.5	8.9±0.4
11	15	8.8±0.8	9.4±0.6	9.3±0.7
12	15	8.6±0.7	9.5±0.5	9.2±0.5
13	15	8.7±0.6	9/8±0.7	9.5±0.8
14	10	8.4±0.5	9.6±0.8	8.3±0.7
15	10	8.4±0.9	9.5±0.6	8.6±0.6
16	10	8.3±0.5	9.5±0.7	8.7±0.7
17	10	7.9±0.8	9.6±0.8	9.0±0.9
18	10	8.6±0.7	10.1±1.0	9.1±0.8
19	10	8.2±0.6	9.7±0.8	8.6±0.7
20	10	8.5±0.8	9.7±0.9	8.7±0.6
21	10	8.5±0.8	9.8±0.7	8.8±0.6
22	10	8.7±0.9	9.7±0.9	8.9±0.7
23	10	8.8±0.7	8.5±0.5	9.1±0.5
24	10	8.4±0.6	8.5±0.6	9.0±0.7
25	10	8.7±0.7	9.0±0.4	9.1±0.5
26	10	8.9±0.5	9.1±0.5	9.0±0.4

Values represent mean ± standard error (g/day/mouse)

Table 4 Food consumption of female mice given ABME orally for 26 weeks

Dosing periods (weeks)	Dose level (mg/kg/day)			
	Female (g)			
	Number of mice	Control	500	3000
1	15	4.3±0.2	4.6±0.3	4.2±0.2
2	15	4.8±0.4	4.9±0.3	4.1±0.3
3	15	4.9±0.3	4.9±0.2	4.7±0.2
4	15	5.3±0.4	5.2±0.3	5.2±0.2
5	15	5.6±0.3	6.1±0.3	5.3±0.4
6	15	5.6±0.3	6.1±0.3	5.9±0.3
7	15	6.0±0.2	6.1±0.3	6.5±0.4
8	15	6.2±0.7	7.2±0.8	6.3±0.5
9	15	6.1±0.5	7.2±0.7	7.0±0.6
10	15	6.3±0.7	7.3±0.8	7.2±0.7
11	15	6.8±0.5	7.9±0.9	6.9±0.7
12	15	6.0±0.4	6.8±0.5	7.1±0.7
13	15	6.4±0.5	7.0±0.7	7.1±0.6
14	10	6.3±0.3	7.2±0.6	6.7±0.4
15	10	6.2±0.5	7.5±0.5	6.9±0.4
16	10	6.7±0.7	6.9±0.6	7.2±0.6
17	10	6.5±0.5	7.0±0.7	6.7±0.6
18	10	6.9±0.9	7.1±0.5	6.8±0.5
19	10	6.4±0.6	7.1±0.5	7.0±0.7
20	10	6.7±0.5	6.8±0.5	7.0±0.4
21	10	6.8±0.7	7.4±0.6	6.7±0.6
22	10	6.6±0.5	7.2±0.7	6.8±0.6
23	10	6.7±0.4	7.0±0.8	7.1±0.5
24	10	6.7±0.3	7.3±0.8	6.6±0.5
25	10	6.9±0.6	7.4±0.5	7.1±0.8
26	10	7.1±0.7	7.6±0.5	7.2±0.7

Values represent mean ± standard error (g/day/mouse)

Table 5 Hematological findings in male mice given ABME orally for 13 weeks

Male											
Dose level (mg/kg/day)	Number of mice	RBC ($\times 10^4/mm^3$)	Ht (%)	Hb (g/dl)	BP ($\times 10^4/mm^3$)	WBC ($\times 10^2/mm^3$)	Differential count (%)				
							L	M	N	E	B
Control	5	778±22	46.8±1.8	15.3±0.3	120±14.7	51±9.4	71.9±2.7	1.0±0.4	26.3±2.6	0.8±0.2	0
500	5	777±20	47.1±1.7	15.0±0.6	115±13.0	50±7.3	70.6±2.5	1.4±0.3	27.2±2.4	0.8±0.1	0
3000	5	780±34	46.1±1.3	15.6±0.3	118±14.1	48±5.7	72.8±2.5	1.0±0.4	25.5±2.5	0.7±0.2	0

Values represent mean ± standard error
RBC (Red blood cell) : TOA Microcell Counter CC-108
Ht (Hematocrit) : Microhematocrit method
Hb (Hemoglobin) : TOA Hemoglobin Counter Hb-100
BP (Blood platelet) : TOA Platelet Counter PL-100
WBC (White blood cell) : TOA Microcell Counter CC-108
L (Lymphocyte), M (Monocyte), N (Neutrophil), E (Eosinocyte) and B (Basocyte) :
Leucocyte ratio (May-Grunwald Giemsa stained method)

Table 6 Hematological findings in female mice given ABME orally for 13 weeks

Female											
Dose level (mg/kg/day)	Number of mice	RBC ($\times 10^4/mm^3$)	Ht (%)	Hb (g/dl)	BP ($\times 10^4/mm^3$)	WBC ($\times 10^2/mm^3$)	Differential count (%)				
							L	M	N	E	B
Control	5	766±33	46.7±1.1	14.9±0.2	117±9.2	49±3.3	76.3±1.6	0.8±0.3	21.5±1.8	1.4±0.3	0
500	5	785±19	47.1±1.0	14.8±0.8	115±7.0	51±3.8	75.2±1.8	1.2±0.2	22.4±2.8	1.2±0.2	0
3000	5	780±28	47.2±1.2	15.1±0.7	123±9.7	46±2.2	76.2±2.8	1.4±0.3	21.5±2.5	0.9±0.3	0

Values represent mean ± standard error
RBC (Red blood cell) : TOA Microcell Counter CC-108
Ht (Hematocrit) : Microhematocrit method
Hb (Hemoglobin) : TOA Hemoglobin Counter Hb-100
BP (Blood platelet) : TOA Platelet Counter PL-100
WBC (White blood cell) : TOA Microcell Counter CC-108
L (Lymphocyte), M (Monocyte), N (Neutrophil), E (Eosinocyte) and B (Basocyte) :
Leucocyte ratio (May-Grunwald Giemsa stained method)

Table 8 Hematological findings in female mice given ABME orally for 26 weeks

Female											
Dose level (mg/kg/day)	Number of mice	RBC ($\times 10^4/mm^3$)	Ht (%)	Hb (g/dl)	BP ($\times 10^4/mm^3$)	WBC ($\times 10^2/mm^3$)	Differential count (%)				
							L	M	N	E	B
Control	10	883±52	44.7±1.3	14.7±0.6	129±7.6	42±4.3	71.9±3.6	1.3±0.4	26.0±3.0	0.8±0.3	0
500	10	920±73	45.1±2.6	15.2±0.7	130±5.3	39±2.3	70.9±2.8	1.1±0.3	27.0±3.2	1.0±0.3	0
3000	10	914±46	44.0±2.1	14.9±0.6	126±3.9	44±3.5	67.4±3.5	1.9±0.5	30.0±3.3	0.7±0.4	0

Values represent mean ± standard error
RBC (Red blood cell) : TOA Microcell Counter CC-108
Ht (Hematocrit) : Microhematocrit method
Hb (Hemoglobin) : TOA Hemoglobin Counter Hb-100
BP (Blood platelet) : TOA Platelet Counter PL-100
WBC (White blood cell) : TOA Microcell Counter CC-108
L (Lymphocyte), M (Monocyte), N (Neutrophil), E (Eosinocyte) and B (Basocyte) :
Leucocyte ratio (May-Grunwald Giemsa stained method)

Table 9 Biochemical findings in male mice given ABME orally for 13 weeks

Male									
Dose level (mg/kg/day)	Number of mice	Glucose (mg/dl)	Urea nitrogen (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Alkaline phoshatase (IU/l)	GOT (IU/l)	GPT (IU/l)	Total cholesterol (mg/dl)
Control	5	127±29	25±4.1	4.6±0.3	1.5±0.3	175±29.3	52±9.7	22±4.3	127±26
500	5	132±30	27±3.7	4.2±0.5	1.4±0.2	198±32.4	54±7.3	26±5.1	130±32
3000	5	116±21	22±3.5	5.0±0.4	1.5±0.3	170±31.9	59±9.8	23±3.4	121±23

Values represent mean ± standard error
* Significantly different from control at $p < 0.05$
Glucose : GLK/G6PDH method
Urea nitrogen : Urease/GLDH method
Albumin : BCG method
Alkaline phosphatase : King-Armstrong method
GOT : NADH-UV (IFCC method)
GPT : NADH-UV (IFCC method)
Total cholesterol : CE/CO/POD method

Table 10 Biochemical findings in female mice given ABME orally for 13 weeks

Female									
Dose level (mg/kg/day)	Number of mice	Glucose (mg/dl)	Urea nitrogen (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Alkaline phoshatase (IU/l)	GOT (IU/l)	GPT (IU/l)	Total cholesterol (mg/dl)
Control	5	133±31	24±2.7	4.6±0.1	1.4±0.2	241±53.3	60±9.8	24±5.1	136±18
500	5	140±29	21±3.4	4.4±0.2	1.3±0.1	221±35.1	57±6.7	21±4.7	120±15
3000	5	128±26	20±2.1	4.5±0.3	1.4±0.1	199±41.4	65±9.7	27±5.6	109±17

Values represent mean ± standard error
* Significantly different from control at $p < 0.05$
Glucose : GLK/G6PDH method
Urea nitrogen : Urease/GLDH method
Albumin : BCG method
Alkaline phosphatase : King-Armstrong method
GOT : NADH-UV (IFCC method)
GPT : NADH-UV (IFCC method)
Total cholesterol : CE/CO/POD method

Table 11 Biochemical findings in male mice given ABME orally for 26 weeks

Male									
Dose level (mg/kg/day)	Number of mice	Glucose (mg/dl)	Urea nitrogen (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Alkaline phoshatase (IU/l)	GOT (IU/l)	GPT (IU/l)	Total cholesterol (mg/dl)
Control	10	102±6.3	22±3.9	4.8±0.3	1.4±0.2	160±22.6	76±10.2	27±5.3	119±23
500	10	96±5.1	21±2.4	4.5±0.2	1.6±0.3	154±20.3	72±9.7	25±4.1	107±15
3000	10	98±5.2	18±2.0	4.9±0.2	1.5±0.2	157±16.5	68±8.1	28±3.6	98±19

Values represent mean ± standard error
* Significantly different from control at $p < 0.05$
Glucose : GLK/G6PDH method
Urea nitrogen : Urease/GLDH method
Albumin : BCG method
Alkaline phosphatase : King-Armstrong method
GOT : NADH-UV (IFCC method)
GPT : NADH-UV (IFCC method)
Total cholesterol : CE/CO/POD method

Table 12 Biochemical findings in female mice given ABME orally for 26 weeks

Female									
Dose level (mg/kg/day)	Number of mice	Glucose (mg/dl)	Urea nitrogen (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Alkaline phoshatase (IU/l)	GOT (IU/l)	GPT (IU/l)	Total cholesterol (mg/dl)
Control	10	93±19	19±2.7	5.1±0.4	1.5±0.2	159±36.0	97±21	37±4.9	110±23
500	10	90±12	22±3.4	4.9±0.3	1.5±0.1	162±41.4	91±34	35±5.3	98±16
3000	10	89±11	20±2.1	5.3±0.3	1.4±0.1	158±29.6	109±42	32±3.7	101±18

Values represent mean ± standard error
* Significantly different from control at $p < 0.05$
Glucose : GLK/G6PDH method
Urea nitrogen : Urease/GLDH method
Albumin : BCG method
Alkaline phosphatase : King-Armstrong method
GOT : NADH-UV (IFCC method)
GPT : NADH-UV (IFCC method)
Total cholesterol : CE/CO/POD method

Table 13 Urinalysis of male mice given ABME orally for 26 weeks

Male												
Dosing period (week)	Dose level (mg/kg/day)	Number of mice	Appearance	pH			Occult blood	Urobilinogen (Ehrlich unit/dl)	Bilirubin (0.4–0.8 mg/dl)	Ketone body	Protein	Glucose
				6	7	8						
13	Control	10	Normal	4	1	0	−	0.1–1	−	−	±～+	−
	500	10	Normal	3	2	0	−	0.1	−	−	−～±	−
	3000	10	Normal	4	1	0	−	0.1	−	−	±～+	−
26	Control	10	Normal	5	0	0	−	0.1–1	−	−	±～+	−
	500	10	Normal	5	0	0	−	0.1	−	−	−～±	−
	3000	10	Normal	4	1	0	−	0.1–1	−	−	−～±	−

pH : pH meter,
Occult blood, Urobilinogen, Bilirubin, Ketone body,
Protein and Glucose : Uro-Labstix (Ames reagent strips for urinalysis)

Table 14 Urinalysis of female mice given ABME orally for 26 weeks

Female													
Dosing period (week)	Dose level (mg/kg/day)	Number of mice	Appearance	pH			Occult blood	Urobilinogen (Ehrlich unit/dl)	Bilirubin (0.4–0.8 mg/dl)	Ketone body	Protein	Glucose
				6	7	8						
13	Control	10	Normal	3	2	0	−	0.1–1	−	−	±～+	−
	500	10	Normal	3	2	0	−	0.1–1	−	−	±～+	−
	3000	10	Normal	4	1	0	−	0.1	−	−	−～±	−
26	Control	10	Normal	4	1	0	−	0.1–1	−	−	±～+	−
	500	10	Normal	5	0	0	−	0.1		−	−～±	−
	3000	10	Normal	4	1	0	−	0.1–1	−	−	±～+	−

pH : pH meter,
Occult blood, Urobilinogen, Bilirubin, Ketone body,
Protein and Glucose : Uro-Labstix (Ames reagent strips for urinalysis)

Table 15 Organ weights in male mice given ABME orally for 13 weeks

Male												
Dose level (mg/kg/day)	Number of mice	Final body wt. (g)	Brain (mg)	Heart (mg)	Lung (mg)	Liver (g)	Kidneys (mg)	Spleen (mg)	Testes (mg)	Thymus (mg)	Pituitary (mg)	Adrenals (mg)
Control	5	46.4±2.7	543±14 (1.170)	163±8 (0.351)	181±15 (0.390)	1.70±0.21 (3.664)	460±29 (0.991)	122±12 (0.263)	344±25 (0.741)	55.8±4.8 (0.120)	2.8±0.2 (0.006)	23.7±3.4 (0.051)
500	5	48.3±3.1	552±17 (1.143)	166±7 (0.344)	187±21 (0.387)	1.55±0.23 (3.209)	477±31 (0.988)	124±23 (0.257)	345±29 (0.714)	51.4±3.2 (0.106)	2.9±0.1 (0.006)	29.1±4.0 (0.060)
3000	5	46.7±2.0	549±15 (1.176)	167±9 (0.358)	173±10 (0.370)	1.84±0.30 (3.940)	462±30 (0.989)	130±9 (0.278)	359±24 (0.769)	54.0±2.2 (0.116)	3.0±0.2 (0.006)	24.5±7.0 (0.052)

Values represent mean ± standard error.
Values in parentheses represent mean comparative organ weights in grams or milligrames per 100g body weight.

Table 16 Organ weights in female mice given ABME orally for 13 weeks

Female												
Dose level (mg/kg/day)	Number of mice	Final body wt. (g)	Brain (mg)	Heart (mg)	Lung (mg)	Liver (g)	Kidneys (mg)	Spleen (mg)	Ovaries (mg)	Thymus (mg)	Pituitary (mg)	Adrenals (mg)
Control	5	36.6±2.0	545±16	152±8	190±11	1.67±0.09	369±18	134±16	13.3±0.9	56.4±5.1	3.1±0.3	12.4±1.3
			(1.489)	(0.415)	(0.519)	(4.563)	(1.008)	(0.366)	(0.036)	(0.154)	(0.008)	(0.034)
500	5	38.0±2.5	557±12	158±4	197±7	1.70±0.21	377±22	146±17	13.5±1.4	59.8±4.0	3.3±0.4	12.6±3.1
			(1.466)	(0.416)	(0.518)	(4.474)	(0.992)	(0.384)	(0.036)	(0.157)	(0.009)	(0.033)
3000	5	39.4±3.1	570±10	159±5	199±13	1.88±0.20	380±21	149±15	14.8±1.8	59.9±7.4	3.5±0.2	13.4±4.5
			(1.447)	(0.404)	(0.505)	(4.772)	(0.964)	(0.378)	(0.038)	(0.152)	(0.009)	(0.034)

Values represent mean ± standard error.
Values in parentheses represent mean comparative organ weights in grams or milligrames per 100g body weight.

Table 17 Organ weights in male mice given ABME orally for 26 weeks

Male												
Dose level (mg/kg/day)	Number of mice	Final body wt. (g)	Brain (mg)	Heart (mg)	Lung (mg)	Liver (g)	Kidneys (mg)	Spleen (mg)	Testes (mg)	Thymus (mg)	Pituitary (mg)	Adrenals (mg)
Control	10	53.1±4.2	564±18 (1.062)	233±12 (0.439)	231±15 (0.435)	2.48±0.24 (4.670)	709±28 (1.335)	134±12 (0.252)	544±15 (1.024)	45.7±3.9 (0.086)	3.0±0.2 (0.006)	26.8±3.5 (0.050)
500	10	54.5±4.9	566±19 (1.039)	246±10 (0.451)	247±20 (0.453)	2.52±0.18 (4.624)	712±30 (1.306)	140±26 (0.257)	544±9 (0.996)	45.4±2.3 (0.083)	3.2±0.2 (0.006)	26.1±2.7 (0.048)
3000	10	53.9±4.3	577±13 (1.071)	239±6 (0.443)	243±17 (0.451)	2.53±0.09 (4.694)	730±29 (1.354)	142±11 (0.263)	553±14 (1.026)	44.0±1.7 (0.082)	3.4±0.2 (0.006)	27.3±3.3 (0.051)

Values represent mean ± standard error.
Values in parentheses represent mean comparative organ weights in grams or milligrames per 100g body weight.

Table 18 Organ weights in female mice given ABME orally for 26 weeks

Female												
Dose level (mg/kg/day)	Number of mice	Final body wt. (g)	Brain (mg)	Heart (mg)	Lung (mg)	Liver (g)	Kidneys (mg)	Spleen (mg)	Ovaries (mg)	Thymus (mg)	Pituitary (mg)	Adrenals (mg)
Control	10	44.8±5.7	566±13	165±6	218±10	2.02±0.15	417±23	147±13	22.7±0.9	72.1±3.4	2.9±0.2	14.3±2.1
			(1.263)	(0.368)	(0.487)	(4.509)	(0.931)	(0.328)	(0.051)	(0.161)	(0.006)	(0.032)
500	10	45.9±4.9	577±10	163±8	209±9	2.11±0.24	412±24	150±17	23.2±1.7	74.6±4.1	3.1±0.3	14.8±3.1
			(1.257)	(0.355)	(0.455)	(4.597)	(0.898)	(0.327)	(0.051)	(0.163)	(0.007)	(0.032)
3000	10	46.3±5.6	581±15	171±7	214±9	2.23±0.33	446±20	154±12	25.5±2.1	75.8±8.3	2.9±0.1	14.4±1.4
			(1.255)	(0.369)	(0.462)	(4.816)	(0.963)	(0.333)	(0.055)	(0.164)	(0.006)	(0.031)

Values represent mean ± standard error.
Values in parentheses represent mean comparative organ weights in grams or milligrames per 100g body weight.

Table 19 Summary of histopathological findings in male mice received daily oral administration of ABME for 13 weeks

Number of individual animal	Control (0)					ABME-500					ABME-3000				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Liver; nuclear hyperplasia	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
cell infiltration	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−
degeneration	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
necrosis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Kidneys; hyaline droplets	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
cell infiltration	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
fibrosis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Spleen; hemosiderin	−	−	±	±	−	−	−	−	−	−	−	−	−	−	−
Heart; cell infiltration	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Thymus; involution	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Brain	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Lungs	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Testis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Pituitary	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Adrenals	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Stomach	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

±; Very slight alteration

Table 20 Summary of histopathological findings in female mice received daily oral administration of ABME for 13 weeks

Number of individual animal	Control (0)					ABME-500					ABME-3000				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Liver; nuclear hyperplasia	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
cell infiltration	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
degeneration	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
necrosis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Kidneys; hyaline droplets	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
cell infiltration	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
fibrosis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Spleen; hemosiderin	−	±	−	−	−	−	−	−	−	±	−	−	−	−	−
Heart; cell infiltration	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Thymus; involution	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Brain	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Lungs	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Ovaries	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Pituitary	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Adrenals	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Stomach	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

±; Very slight alteration

Table 21 Summary of histopathological findings in male mice received daily oral administration of ABME for 26 weeks

Number of individual animal	Control (0)					ABME-500					ABME-3000				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Liver; nuclear hyperplasia	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
cell infiltration	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
degeneration	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
necrosis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Kidneys; hyaline droplets	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
cell infiltration	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
fibrosis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Spleen; hemosiderin	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Heart; cell infiltration	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Thymus; involution	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Brain	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Lungs	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Testis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Pituitary	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Adrenals	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Stomach	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

±; Very slight alteration

Table 22 Summary of histopathological findings in female mice received daily oral administration of ABME for 26 weeks

Number of individual animal	Control (0)					ABME-500					ABME-3000				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Liver; nuclear hyperplasia	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
cell infiltration	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
degeneration	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
necrosis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Kidneys; hyaline droplets	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
cell infiltration	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
fibrosis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Spleen; hemosiderin	−	±	−	−	−	−	−	−	−	−	−	−	±	−	−
Heart; cell infiltration	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Thymus; involution	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Brain	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Lungs	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Ovaries	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Pituitary	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Adrenals	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Stomach	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

±; Very slight alteration

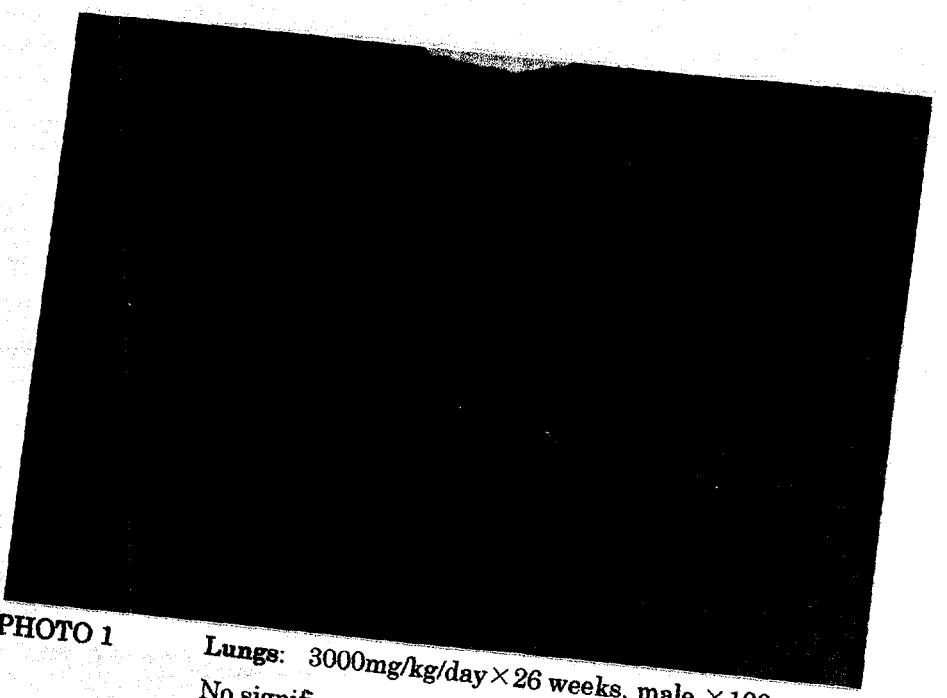


PHOTO 1 **Lungs:** 3000mg/kg/day × 26 weeks, male, × 100, No significant change (Not different from treated group of 3000mg/kg/day × 13 weeks and control group)

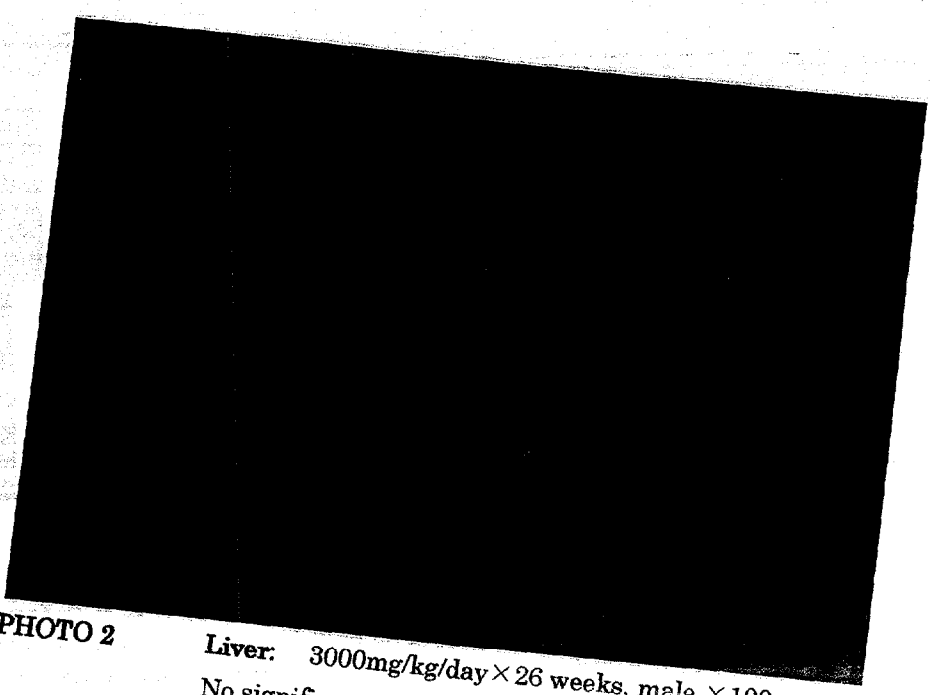


PHOTO 2 **Liver:** 3000mg/kg/day × 26 weeks, male, × 100, No significant change (Not different from treated group of 3000mg/kg/day × 13 weeks and control group)

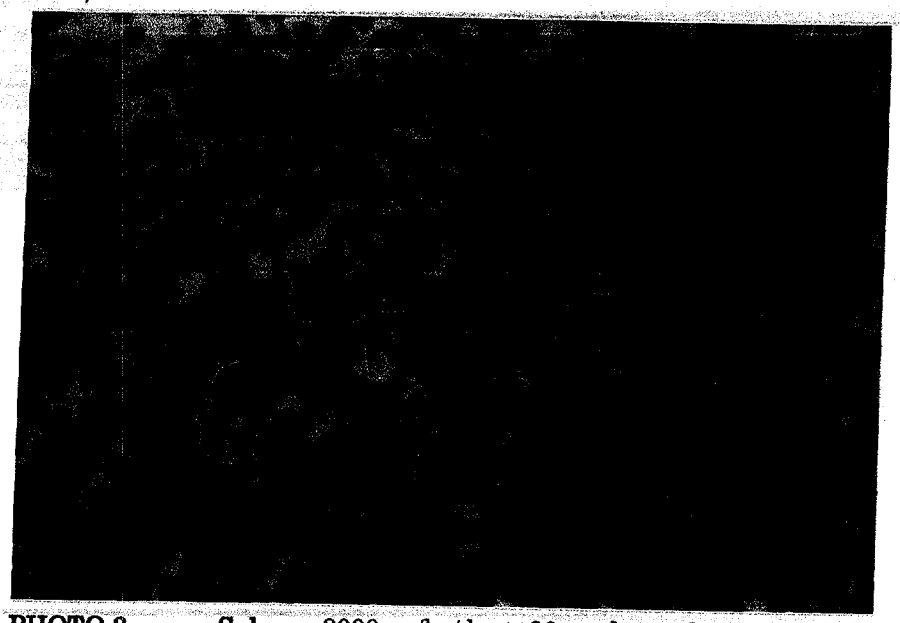


PHOTO 3 **Spleen**: 3000mg/kg/day×26 weeks, male, ×400,
No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

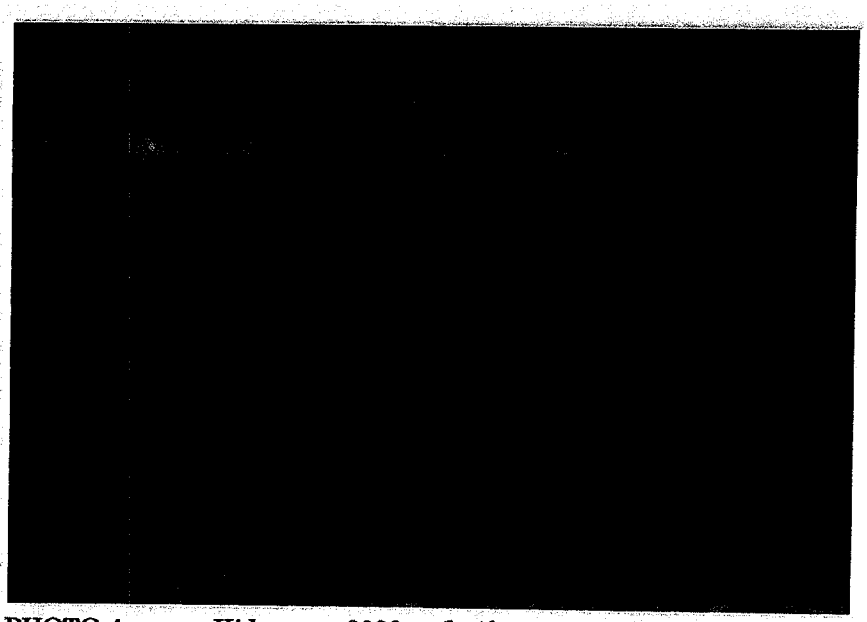


PHOTO 4 **Kidneys**: 3000mg/kg/day×26 weeks, male, ×100,
No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

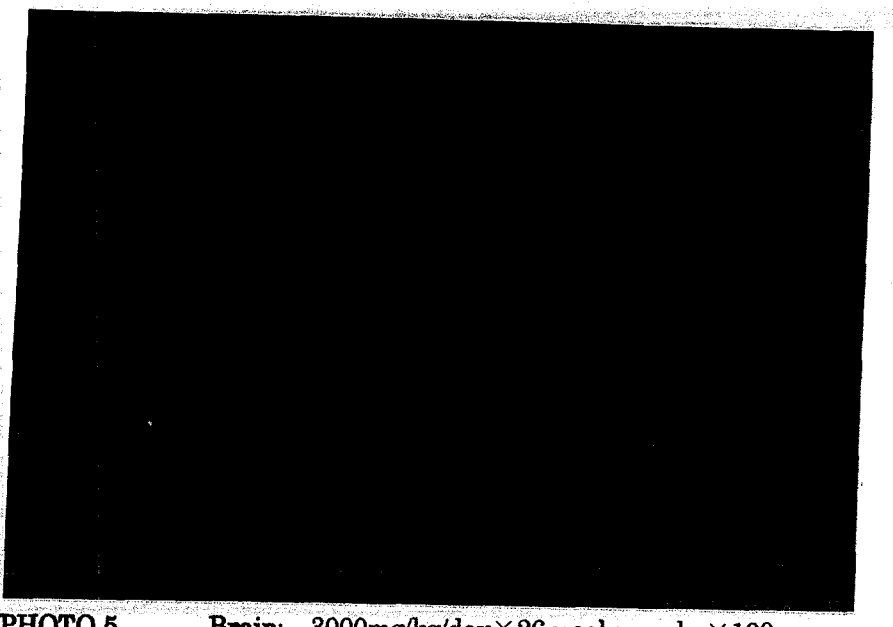


PHOTO 5 **Brain**: 3000mg/kg/day×26 weeks, male, ×100, No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

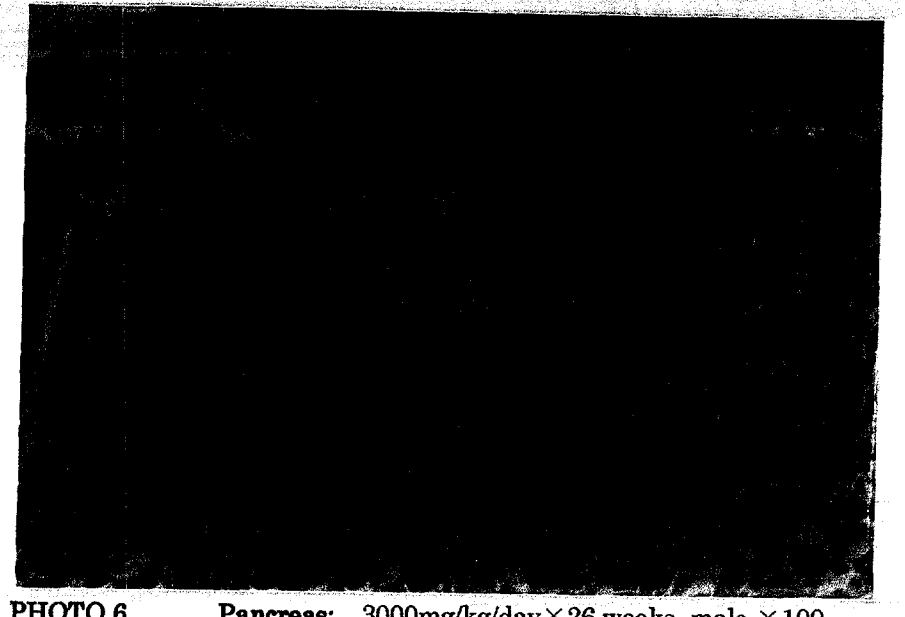


PHOTO 6 **Pancreas:** 3000mg/kg/day×26 weeks, male, ×100, No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

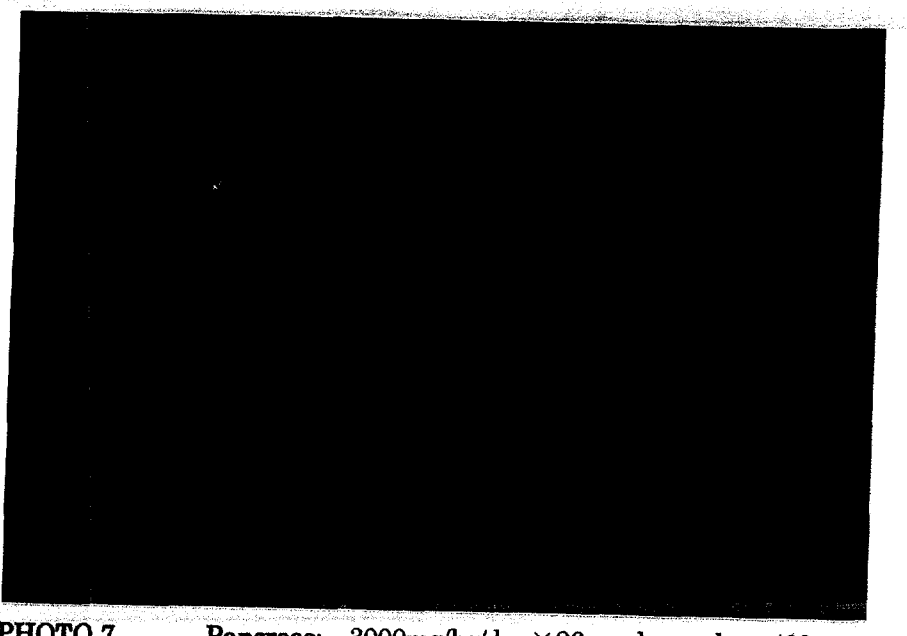


PHOTO 7 **Pancreas:** 3000mg/kg/day×26 weeks, male, ×400, No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

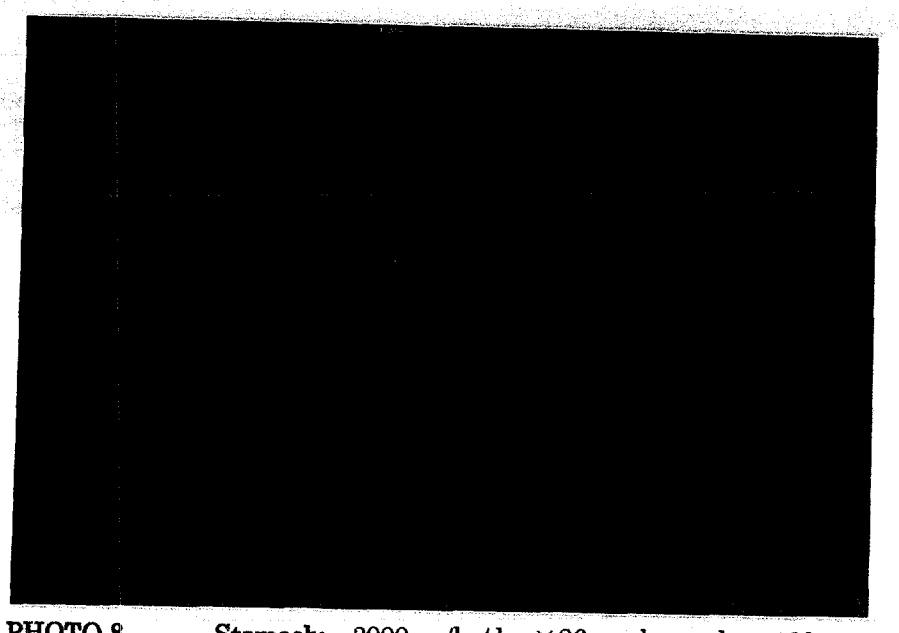


PHOTO 8 **Stomach:** 3000mg/kg/day×26 weeks, male, ×100, No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

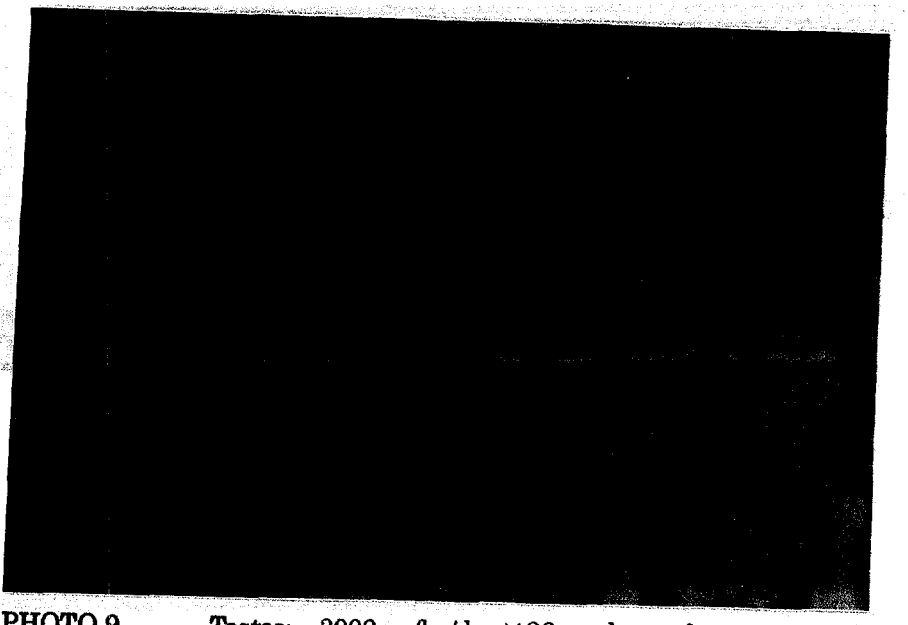


PHOTO 9 **Testes:** 3000mg/kg/day×26 weeks, male, ×100, No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

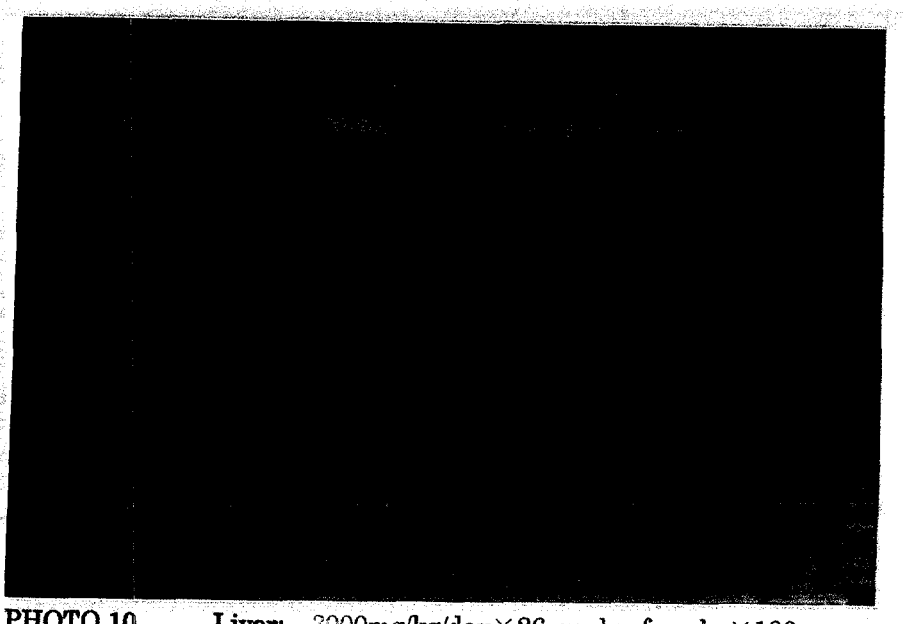


PHOTO 10 **Liver:** 3000mg/kg/day×26 weeks, female, ×100, No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

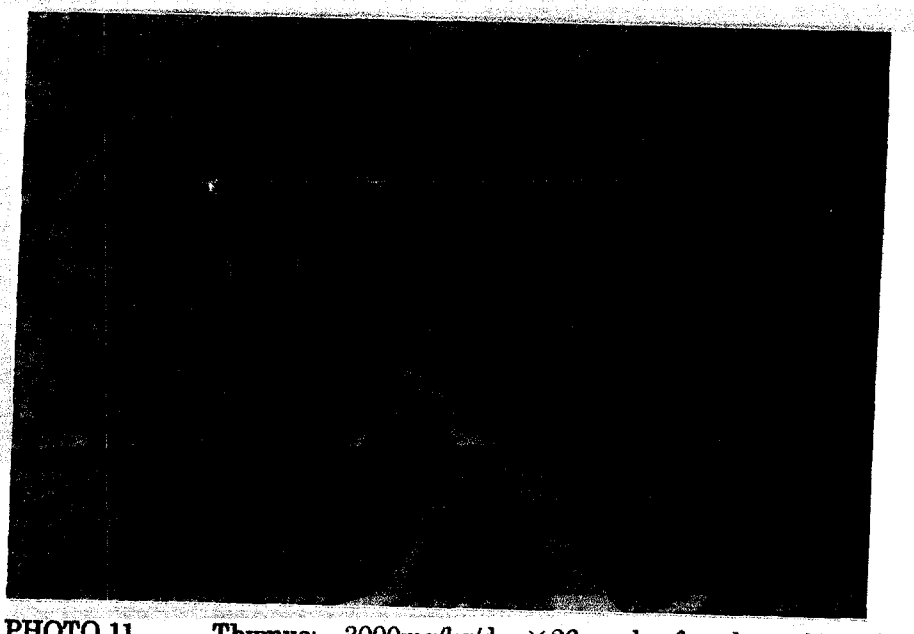


PHOTO 11 **Thymus**: 3000mg/kg/day×26 weeks, female, ×100, No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

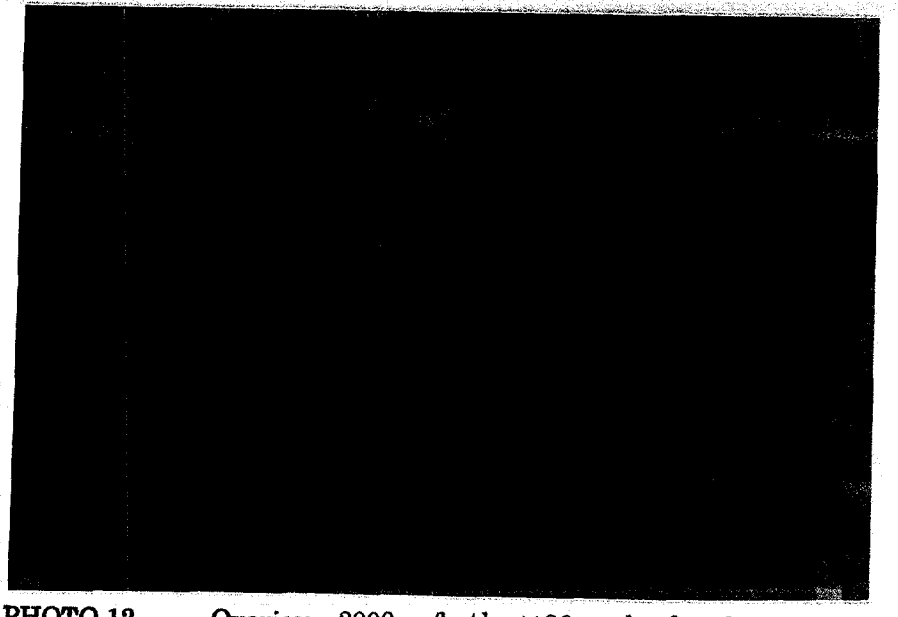


PHOTO 12 **Ovaries**: 3000mg/kg/day×26 weeks, female, ×100, No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

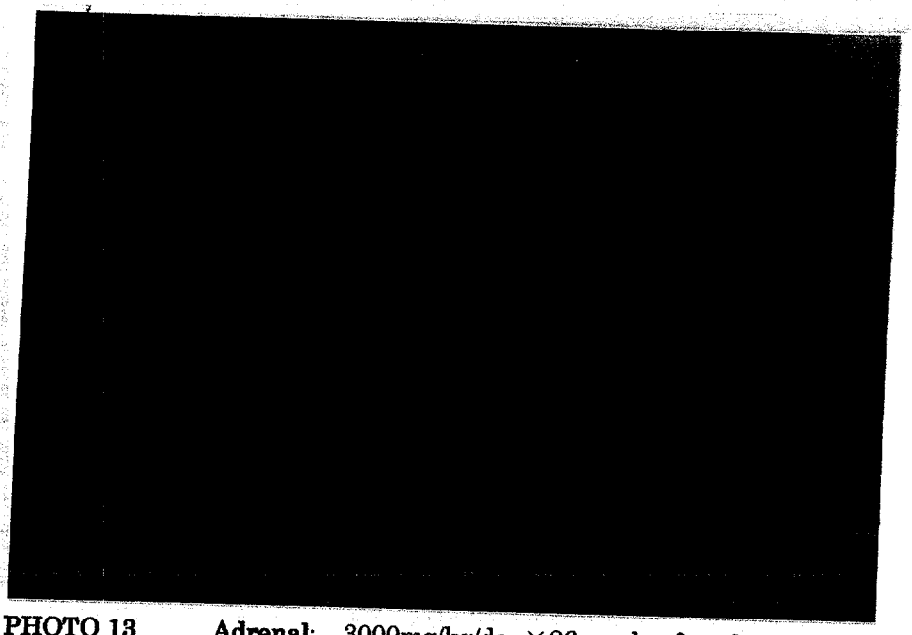


PHOTO 13 **Adrenal**: 3000mg/kg/day×26 weeks, female, ×100, No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)

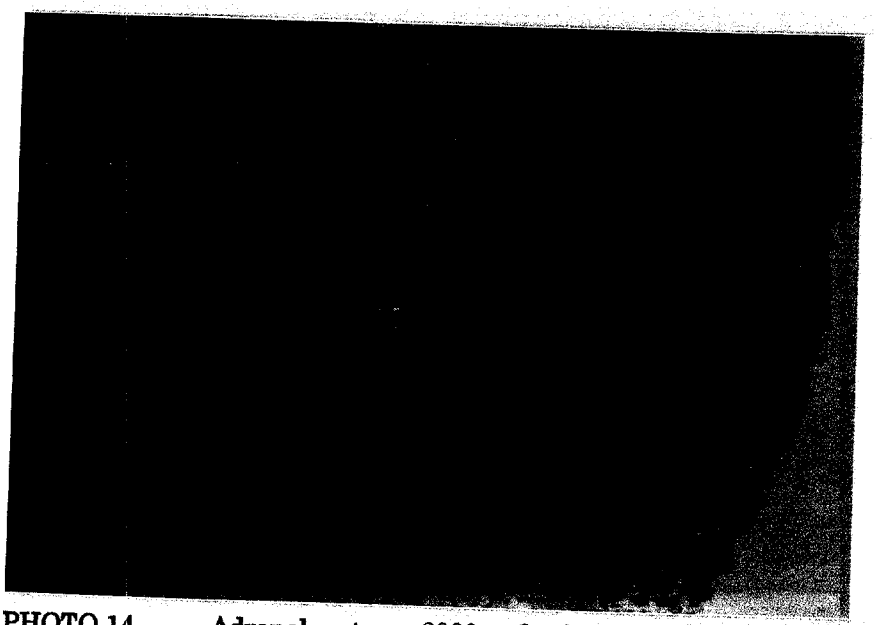
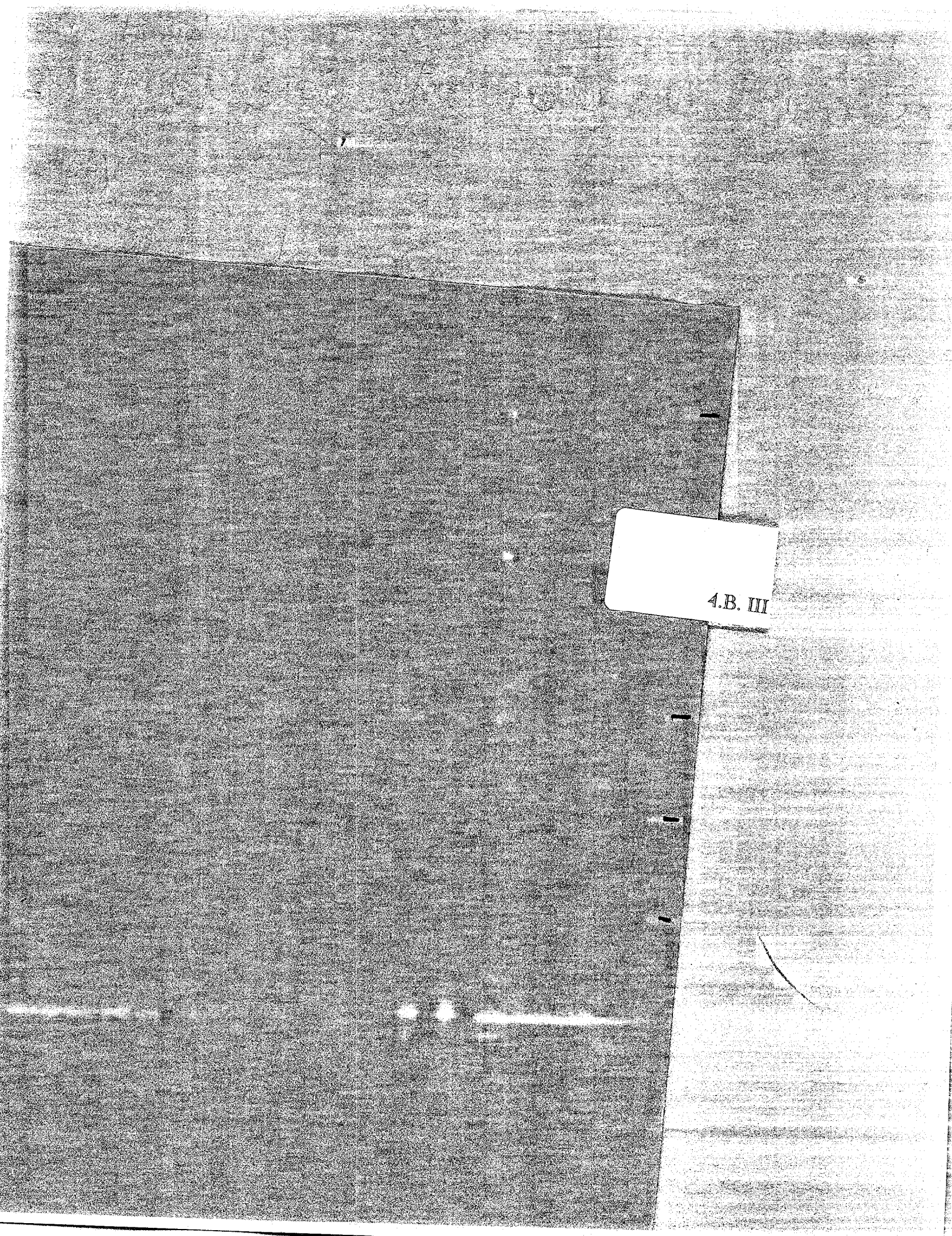


PHOTO 14 **Adrenal cortex:** 3000mg/kg/day×26 weeks, female, ×100, No significant change (Not different from treated group of 3000mg/kg/day×13 weeks and control group)


4.B. III

Safety of Cultured *Agaricus blazei* Murrill (Iwade Strain 101) (Japanese name ; Himematsutake) Preparation, ABME, for Humans in Relatively Long Term Oral Administration.

Shiro Suzuki, M.D., Ph.D.
Tsu Health Clinic
799-7, Kannonji-cho, Tsu, Mie, 514-0062, JAPAN

IWADE RESEARCH INSTITUTE OF MYCOLOGY CO.,LTD.
1-9, SUEHIRO-CHO, TSU, MIE, 514-0012, JAPAN TEL : +81 59 228 5786/FAX : +81 59 224 4661 [e-mail]iwaderim@ztv.ne.jp

November, 17, 1999

Letter of Confirmation

To whom it may concern:

This is to confirm that the test substance, ABME (*Agaricus blazei* Murrill Extract) prepared from cultured *Agaricus blazei* Murrill[Iwade Strain 101], (Japanese name; "Himematsutake"), used for 12-week human study, was identical with the substance, ABME (*Agaricus blazei* Murrill Extract), which has been applied by Iwade Research Institute of Mycology Co., Ltd., as a new dietary ingredient to Food and Drug Administration in the U.S.A.

This is also to confirm that the above mentioned 12-week human study was conducted by Dr. Shiro Suzuki, former professor of 3rd Internal Medicine Department at Mie University School of Medicine, who currently practices at Tsu Health Clinic in Mie, Japan.

Toshimitsu Sumiya

Toshimitsu Sumiya
President
Iwade Research Institute
of Mycology Co., Ltd.
1-9 Suehiro-cho, Tsu
Mie-pref., Japan

Introduction

Several kinds of mushrooms have been used for the maintenance of health or therapy for some disease, for instance, Kofuki Sarunokoshikake or Maitake have been used as diuretics or carcinostatic substance. Among these mushrooms, Japanese researchers, Dr. Iwade et al., noticed that *Agaricus blazei* Murrill (Iwade Strain 101) had most potent anticancer activity in animal experiment and that the nature of the activity was immune modifying one by its polysaccharide, D-glucan. Recently new method to extract D-glucan rich fraction from *Agaricus blazei* Murrill Strain was developed, and resultant extract was named ABME (*Agaricus blazei* Murrill Extract). This report describes the safety of ABME for humans in relatively long term, 12 weeks, oral administration.

Methods of study

ABME is mucous dark brown fluid. Nine persons administered orally daily dose of 30ml of ABME 3 times a day, 10 ml each at morning before breakfast, after lunch, and at night before sleep, for 12 weeks. At the beginning of the test and every other week thereafter, blood pressure estimation, urinalysis, and hematological and biochemical examination of the blood were undertaken. Measurement of body weight was performed at the beginning and the end of the test. Questionnaire for any complaints during administration was done every other week. Nine persons, 4 males and 5 females with age range from 29 to 67, were explained the details of test schedules and the purpose of the test, then all persons gave consent to enroll the test. Table Ⅰ showed the age and sex of nine persons including body weight and other special feature if any. Table Ⅱ showed the testing items. All the items were tested at the beginning and every 2 weeks thereafter, but HbA1c estimation was performed in 4 weeks interval.

Results

Subjective complaints and general condition

During the course of study any complaints atributing ABME administration were not observed. Body weight and blood pressure estimation and urinalysis showed no significant changes during the observation period.

Results of laboratory investigation

Serum total protein and albumin

The change of serum total protein was showed in Fig. 1. All the data were within normal range and no specific change was observed during administration. Serum albumin levels (Fig. 2) were also the same showing no special change during

observation period.

The change of ZTT levels was shown in Fig. 3. There are no special changes during administration indicating no special change of serum protein subfraction.

The change of serum enzyme levels such as GOT, GPT, LDH, ALP, LAP, γ-GTP were shown in Fig. 4, 5, 6, 7, 8, 9. All the data showed no adverse effect of ABME on liver function. Rather GPT and γ-GTP of 67-year-old male person having diabetes showed improving during ABME administration.

The change of serum lipids such as total cholesterol and triglyceride showed in Fig. 10, 11, and there was no special change during administration, i.e. not only persons having initial hyperlipidemia but also persons having normal range of lipids showed no special change during observation period.

The change of BUN, creatinine and uric acid were shown in Fig. 12, 13, 14, and there was no significant change of each value during administration. In a case of 34-year-old male slight rise BUN was observed at 10 week's bled, but 2 weeks later it returned to normal suggesting temporary rise due to unknown cause.

The change of HbA1c levels which was estimated every 4 weeks interval was shown in Fig. 15, and also shown no significant change at all including one case of diabetes having elevated HbA1c levels of 6 %.

Hematological data were shown in Fig. 16, 17, 18, 19, 20, each the change of RBC, WBC, hemoglobin, hematocrit, and platelet, indicating no significant change during administration. Leucocyte differential count was also estimated, and no significant change was observed (data not shown).

Conclusion

Oral ABME administration of 30 ml a day for 12 weeks in 9 persons showed no adverse effect on hematology, enzyme biochemistry, urinalysis, and kidney function. Rather initial elevation of GPT and γ-GTP of one case showed improvement during administration. Therefore the safety of oral administration of ABME was confirmed in relatively long term.

Table I Nine persons enrolled to the test

	Sex	Age	Body weight		Blood pressure		Others
			Before	After	Before	After	
K.M.	M	49	65.8	65.7	150/ 91	136/ 89	Low grade hypertension
K.S.	M	67	58.3	58.9	156/ 95	177/100	HT and IGT
H.N.	M	49	82.3	81.0	157/101	147/97	HT and Hyperlipidemia
Y.I.	M	34	81.0	80.9	142/ 90	135/ 81	
A.M.	F	33	60.4	57.9	131/ 78	104/ 47	
K.S.	F	65	46.1	44.5	135/ 83	111/ 68	
S.M.	F	42	54.9	54.0	117/73	102/ 61	
E.S.	F	29	45.8	46.6	93/ 59	106/ 58	
S.K.	F	65	47.5	47.6	132/73	124/ 76	

Table II Tests performed during ABME administration

Blood pressure, Body weight, Urinalysis, Complete blood count and WBC differential count, Total protein, Albumin, A/G, ZTT, ALP, LAP, GOT, GPT, LDH, γ-GTP, Total cholesterol, Triglyceride, BUN, Creatinine, Uric, acid, HbA1c (every 4 weeks)

End of report

Fig. 1 Serum total protein

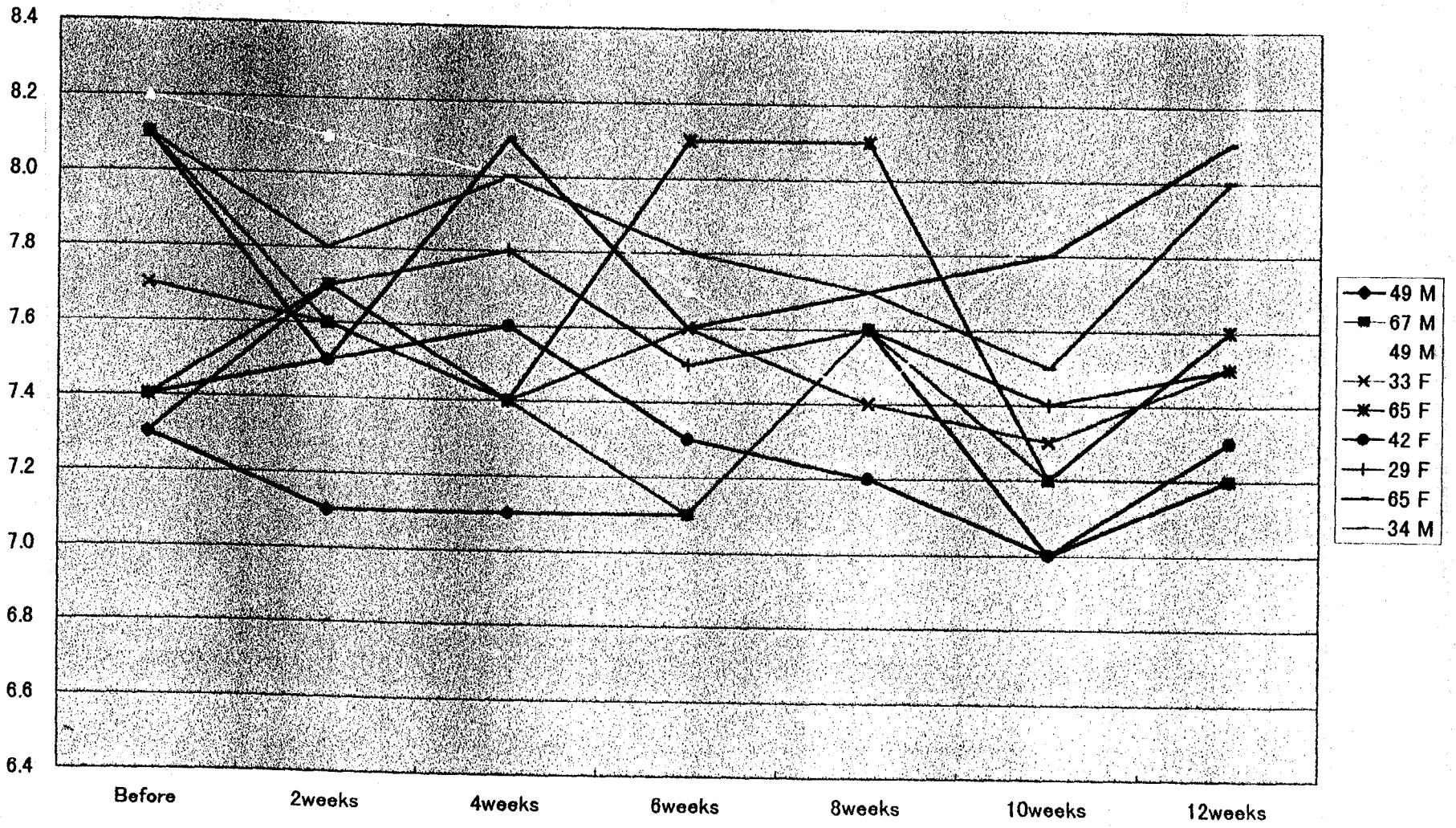


Fig. 2 Albumin

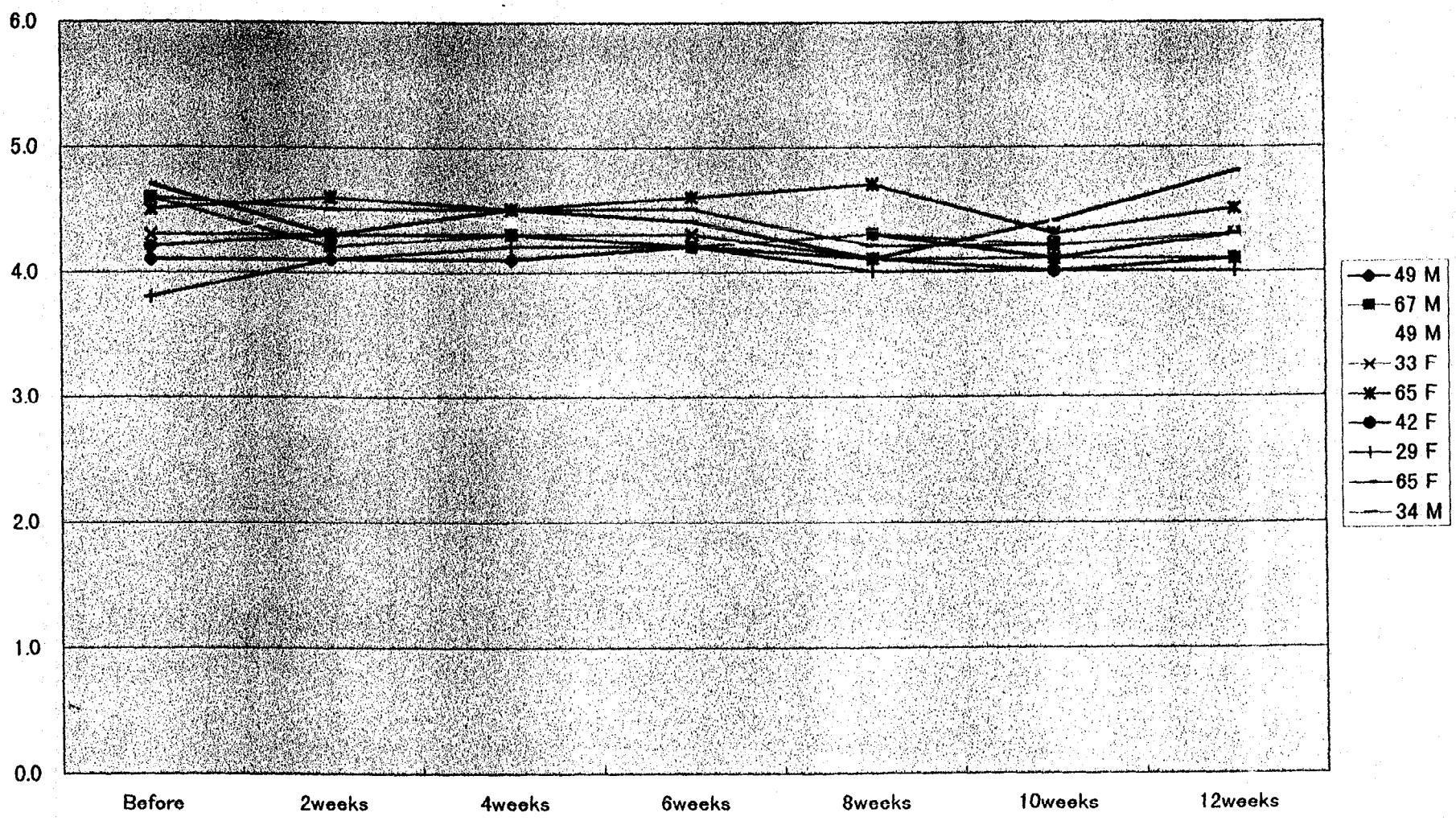


Fig. 3 ZTT

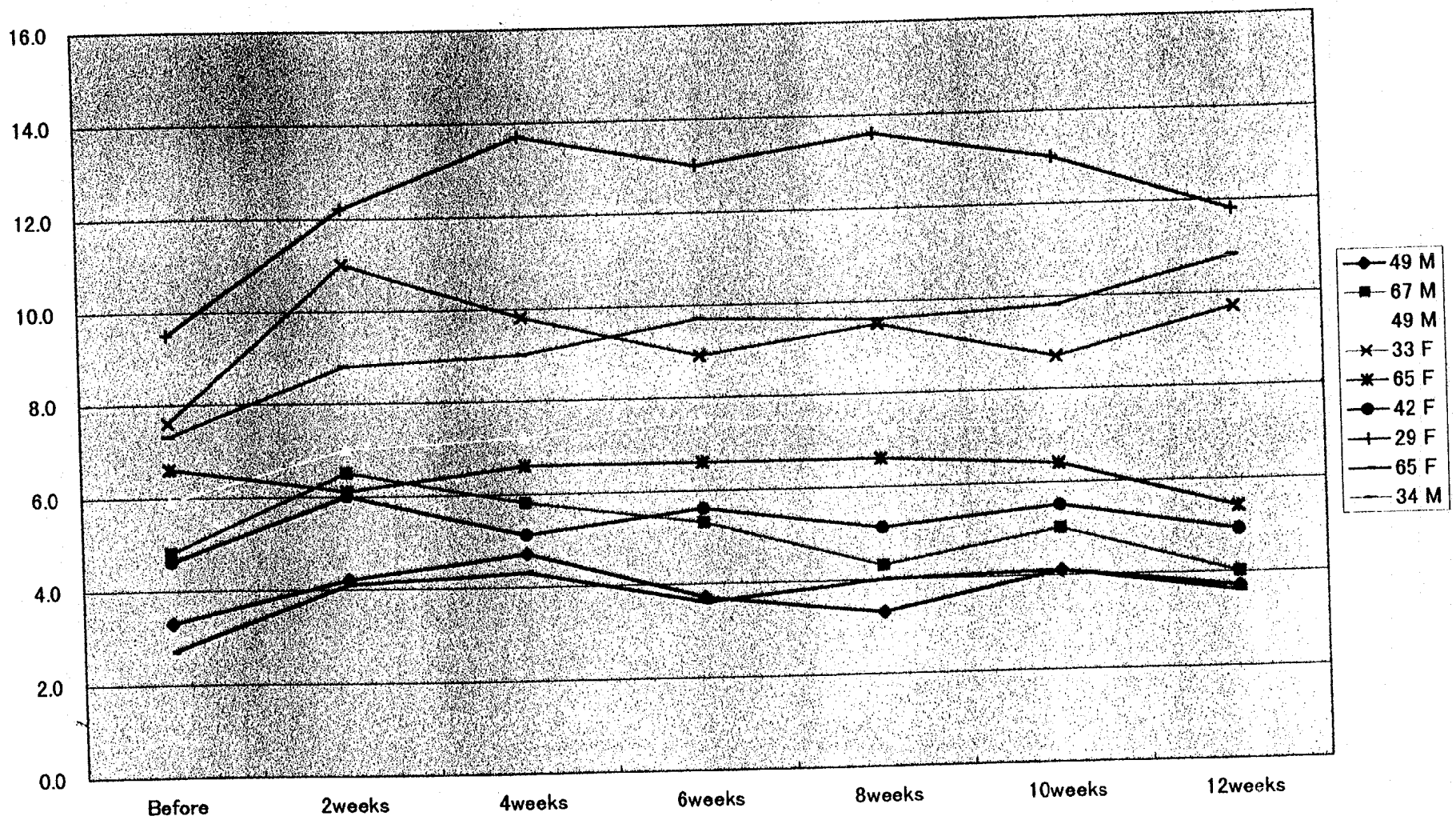

16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Before
2weeks
4weeks
6weeks
8weeks
10weeks
12weeks
49 M
67 M
49 M
33 F
65 F
42 F
29 F
65 F
34 M

Fig. 4 ALP

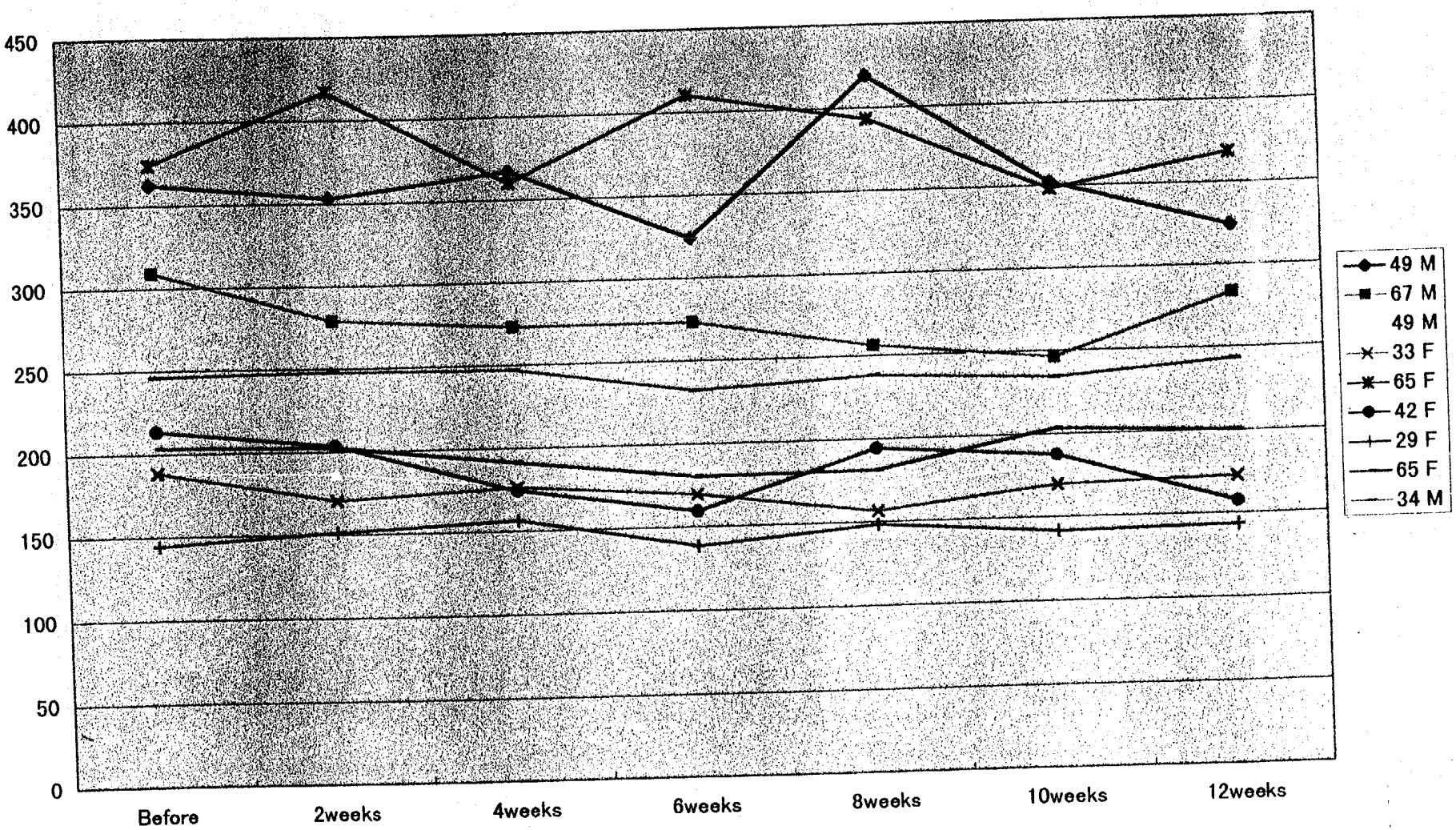

450
400
350
300
250
200
150
100
50
0
Before
2weeks
4weeks
6weeks
8weeks
10weeks
12weeks
49 M
67 M
49 M
33 F
65 F
42 F
29 F
65 F
34 M

Fig. 5 LAP

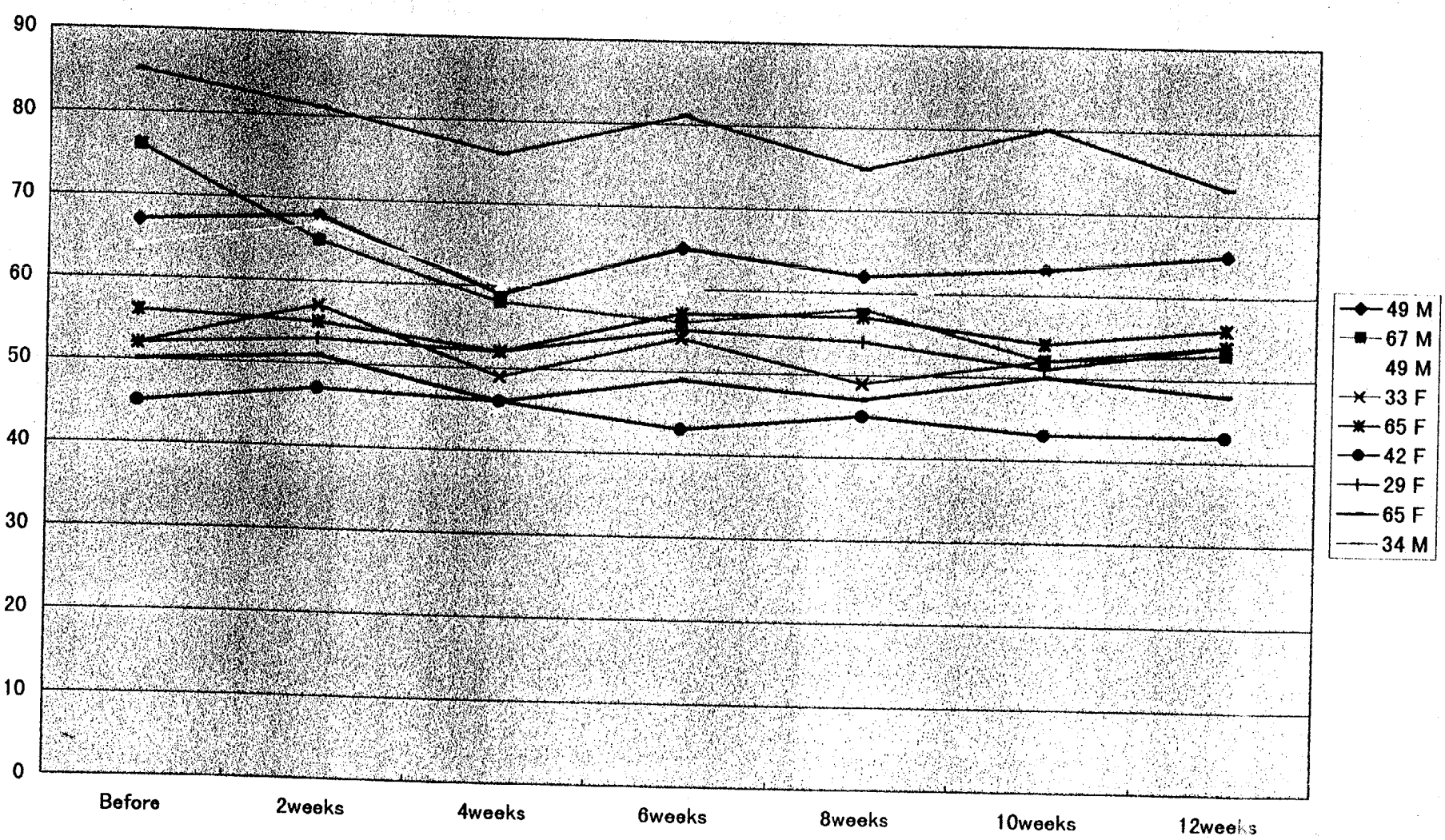

90
80
70
60
50
40
30
20
10
0
Before
2weeks
4weeks
6weeks
8weeks
10weeks
12weeks
49 M
67 M
49 M
33 F
65 F
42 F
29 F
65 F
34 M

Fig. 6 GOT

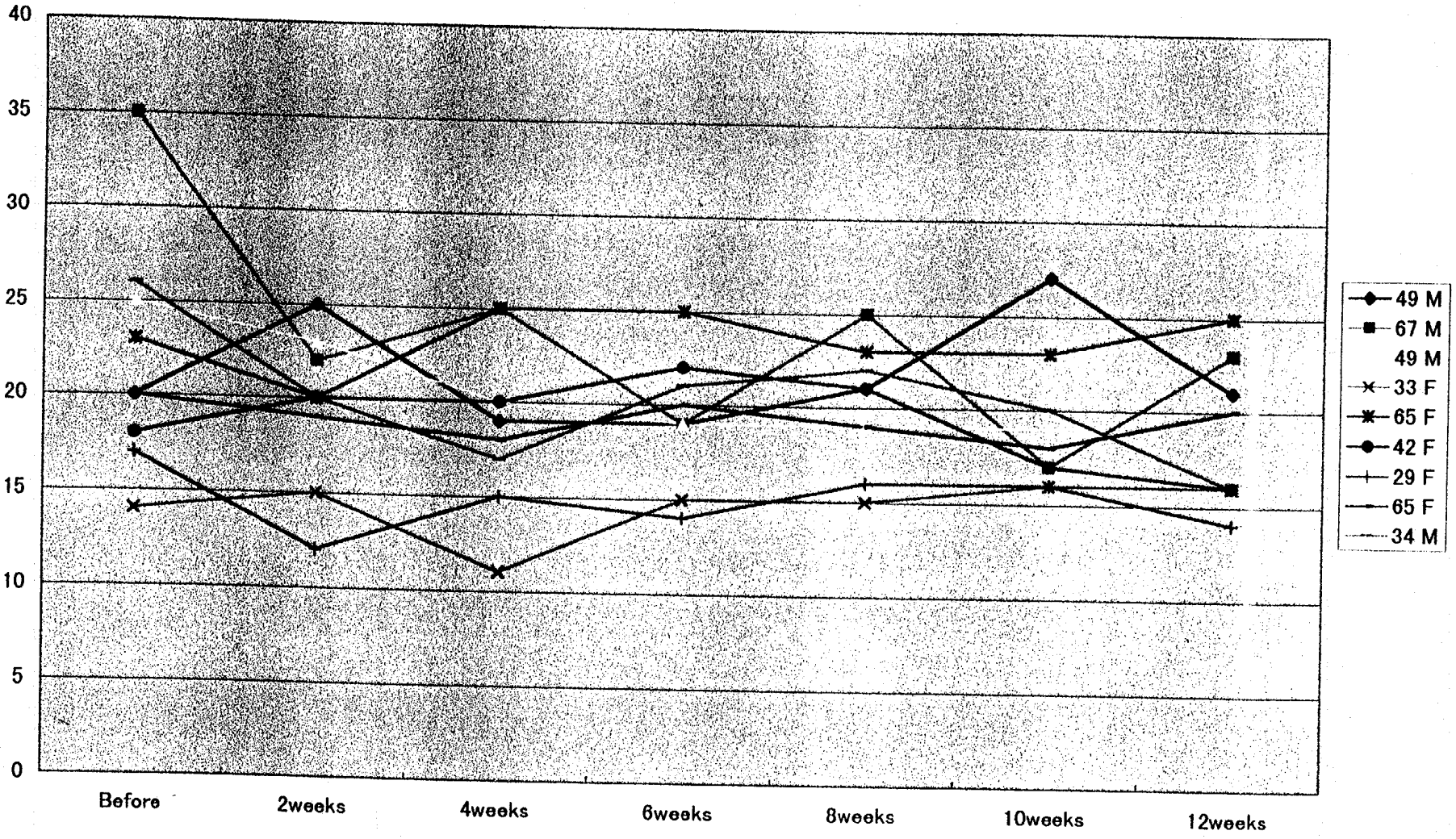

40
35
30
25
20
15
10
5
0
Before
2weeks
4weeks
6weeks
8weeks
10weeks
12weeks
49 M
67 M
49 M
33 F
65 F
42 F
29 F
65 F
34 M

Fig. 7 GPT

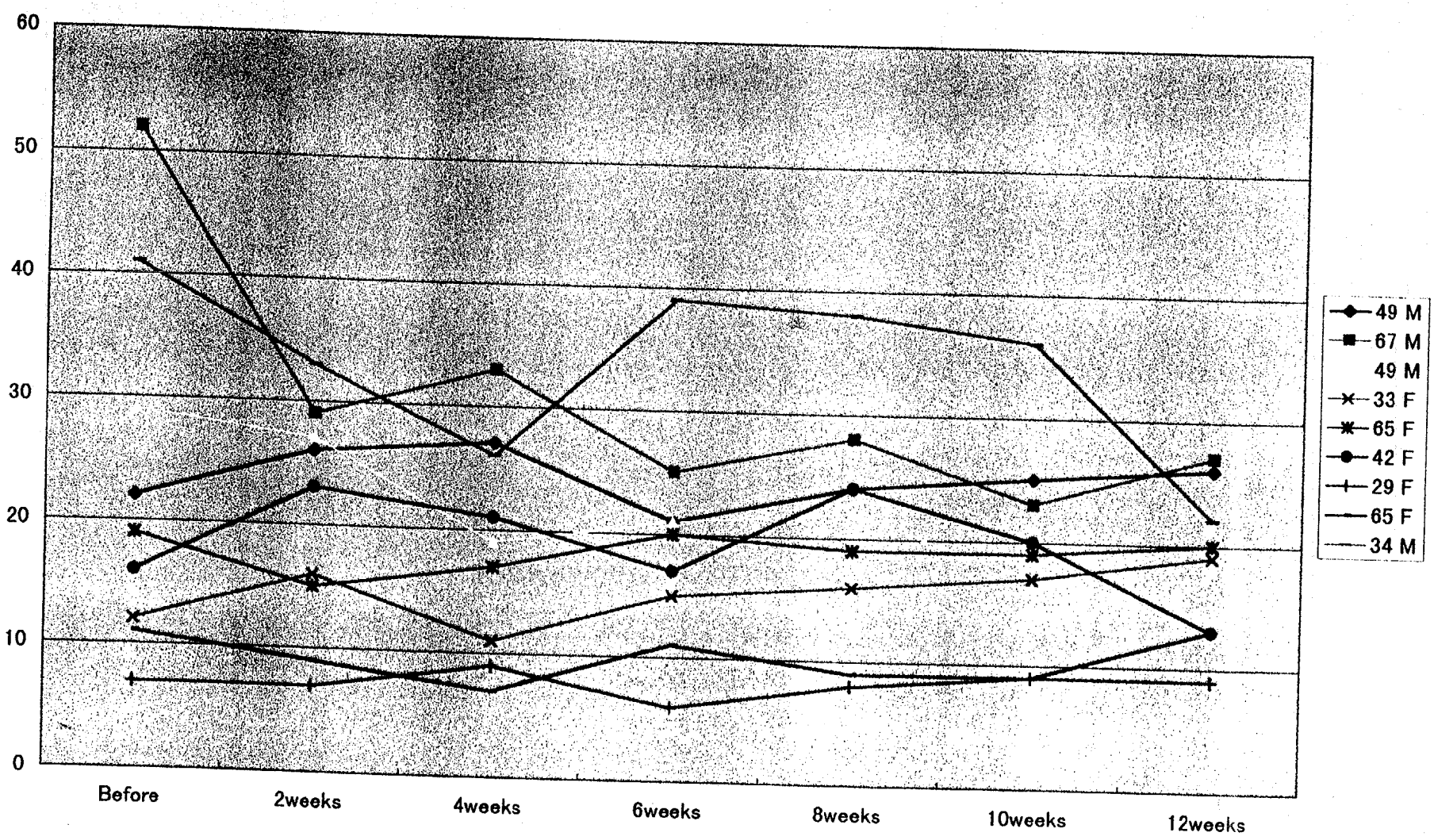

60
50
40
30
20
10
0
Before
2weeks
4weeks
6weeks
8weeks
10weeks
12weeks
49 M
67 M
49 M
33 F
65 F
42 F
29 F
65 F
34 M

Fig. 8 LDH

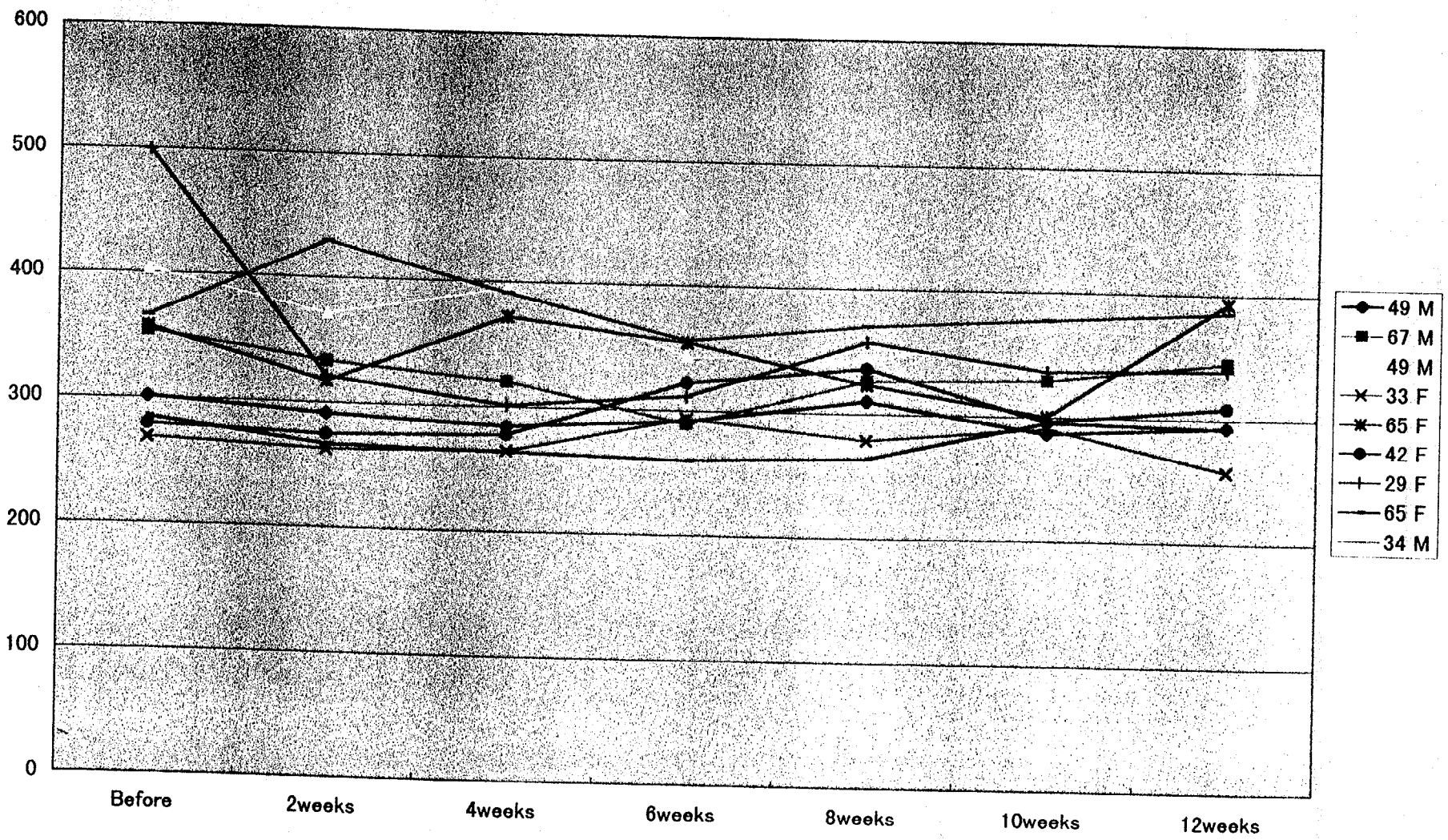

600
500
400
300
200
100
0
Before
2weeks
4weeks
6weeks
8weeks
10weeks
12weeks
49 M
67 M
49 M
33 F
65 F
42 F
29 F
65 F
34 M

Fig. 9 γ—GTP

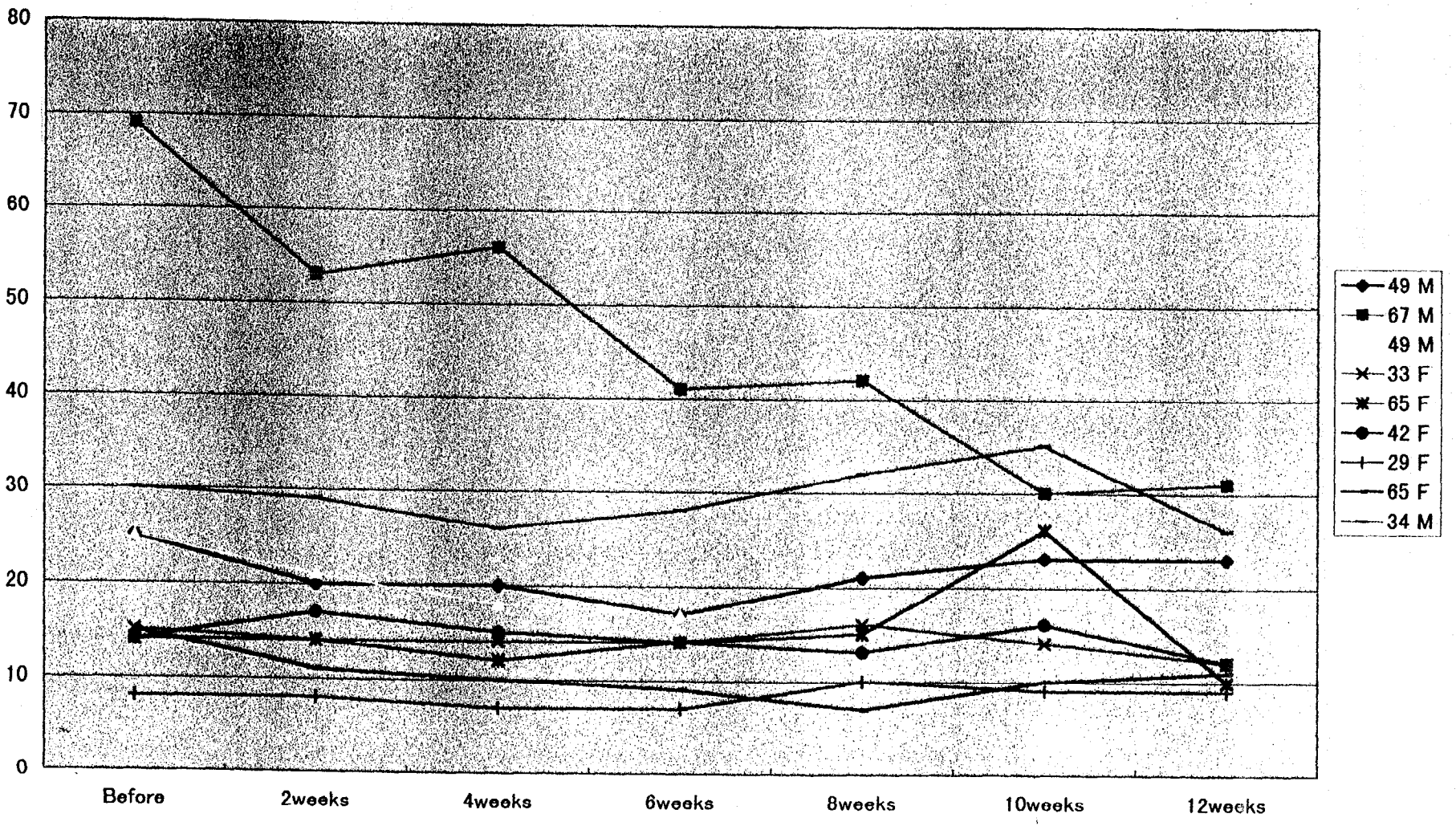

80
70
60
50
40
30
20
10
0
Before
2weeks
4weeks
6weeks
8weeks
10weeks
12weeks
49 M
67 M
49 M
33 F
65 F
42 F
29 F
65 F
34 M

Fig. 10 Total cholesterol

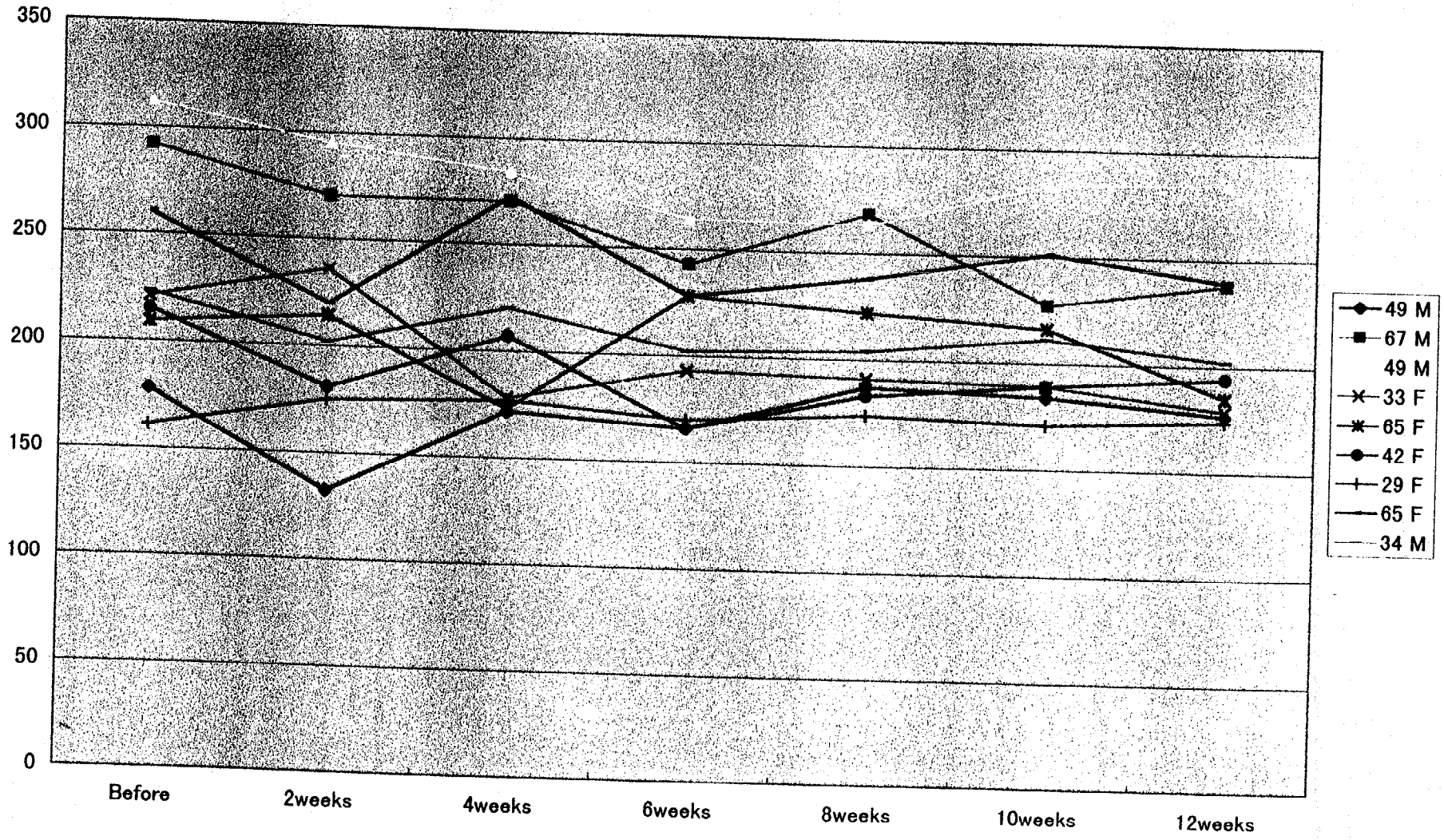


Fig. 11 Triglyceride

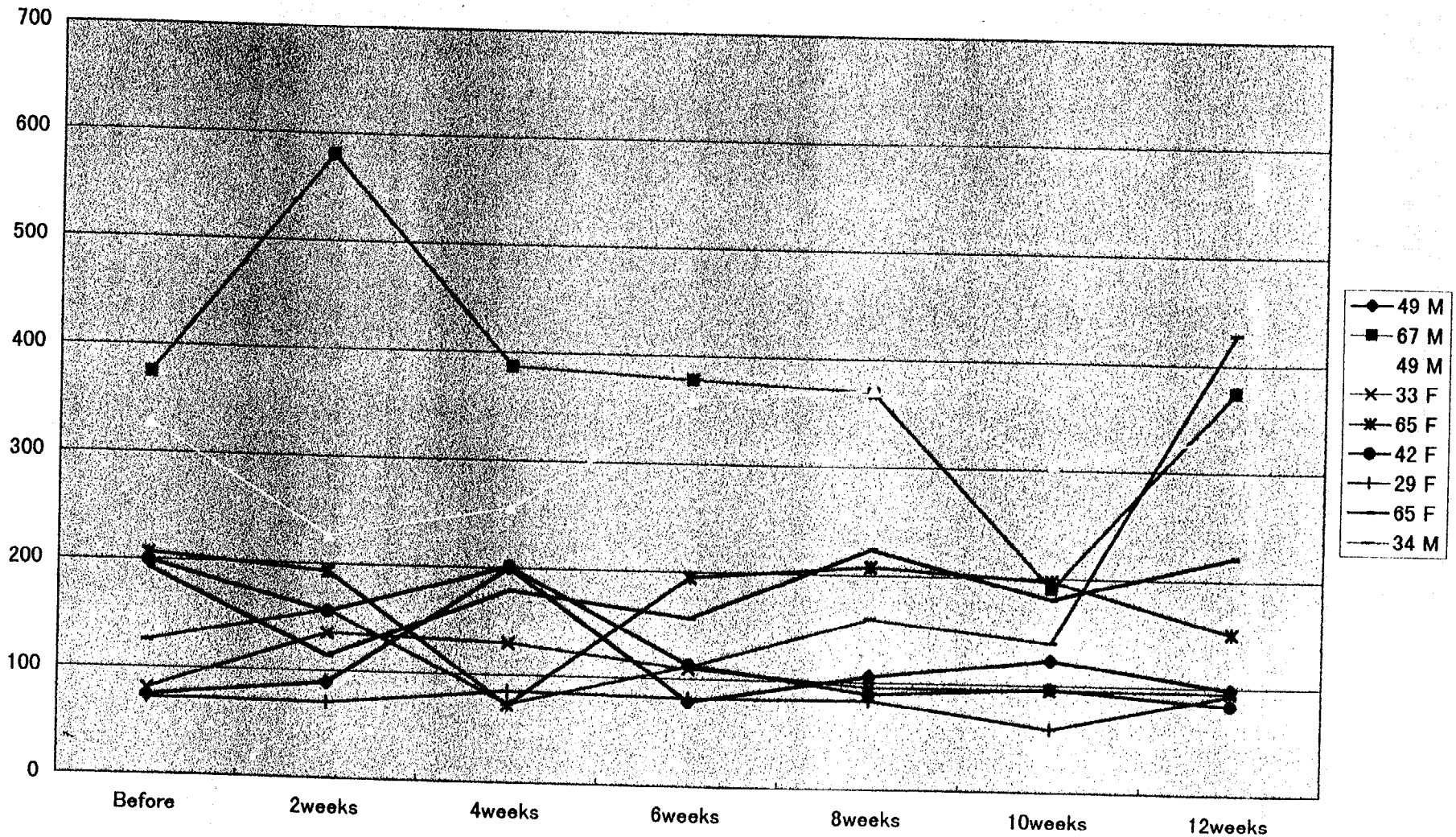


Fig. 12 BUN

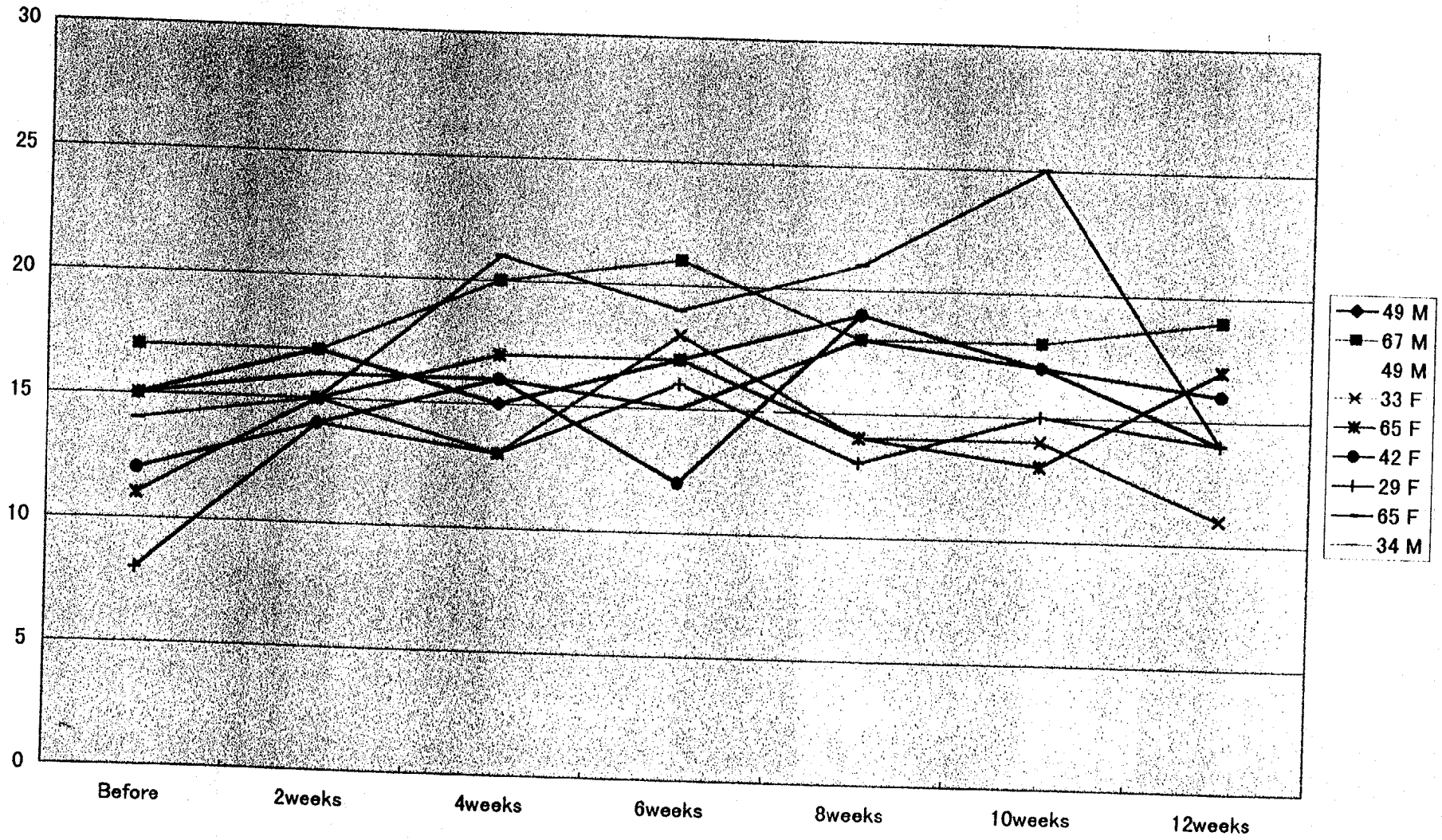

30
25
20
15
10
5
0
Before
2weeks
4weeks
6weeks
8weeks
10weeks
12weeks
49 M
67 M
49 M
33 F
65 F
42 F
29 F
65 F
34 M

Fig. 13 Creatinine

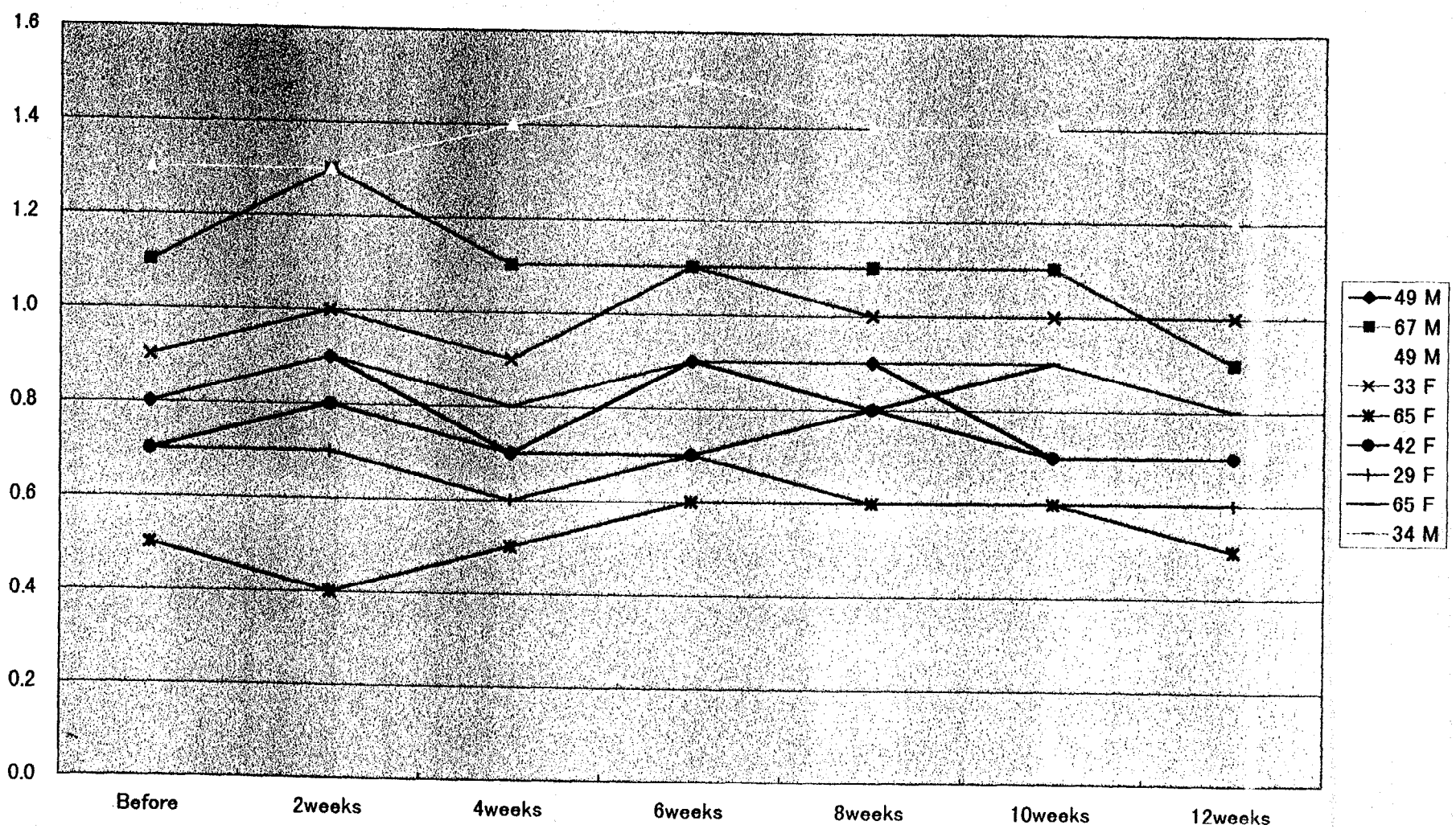


Fig. 14 Uric acid