Cancer Induction in Mice by Feeding the Raw False Morel Mushroom

Gyromitra esculenta

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ABSTRACT

One of the false morel mushrooms, Gyromitra esculenta, was administered p.o. to Swiss mice that were 6 weeks old at the beginning of the experiment. The mushrooms were fed to the mice for 3 days and were followed by a semisynthetic diet for 4 days each week for life. The treatment induced tumors in the lungs, nasal cavity, blood vessels, forestomach, glandular stomach, cecum, and liver in the following incidences: 80, 10, 50, 16, 4, 28, and 6% in females, and 70, 12, 32, 18, 20, 22, and 2% in males. The light microscopic examination revealed the typical appearance of adenomas and adenocarcinomas of the lungs, adenomas and adenocarcinomas of the forestomach, adenomas and adenocarcinomas of the glandular stomach, polypoid adenomas and adenocarcinomas of the cecum, and hepatomas. The work demonstrates the carcinogenic action of the raw G. esculenta mushroom.

INTRODUCTION

Gyromitra esculenta (Fig. 1) is a wild edible mushroom belonging to the false morel mushroom family. On the North American continent, 11 such mushroom species exist, while in Europe only 2–3 are known (1). It is estimated that over 100,000 people annually consume this fungus in the United States.2 G. esculenta is also eaten in substantial quantities in other continents, particularly in Northern and Eastern Europe (2, 3). The consumption is more prevalent in rural areas, although the mushroom is also marketed in canned and dried forms in certain parts of the world (2, 3).

Systematic carcinogenesis investigations have been conducted in animals with six hydrazine ingredients of this mushroom in this laboratory. These studies clearly demonstrated the highly carcinogenic nature of these compounds which induced cancers in nearly a dozen organs and tissues of mice and hamsters (4–7).

The present work was undertaken to ascertain the possible carcinogenicity of the raw G. esculenta by p.o. lifelong intermittent administration in mice.

MATERIALS AND METHODS

Swiss albino mice from a colony randomly bred by us since 1951 were used. They were housed in plastic cages on granular cellulose bedding, separated by sex into groups of 5, and given Wayne Lab-Blox diet in regular pellets (Allied Mills, Inc., Chicago, IL) until 6 weeks of age. Afterwards the mice were given a semisynthetic diet (8) prepared by us. Tap water was provided ad libitum from birth until natural death.

Dr. Heikki Pyysalo and his colleagues collected the GE mushroom (Fig. 1) in southern and eastern Finland. A mycologist (Dr. Mauri Korhonen) taxonomically identified all the specimens as GE by using the guidelines of Harmaja and Weber (9, 10). There is only one species in this region. The mushrooms were stored in dry ice after harvest. Then they were deep-frozen at −25°C. Within 2 weeks, the mushrooms were shipped frozen with dry ice to Omaha by air. Upon arrival, the mushrooms were inspected, found to be completely frozen, and placed in a deep freeze at −15°C in the Eppley Institute. On each feeding day, an appropriate amount was defrosted and given to the animals.

Several samples, approximately 30 g, of the fresh mushroom were thawed, placed in a Soxhlet thimble, and continuously extracted with diethyl ether for 24 h. The ether extract was then dried over sodium sulfate, concentrated to 5 ml by distillation, and analyzed for gyromitrin and N-methyl-N-formylhydrazine by gas chromatography (DB-1, 70°C). The concentration of gyromitrin and N-methyl-N-formylhydrazine were 44 and 4 mg/kg, respectively.

Toxicity studies were carried out with GE prior to the chronic experiment. GE was fed ad libitum for 4.5, 4, 3.5, 3, and 2.5 days each week, followed by semisynthetic diet for the rest of the week for a total of 70 days. Each group consisted of 8 animals (4 female, 4 male). Taking into account four parameters (survival rates, body weights, dose of mushroom, and histological changes), feeding GE for 3 days was found to be suitable for the chronic experiment. This toxicity technique was developed in this laboratory (11).

The chronic experimental groups and the controls were the following:

Group 1. GE was fed ad libitum for 3 days, followed by semisynthetic diet for 4 days of each week for the life span of 50 female and 50 male mice that were 6 weeks (48 days) old at the beginning of the experiment.

Group 2. Consisted of 50 female and 50 male mice, 6 weeks (44 days) old at the beginning of the experiment. They served as untreated controls.

The experimental and control animals were carefully checked and weighed weekly, and the gross pathological changes were recorded. The animals either were allowed to die or were killed with ether when found in poor condition. Complete necropsies were performed on all animals. All organs were examined macroscopically and were fixed in 10% buffered formalin. The liver, spleen, kidneys, bladder, thyroid, heart, pancreas, testes, ovaries, preputial and clitoral glands, brain, nasal turbinals, forestomach, glandular stomach, duodenum, jejunum, ileum, cecum, colon, rectum, at least four lobes of the lungs of each mouse, and organs showing gross pathological changes were studied histologically. Sections from these tissues were stained routinely with hematoxylin and eosin and examined by light microscopy.

Statistical Analysis. The statistical analysis was performed by using the Fisher's exact test for 2 x 2 tables (12).

RESULTS

The survival rates of GE-treated and control mice are recorded in Table 1. The data show that the mushroom treatment had no substantial effect on survival when compared with the
untreated controls. In addition, the average weekly body weight curves of the treated and untreated mice have not shown any significant difference until 100 week of age. Afterwards, the body weights of the untreated mice were significantly higher than of the corresponding treated groups.

The number, percentages of mice with tumors, and their ages at death (latent periods) are summarized in Table 2. The treatment induced tumors in the lungs, nasal cavity, blood vessels, forestomach, glandular stomach, cecum, and liver, which are described in detail below.

Lung Tumors. Of the treated females, 40 (80%; P < 0.0001) developed 117 neoplasms in this organ. Of these, 24 mice had 62 adenomas, 2 mice had 2 adenocarcinomas, and 14 mice developed 31 adenomas and 22 adenocarcinomas. In the treated males, 35 (70%; P < 0.0001) developed 81 lung tumors. Of these, 22 mice had 42 adenomas, 2 mice had 2 adenocarcinomas, and 11 mice developed 24 adenomas and 13 adenocarcinomas.

In the untreated control females, 14 (28%) developed 15 neoplasms in this organ. Of these, 9 mice had 10 adenomas and 5 mice developed 5 adenocarcinomas. In the untreated control males, 19 (38%) developed 31 lung tumors. Of these, 11 mice had 17 adenomas, 4 mice had 4 adenocarcinomas, and 4 mice developed 5 adenomas and 5 adenocarcinomas.

Macroscopically and histologically all lung tumors were similar to those described earlier (13, 14).

Tumors of the Nasal Cavity. Of the treated females, 5 (10%; P < 0.04) developed 5 neoplasms in this tissue. Of these, 4 were classified as adenomas and the remaining 1 as an adenocarcinoma. In the treated males, 6 (12%; P < 0.02) developed 6 nasal cavity tumors. Of these, 5 were classified as adenomas and 1 as an adenocarcinoma. No nasal cavity tumors were found in the untreated controls.

The epithelial layer of the nasal cavities gradually increased in thickness, presenting the appearance of hyperplasia. The benign adenomas usually were circumscribed and the glandular formations were lined by the cuboidal and columnar cells (Fig. 2). In other instances, the olfactory epithelium developed into the characteristic patterns of adenocarcinoma, which were usually larger than the benign tumor; they showed moderate invasiveness, although no metastasis was observed.

Otherwise, grossly and histopathologically, these tumors were similar to those described by other investigators (15).

Tumors of Blood Vessels. Of the treated females, 25 (50%; P < 0.0002) developed vascular tissue tumors. Of these, 12 mice had hemangiomas in livers, 10 mice had hemangiosarcomas in livers, 1 mouse had a hemangioma in spleen, 1 mouse had a hemangioma in ovary, and 1 mouse developed a hemangiosarcoma in lungs. Of the treated males, 16 (38%; P < 0.001) developed blood vessel neoplasms. Of these, 9 mice had hemangiomas in livers and 7 mice developed hemangiosarcomas in livers.

In the untreated control females, 7 (14%) developed vascular tissue tumors. Of these, 3 had hemangiomas in livers, 1 had a hemangiosarcoma in liver, 2 had hemangiomas in ovaries and 1 mouse developed hemangiomas in the uterus and s.c. tissue. In the untreated control males, 3 (6%) developed blood vessel tumors. Of these, 2 had hemangiomas in livers and 1 mouse developed a hemangiosarcoma in liver.

In other respects, grossly and histopathologically, these tumors were similar to those found in earlier experiments in this laboratory (16, 17).

Tumors of the Foregut. Of the treated females, 8 (16%; P < 0.005) developed 8 squamous cell papillomas of this organ. Of the treated males, 9 (18%; P < 0.003) developed 16 neoplasms in this organ. Of these, 8 mice had 15 squamous cell papillomas and the remaining mouse developed a squamous cell carcinoma. No forestomach tumor was found in the untreated controls.

Otherwise grossly and histologically, these tumors were similar to those reported earlier in this laboratory (18).

Tumors of the Glandular Stomach. Of the treated females, 2 (4%) developed 2 adenocarcinomas in this organ. Of the treated males, 10 (20%; P < 0.002) developed 11 tumors in this organ. Of these, 1 mouse had 2 adenomas and 9 mice developed 9 adenocarcinomas in the glandular stomach.

Most of the glandular stomach tumors grew into the lumen of the organ, although sometimes they invaded the wall of the stomach. These lesions often exhibited irregular sizes and shapes of the gastric glands lined by cuboidal or columnar cells (Fig. 3). In some cases, the tumor was well circumscribed and was diagnosed as an adenoma. In other instances, particularly when the neoplastic glands invaded the muscularis layer, the lesion was classified as an adenocarcinoma. No glandular stomach tumors were found in the control animals.

In other respects macroscopically and histopathologically, these tumors were similar to those described earlier in this laboratory (19).

Tumors of the Cecum. Of the treated females, 14 (28%;

### Table 1  Treatment and survival rates in GE-treated and control Swiss mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Initial no. and sex of mice</th>
<th>No. of survivors (age in wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>1</td>
<td>GE p.o. for 3 days each week for life</td>
<td>50F</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50M</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Untreated controls</td>
<td>50F</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50M</td>
<td>50</td>
</tr>
</tbody>
</table>
FALSE MOREL AND CANCER

Fig. 2. Adenoma of the nasal cavity. The lesion consists of columnar cells arranged in a single layer around irregular glandular spaces. H & E, x 80.

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<ref>Fig. 2. Adenoma of the nasal cavity. The lesion consists of columnar cells arranged in a single layer around irregular glandular spaces. H & E, x 80.\n</ref>

P < 0.001) developed 23 tumors in this organ. Of these, 13 had 22 polypoid adenomas and 1 mouse developed an adenocarcinoma. Of the treated males, 11 (22%; P < 0.05) developed 22 tumors in this organ. Of these, 10 mice had 18 polypoid adenomas and 1 mouse developed 3 polypoid adenomas and 1 adenocarcinoma.

In the untreated control females, 4 (8%) developed 5 polypoid adenomas in this organ. In the untreated control males, 4 (8%) developed 5 polypoid adenomas in the cecum.

These growths obtruded into the lumen of the cecum, showing polypoid or cauliflower-like appearances, often obstructing partially or completely the intestine. Histologically, the polypoid adenomas consisted of irregular and deformed tubules and acini lined by the neoplastic epithelium. These structures spread upward around the central fibrous stalk (Fig. 4). In adenocarcinomas, evidence of infiltrative activity was obvious. The penetration of muscularis mucosae and invasions of submucosa were often present.

Otherwise grossly and histologically, these tumors were similar to those published previously in this laboratory (18).

Liver Tumors. Of the treated females, 3 (6%) developed benign hepatomas. Of the treated males, 6 (12%; P < 0.06) developed benign hepatomas.

In the untreated control males, one mouse developed a benign hepatoma while no such tumor was observed in the corresponding females.

In other respects macroscopically and histologically, the liver tumors were similar to those described previously in this laboratory (16).

Other Tumors. In a number of instances, other types of neoplasms were observed in the treated animals shown in Table 2. Since these neoplasms occurred in low incidences, their appearance cannot be attributed to treatment.

DISCUSSION

This experiment shows that the lifelong administration from 6 weeks of age of GE for 3 days, followed by a semisynthetic diet for 4 days each week, induced tumors in seven organs and tissues of Swiss mice.

Methylhydrazine, an ingredient of this mushroom, when given p.o. in the drinking water to mice, induced lung neoplasms, and also induced tumors of the cecum and malignant histiocytomas in hamsters. N-Methyl-N-formylhydrazine administered under identical conditions as methylhydrazine caused tumors of the liver, lungs, blood vessels, bile ducts, and gallbladder in mice, and tumors of the liver, gallbladder, bile ducts, and malignant histiocytomas in hamsters. Acetaldehyde methylformylhydrazone induced tumors of the lungs, forestomach, and preputial and clitorial glands in mice. Pentanal methylformylhydrazone and hexanal methylformylhydrazone each elicited the development of tumors of the lungs, liver, and preputial glands in Swiss mice (4, 6). Finally, 3-methylbutanal methylformylhydrazone induced tumors in the lungs, liver, gallbladder, preputial glands, and thyroid in mice (5). The present administration of GE mushroom induced seven types of neoplasms in mice. Those of the lungs, blood vessels, forestomach, and liver were identical to those found in mice treated with the six chemical ingredients of this mushroom. In addition, tumors were induced in the nasal cavity, glandular stomach, and cecum of GE-fed mice. This discrepancy could be due to the additional, thus far unstudied, carcinogenic chemicals that may be present in GE.

Medical literature has reported poisoning cases from G. esculenta. Fatalities following the consumption of this fungus have been reported in various countries (2). Recently, a review
Table 2 Treatment and tumor incidences in GE-treated and control Swiss mice

<table>
<thead>
<tr>
<th>Effective Group 1 Treatment (G) p.o. for 3 days each week for life</th>
<th>Lungs</th>
<th>Nasal cavity</th>
<th>Blood vessels</th>
<th>Forestomach</th>
</tr>
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<tbody>
<tr>
<td>50 F</td>
<td>40</td>
<td>80</td>
<td>94 (66-122)</td>
<td>5</td>
</tr>
<tr>
<td>50 M</td>
<td>35</td>
<td>70</td>
<td>97 (56-140)</td>
<td>6</td>
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</table>

For untreated controls (Group 2):
- 50 F: 14, 28, 90 (58-115)
- 7, 14, 97 (60-129)
- 50 M: 19, 38, 88 (53-120)
- 3, 6

**Description:**
- Average and range in weeks.
- Numbers in parentheses, age at death in weeks.

Describing the toxic syndrome attributed to *G. esculenta* poisonings and the possible mode of action of the toxins was published (3).

Several investigators worldwide analyzed the *G. esculenta* mushroom for hydrazines. The following types and amounts were identified in it: acetaldehyde MFHO, 49.9 mg/kg; propanal MFHO, 1.0 mg/kg; butanal MFHO, 0.6 mg/kg; 3-methylbutanal MFHO, 2.2 mg/kg; pentanal MFHO, 0.8 mg/kg; hexanal MFHO, 1.4 mg/kg; octanal MFHO, 0.2 mg/kg; trans-2-octenal MFHO, 0.6 mg/kg; cis-2-octenal MFHO, 0.3 mg/kg; N-methyl-N-formylhydrazine, 500 mg/kg (by dry weight); and methylhydrazine, 14 mg/kg (20-23). It has been shown that boiling decreases the levels of hydrazines in the mushroom (23).

In addition to the *G. esculenta*, other mushroom species also contain carcinogenic hydrazines. Some of the species, including *Gyromitra gigas*, *Helvella crispa*, and *H. lacunosa*, which also belong to the Helvellaceae family, as does the *G. esculenta* (24, 25). Other mushrooms, such as the *Cyahtipodia macropus*, *Leptopodia elastica*, *Otidea onotica*, *Cudonia circinans*, *Leotia lubrica*, *Spathularia flavida*, and *Neohulgaria pura*, are members of other mushroom families (24, 25). In an earlier study, feeding of the cultivated mushroom in the Western hemisphere, *Agaricus bisporus*, induced tumors in four organs and tissues of Swiss mice (26). Subsequently, in another laboratory, a diet containing a 30% dry powder of *A. bisporus* was given for 500 days to 20 female rats. The treatment had no tumorigenic effect in the animals (27). This study can be criticized because the dry mushroom probably contained less carcinogens (no measurement was taken) than the fresh; it was given for a shorter time (500 days versus life span); only one sex of animals was treated; too few rats were used; and, above all, humans rarely consume dry *A. bisporus*. It is also known that several other relatives of this fungus, all belonging to the *Agaricaceae* family, contain carcinogenic hydrazines (28). To date, 22 mushroom species are known to contain 11 carcinogenic hydrazine analogues and diazonium ions (7).

The *G. esculenta* mushroom fed in the present experiment was obtained from Finland and was analyzed periodically for hydrazines. The concentrations of hydrazines were similar to...
the values found in the North American *G. esculenta*, which were reported previously.

Interestingly, the body weight of the animals fed the GE mushroom for nearly 2 years increased steadily. This means that the treated mice obtained a nourishing diet during the main course of the experiment just like the untreated control animals.

The uncooked *G. esculenta* mushroom was found to be carcinogenic in the present experiment. Earlier studies also have shown that six hydrazine ingredients of this fungus acted similarly in rodents. Because this mushroom also is eaten uncooked, humans should abstain from the consumption of the raw form of this species.

## REFERENCES


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