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## Blood Pressure-Lowering Activity Present in the Fruit Body of *Grifola frondosa* (Maitake). I

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The fruit body of *Grifola frondosa* (maitake), Basidiomycetes, was confirmed to contain a substance with blood pressure-lowering activity. When powdered fruit body of maitake was given orally to spontaneously hypertensive rats (SHR), blood pressure reduction was observed, in contrast to the control group in which the blood pressure increased with ageing. Ether-soluble (ES) and hot water-soluble (WS) fractions were prepared from the fruit body and their hypertensive action was examined. Blood pressure-lowering activity was found when the dried ES fraction was administered orally at 30 mg/kg, but the WS fraction was inactive. Thus, the ES fraction was further separated into acetone-soluble (ES-AS) and insoluble (ES-AP) fractions. Administration of 20 mg/kg dried material caused a blood pressure reduction of about 45 mmHg in the former case and 65 mmHg in the latter as compared with the control (about 220 mmHg). When the ES-AP fraction was separated to 5 subfractions by thin layer chromatographic analysis, the most potent activity was observed in the subfraction which was detected on the spot of  $R_f=55.3$ . When authentic antihypertensive agents were examined in combination with maitake, a hypotensive effect was observed on administration of reserpine. This suggests that the blood pressure-lowering effect of maitake was brought about by a mechanism other than sympatholytic action.

**Keywords**—*Grifola frondosa* (maitake); blood pressure; blood pressure-lowering activity; SHR

The presence of various biologically active materials in the fruit bodies of Basidiomycetes has been known for a long time. Crestine, obtained from *polystictus* in the family of Polyporaceae, and lentinan, obtained from Shiitake (*Lentinus edodes*), have already been used clinically as carcinostatic materials, it is also reported and the fruit bodies of *Lentinus edodes* and *Ganoderma lucidum* possess blood pressure-lowering activity.<sup>2-4)</sup> On the other hand, Yadomae *et al.*<sup>5)</sup>, Mizuno *et al.*<sup>6)</sup> and the authors<sup>7)</sup> have found a polysaccharide of lentinan-like structure in the fruit body of *Grifola frondosa* and showed that it has antitumor activity on intraperitoneal administration. However, no report on other biological activities of the edible mushrooms has appeared. Thus, the present experiment was carried out to examine whether a material showing blood pressure-lowering activity is present in the fruit body of *Grifola frondosa* (maitake), as in *Lentinus edodes* (shiitake) and *Ganoderma lucidum* (mannentake).

### Materials and Methods

**Substance**—Powdered fruit bodies of maitake (200 g, passed through 60 mesh or less) were repeatedly extracted under reflux with 1200 ml of diethyl ether at 50 °C. After filtration, the filtrate was dried *in vacuo*, giving 4 g of the ether-soluble fraction (ES fraction). The residue (EP fraction) was extracted with 3000 ml of hot water at 120 °C by the autoclave method to obtain 60 g of the dried hot water-soluble fraction (WS fraction) and hot water-insoluble residue (WP fraction). The ES fraction was further treated with 700 ml of acetone to obtain 2.7 g of the dried acetone-soluble (ES-AS) fraction and 0.9 g of the dried acetone-insoluble (ES-AP) fraction. Soluble materials in every extracted fraction were obtained by centrifugation. This fractionation procedure is illustrated in Fig. 1.

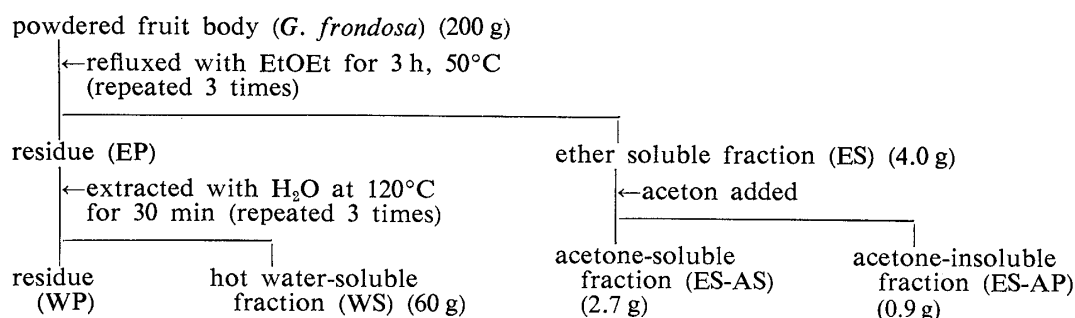


Fig. 1. Fractionation Procedure

(g): dry weight.

**Animals**—Six-week-old male spontaneously hypertensive rats (SHR), obtained from Charles River Japan, were bred on a commercial rat feed, CRF-1, (Oriental Yeast Co., N feed) in an animal experimental room (27°C) and those in which the blood pressure reached 180–220 mmHg were used (around 10 weeks old).

**Preparation of Feed Containing Powdered Maitake or Extracted Substance**—Either the dried powder of maitake fruit body at a ratio of 20 or 30% (w/w) or extracted EP or WP fraction at 20% was mixed with commercial rat feed. Each mixture was well kneaded with distilled water. The paste was cut into pieces (3 × 3 × 3 cm) and dried at 80°C for about 20 h. The completely dried feeds were used as G feed, EP feed and WP feed, respectively. A mixture of 1000 g of CRF-1 and 800 ml of WS fraction (containing 2.7 g of sugar) was used as WS feed. ES feed was prepared homogeneously from 1000 g of the commercial solid feed and 4.0 g of ES fraction and the mixture was dried sufficiently.

**Thin Layer Chromatographic (TLC) Analysis**—TLC analysis was conducted to separate constituents in the ES-AP fraction. The ES-AP fraction (100 µg) was spotted on a silica gel plate (Kieselgel 60 Art 5721: Merck), and developed with chloroform–methanol–water (130:50:8) mixture for 1.5 h. After drying, No. 1–5 subfractions of ES-AP fraction were detected by spraying 30% sulfuric acid.

**Measurement of Blood Pressure**—The cutaneous blood vessels of a rat were dilated preliminarily by warming the animal in a thermal box at 37 ± 2°C for 10 min. Then, the systolic blood pressure in the caudal artery was recorded using a rat blood pressure measurement apparatus (FK-A 93, Kyoto University system).<sup>8)</sup>

In the experiments in which prepared feed was given, the blood pressure of rats kept on normal feed (N feed) was taken as the control. When the samples were administered by gavage, after fasting for 18 h, blood pressure was measured twice with a one-hour interval and the second value was taken as the control. Immediately after the second measurement, each extracted fraction was administered by gavage to the rats and the blood pressure was measured at 1, 3, 5 and 7 h thereafter.

**Statistical Treatment**—Student's *t*-test was used to examine the significance of differences among groups.

## Results

### Blood Pressure-Lowering Activity in G Feed

First, whether blood pressure-lowering activity was present in the powder of maitake was examined. Male SHR (10 weeks old) were bred on G feed containing 20 and 30% (w/w) of the powdered fruit body of maitake. As shown in Fig. 2, at both doses, blood pressure was reduced by about 40 mmHg from that of the control group (about 220 mmHg) bred on N feed on the 7th day. This blood pressure reduction was significant. The reduction was maintained until discontinuation of G feed and, as soon as N feed was started, the value increased to the control level. However, a blood pressure drop of about 40 mmHg was again observed when G feed was subsequently given. Body weight and food and water consumption of rats in this study are indicated in Figs. 3 and 4. These results indicated that a substance possessing hypotensive activity is present in maitake. Thus, the effects obtained by the combined use of authentic antihypertensive agents having various action mechanisms together with G feed was studied.

### Effect of the Combined Use of an Antihypertensive Agent with G Feed

SHR with a blood pressure of around 170 mmHg (obtained by giving G feed for 7 d) were

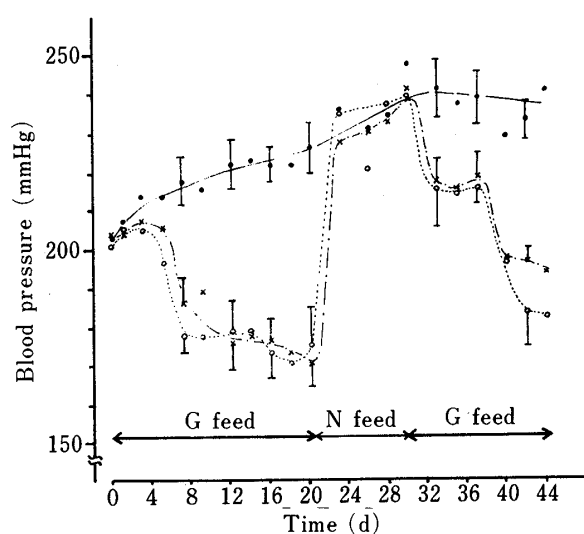


Fig. 2. Blood Pressure-Lowering Activity of G Feed

Five male SHR (10 weeks old) were bred on G feed containing 20 and 30% (w/w) of maitake.  
 ●—●, N feed; ×—×, 20% G feed; ○—○, 30% G feed.

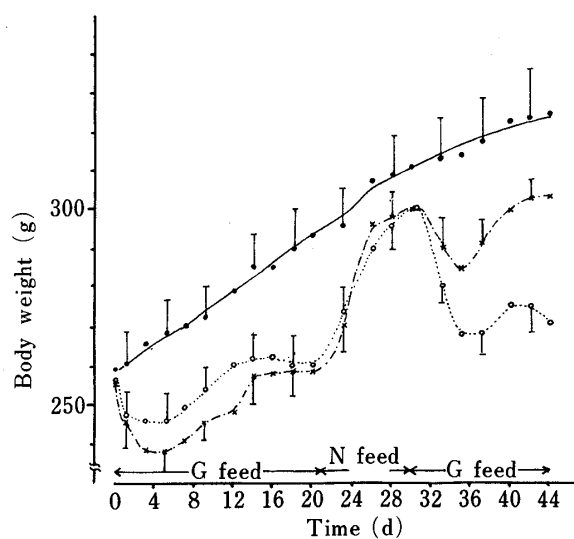


Fig. 3. Profile on Body Weight of SHR Given G Feed

Five rats were used in this experiment.  
 ○—○, N feed; ×—×, 20% G feed; ○—○, 30% G feed.

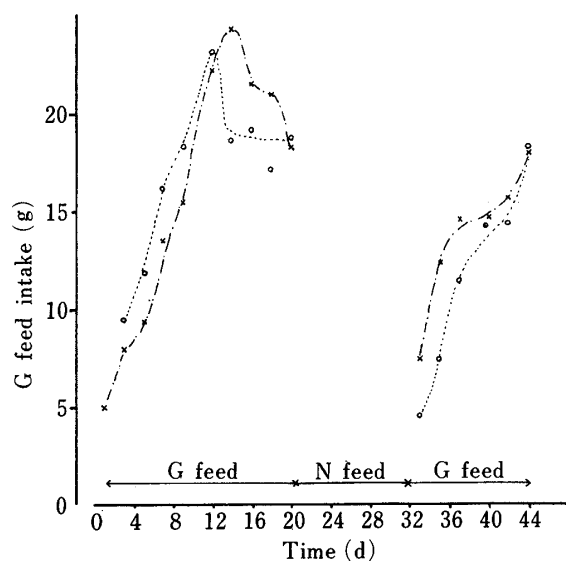


Fig. 4. Profile of Consumption by G Feed in SHR

Intake of N feed in a day was  $18.1 \pm 0.7$  g/rat and that of water in a day was  $26 \pm 3$  ml/rat (average values of 5 rats).  
 ×—×, 20% G feed; ○—○, 30% G feed.

administered with 4 kinds of antihypertensive agents (hexamethonium chloride (suspended in distilled  $H_2O$ ), hydralazine hydrochloride, hydrochlorothiazide and reserpine (suspended with 0.2% CMC-Na), Sigma) orally at 1 mg/kg. As shown in Fig. 5, further reduction in blood pressure from the level obtained with G feed alone was observed only when reserpine was given. These results suggest that the action mechanism of the antihypertensive material in maitake differs from that of reserpine, which is sympatholytic. Further work on the action mechanism is planned.

#### Blood Pressure-Lowering Action of ES, EP, WS and WP Feed

To examine which component of maitake fruit body showed blood pressure-lowering activity, ES, EP, WS and WP fractions were separated as shown in Fig. 1. Then, feeds containing the respective fractions (4 g of ES, 195 g of EP, 60 g of WS and 135 g of WP) were prepared. As shown in Fig. 6, when these feeds were given to SHR, the ES feed gave a blood

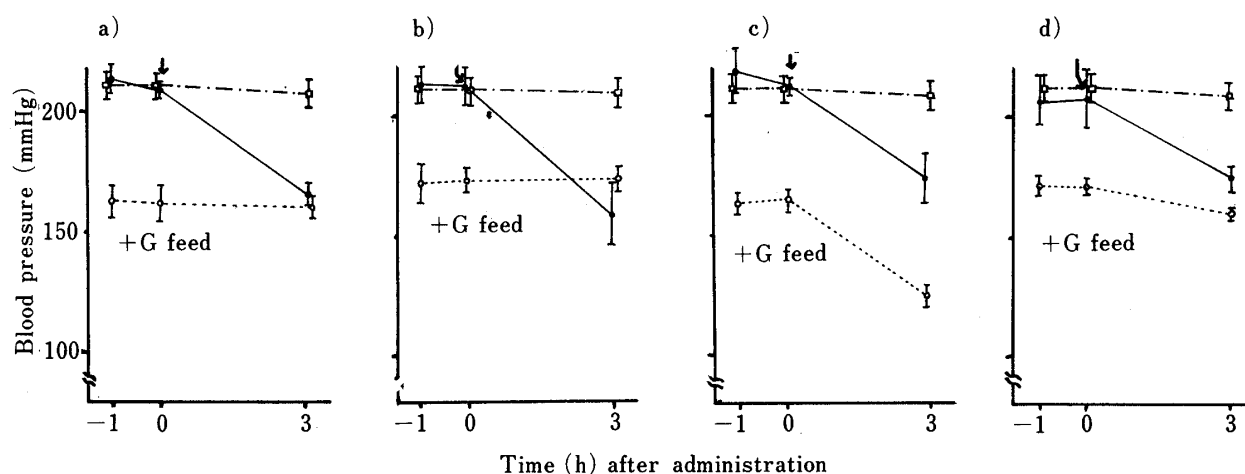


Fig. 5. Profiles of Blood Pressure on Combined Administration of Authentic Antihypertensive Agents with G Feed

a) Hexamethonium chloride, b) hydralazine dihydrochloride, c) reserpine, d) hydrochlorothiazide.

Six SHR, the blood pressure of which was reduced to 170 mmHg by feeding on G feed (○-----○), were given 4 kinds of antihypertensive agent by orally at 1 mg/kg each. This experiment was repeated twice (3 rats each time).

□-----□, non-treated (control) high blood pressure SHR; ●-----●, antihypertensive agent-treated high blood pressure SHR.

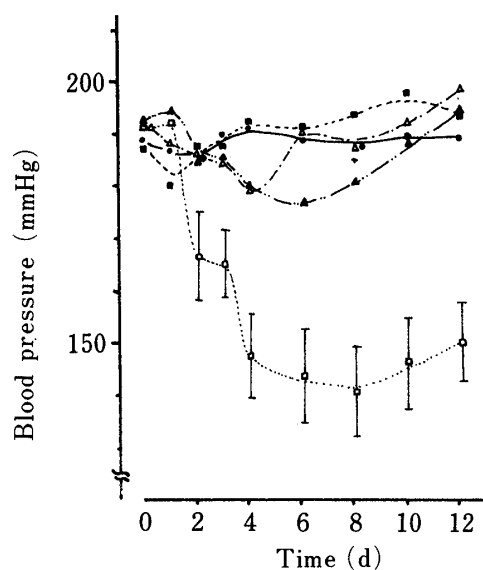


Fig. 6. Profile of Blood Pressure-Lowering by ES, EP, WS and WP Feeds

Each extracted fraction was mixed with CRF-1 feed and given to 5 SHR. This intakes of feed and water per day were about  $15.8 \pm 2.5$  g/rat and  $28 \pm 4$  ml/rat. The body weight did not decrease markedly when this feed was given.

○-----○, N feed; □-----□, ES feed; ■-----■, EP feed; △-----△, WS feed; ▲-----▲, WP feed.

pressure reduction of about 50 mmHg on the 2nd day; this differed significantly from the control value in the group given N feed (CRF-1). The blood pressure-lowering effect continued for the whole period of ES feed administration. On the other hand, the hot water-extracted fraction (WS) was inactive, even though the same fraction of *Lentinus edodes* and *Ganoderma lucidum* possessed the activity. The ES fraction, which was expected to contain lipid components principally, was examined in detail.

#### Blood Pressure-Lowering Activity in the ES Fraction

Since feed consumption was reduced when ES fraction was incorporated in the feed, a fixed dose per body weight was administered by oral gavage. Three doses (36, 72 and 360 mg/kg) of the ES fraction suspended in 33% propylene glycol were given to the rats after 18 h of fasting. In the group given propylene glycol alone, no change in blood pressure

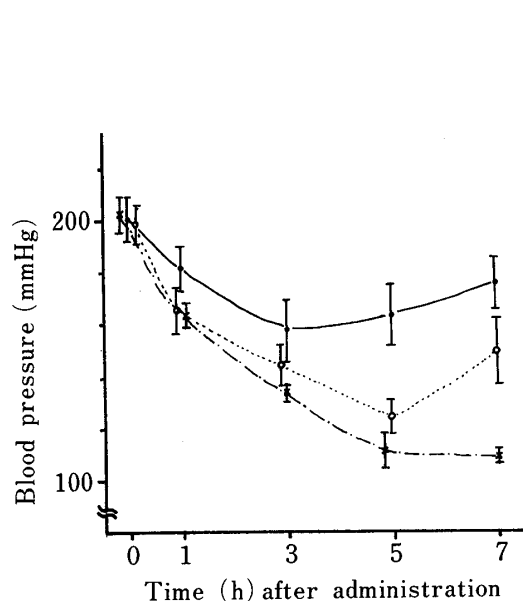


Fig. 7. Blood Pressure-Lowering Activity of the ES Fraction on Oral Administration

Three doses of the ES fraction suspended in 33% propylene glycol were given to 5 rats after 18 h of fasting.

●—●, 36 mg/kg; ○---○, 72 mg/kg; ×---× 360 mg/kg.

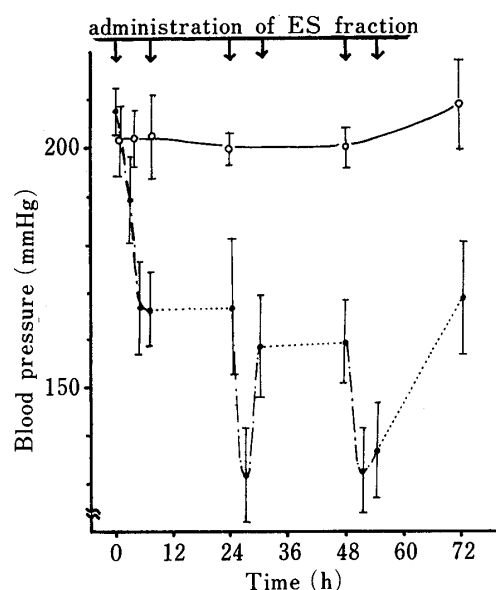


Fig. 8. Blood Pressure-Lowering Activity on Successive Administration of the ES Fraction

When 140 mg/kg of ES fraction was administered to 4 rats orally twice a day, about 50 mmHg reduction of blood pressure was observed.

○—○, control; ●---●, ES fraction.

occurred. As shown in Fig. 7, blood pressure reduction was already observed within 1 h of the administration and the extent was about 90 mmHg at 360 mg/kg and 40 mmHg at 36 mg/kg, being dose-dependent. The duration of blood pressure reduction was longer at the large dose. However, as the hypotensive action of the ES fraction was not durable in spite of its immediate effect, the fraction was administered twice a day for several days. Thus, 140 mg/kg of the ES fraction was given at 9:00 a.m. and 5:00 p.m. for 3 d, and the changes of blood pressure were followed. The result is shown in Fig. 8. A blood pressure reduction of about 50 mmHg was caused by the first administration and, as the second dose followed when the effect started to diminish, hypotension was sustained even after 18 h. A mean value of 70 mmHg reduction with a deviation of about 40 mmHg was observed in comparison with the control group.

#### Blood Pressure-Lowering Activity in the ES-AS and ES-AP Fractions

The components of the ES fraction, which has a hypotensive effect were examined. The ES fraction was further separated into the acetone-soluble (ES-AS) and insoluble (ES-AP) fractions and 30 mg/kg of each fraction was given to rats orally. A blood pressure reduction of about 45 mmHg was observed after the administration of ES-AS fraction, while the reduction was about 65 mmHg by the ES-AP fraction, as shown in Fig. 9. It is considered that lipid and phospholipid are the principal constituents in the ES-AS and ES-AP fractions. The content of ES-AS fraction was approximately 3 times that of ES-AP fraction, but as the ES-AP fraction showed a stronger hypotensive effect, this fraction was examined in detail.

#### TLC Analysis of the ES-AP Fraction

In TLC analysis of the ES-AP fraction with chloroform-methanol-water (130:50:8) solvent, 8 components were detected. Each component was collected and extracted with a chloroform-methanol mixture (1:1). The hypotensive effects of lipid-like substances in ES-AP No. 1—5 subfractions ( $R_f$  values: No. 1=26.7, No. 2=38.5, No. 3=55.3, No. 4=57.5 and No. 5=62.1), obtained in relatively large amounts, were examined.

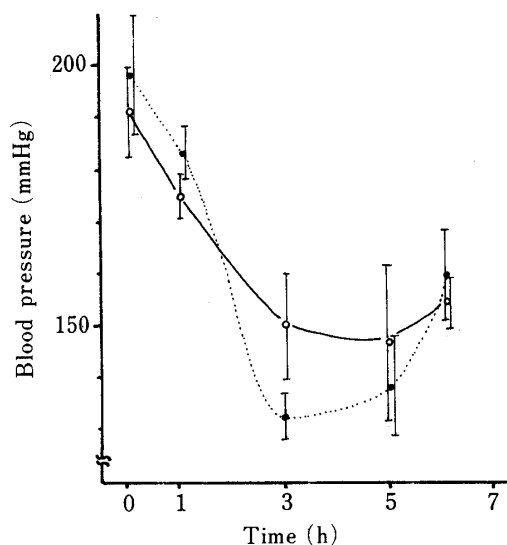


Fig. 9. Blood Pressure-Lowering Activity in the ES-AS and ES-AP Fraction

When 30 mg/kg of acetone-soluble (ES-AS) and insoluble (ES-AP) materials from ES fraction were given to 3 rats, blood pressure reductions of about 45 and 65 mmHg, respectively, were observed.

○—○, ES-AS fraction; ●---●, ES-AP fraction.

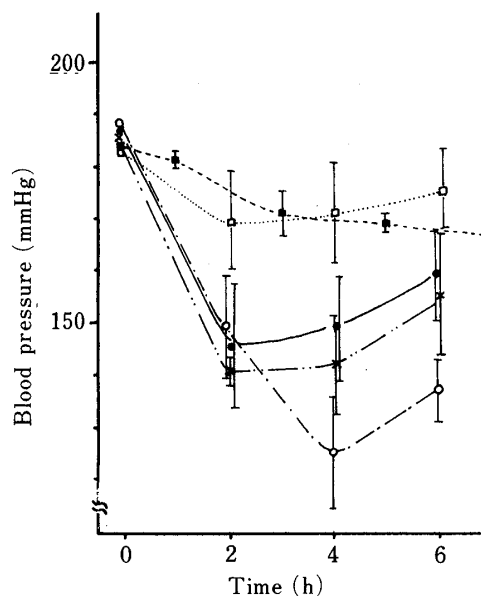


Fig. 10. Blood Pressure-Lowering Activities in the ES-AP No. 1—5 Subfractions

When 20 mg/kg of purified No. 1—5 subfractions from ES-AP fraction were administered, hypotensive effects were recognized in the cases of No. 2—4 ES-AP subfractions.

□---□, ES-AP No. 1; ●---●, ES-AP No. 2; ○---○, ES-AP No. 3; ×---×, ES-AP No. 4; ■---■, ES-AP No. 5.

### Blood Pressure-Lowering Activities in the ES-AP No. 1—5 Subfractions

When 20 mg/kg of each extracted subfraction was administered orally to rats, hypotensive effects were found in the cases of ES-AP No. 2—4 subfractions. In particular, the ES-AP No. 3 subfraction produced a blood pressure reduction of about 60 mmHg.

### Discussion

The fungi which belong to Basidiomycetes have been used as herbal medicines for a long time. Recently, studies on the biological activities of edible mushrooms have been conducted as their artificial cultivation has become possible. It has been found that shiitake and maitake are effective against essential hypertension.<sup>2,3)</sup> Thus, we examined the hypotensive action of maitake, a mushroom belonging to basidiomycetes. First, we tried to mix powder or maitake fruit body into the feed. Rats refused to take the G food initially and a drop in body weight was observed. However, they gradually got used to the G feed and, after 10 d, they consumed the same amount (about 20 g) as the control per animals per day. The body weight also started to increase at the same rate as in the control. During this breeding period, the blood pressure of the control group increased with age while significant hypotension was observed in the G feed group on the 7th day. Although this effect continued as long as G feed was given, the blood pressure increased to the control level immediately after switching the rats to N feed. Blood pressure reduction was again observed on the 3rd day when the rats were put back on G feed. This result indicates that maitake contains a substance which has an immediate and sustained hypotensive action when taken consistently.

As numerous mechanisms of blood pressure adjustment are known, we examined the combined effect of maitake with 4 types of antihypertensive agents having different mechanisms to elucidate the mechanism of hypotensive action of maitake. Rats in which the

blood pressure had already been decreased by G feed were given hexamethonium chloride, an autonomic ganglion blocking agent, hydralazine hydrochloride, a direct smooth muscle relaxant of blood vessels, reserpine, a sympatholytic agent, and hydrochlorothiazide, a diuretic antihypertensive agent. No hypotensive effect was seen except with reserpine. This result suggests that the substance in maitake causes hypotension by a mechanism other than sympatholytic action, but further work is necessary to confirm this. The fruit body of maitake was fractionated to examine which constituents showed the activity. When rats were given feeds containing fractions extracted from maitake, significant hypotension was observed only with ES feed. Interestingly, in the hot water extracted fraction, which was reported to possess activity in the cases of *Lentinus edodes*, *Ganoderma lucidum* and a *Polypore*, no such effect was detected.

Next, the ES fraction was administered to rats by gavage. As the ES fraction was insoluble water, it was given as a suspension in 33% propylene glycol. Reduction in blood pressure was observed immediately after administration, and its duration depended on the dose. These results suggested that a substance of relatively low molecular weight, which might be easily metabolized, in the ES fraction was causing the hypotensive effects. Furthermore, a blood pressure reduction of 70 mmHg on average was observed on continuous administration of the ES fraction with a peak at 3–5 h postadministration. The 33% propylene glycol solution did not affect the blood pressure-lowering activity. When the ES fraction was separated into an acetone-soluble (ES-AS) and insoluble (ES-AP) fractions both fractions showed the effect. Because a stronger effect was obtained with the ES-AP fraction, this fraction was separated into 8 subfractions by TLC with chloroform–methanol–water (130:50:8) mixture as the developing solvent. Of these, ES-AP No. 1–5, which were obtained in relatively large amounts, were examined for blood pressure-lowering activity. Lowering activity was caused by the ES-AP No. 2 ( $R_f=38.5$ ), No. 3 ( $R_f=55.3$ ) and No. 4 ( $R_f=57.5$ ) subfractions. In particular, the activity of the ES-AP No. 3 subfraction was the strongest. These results revealed that the fruit body of maitake possessed a blood pressure-lowering activity in the ether-extracted fraction, unlike other Basidiomycetes, the hot water-extracted fractions of which were reported to be hypotensive. This effect was quick, yet its duration was short. Further work to isolate the hypotensive agent, which is present in the ether-soluble, acetone-insoluble fraction, is in progress.

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