

Damage Caused by Two Finnish Mushrooms, *Cortinarius speciosissimus* and *Cortinarius gentilis* on the Rat Kidney

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The toxicity of two mushroom species found in Finland, *Cortinarius speciosissimus* and *Cortinarius gentilis*, on the rat was studied. Dried, homogenized mushroom was given orally by stomach tubing. A dose of 500 mg dried mushroom/kg body weight, was used.

It was demonstrated that both species caused renal damage. No damage could be shown in other organs. The renal histopathological changes corresponded to those of tubulo-interstitial nephritis. The sensitivity of different individuals to the fungal toxins varied greatly.

Introduction

Numerous fatal mushroom poisonings occur yearly in Europe, for which *Amanita phalloides* and *Amanita virosa* have been mainly responsible. Due to the rarity of *A. phalloides*, the most feared poisonous toadstool in Finland is *A. virosa*. The toxicity of other fungi is generally considered to be slight. The public has, thus, generally only been warned to beware of white mushrooms.

However, in the last few years several cases of rare types of mushroom poisonings have been made public in Finland. A latent period of several days and renal toxicity have been characteristic of the poisonings¹. These poisonings resemble those occurring in Poland in the 1950's which were caused by *Cortinarius orellanus*^{2,3}. *C. orellanus* is not known to be found in Finland. Thus, it is suspected that in Finland these poisonings have been caused by *C. speciosissimus* which closely resembles *C. orellanus*, and which is said to contain the same kind of toxins as *C. orellanus*⁴.

Identification of the fungus causing poisoning is often difficult, especially if the latent period is several days. It is possible that the mushrooms eaten by the patient may have included a toxic species that is not present in the remaining sample. As mushroom poisoning caused by the genus *Amanita*

has been successfully studied by means of animal experiments, we wanted to demonstrate poisoning by *Cortinarius speciosissimus* with specimens collected in Finland. As the genus *Cortinarius* consists of a great many of species, it is possible that it includes many poisonous species in Finland as well. For this reason the other member of the genus, *C. gentilis*, which resembles *C. speciosissimus*, was included in the animal toxicity experiments.

Materials

Two each other resembling orange brown *Cortinarius* species with yellow zones in the stem were used: *C. speciosissimus* Kühn. & Romagn. and *C. gentilis* Fr. The material was collected in Eastern Finland near the city of Kuopio in the district of North Savo (Savonia borealis, Sb). The collections and localities are more closely described below.

Cortinarius speciosissimus Kühn. & Romagn.

Two collections (Heli Heikkilä Nos. 72 and 75) from the same locality were used: Sb, Siilinjärvi, Toivala, School of Forestry; 20. VIII. 1974 and 10. IX. 1974.

Cap: width mostly 4–6 cm, bright orange brown, bell-shaped or conical, expanding with age, retaining a distinct acute umbo even when mature, non-hygrophanous, with a mat surface.

Gills: broad, distant, colour of the cap.

Stem: length ca. 7–9 cm, thickness 0.6–1.5 cm, often slightly swollen towards the base, orange brown with a few somewhat diffuse yellow zones,

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which disappear easily by touching or by drying. Spores: ellipsoid to almond-shaped, minutely warty, $8.5 - 12 \times 6.5 - 9 \mu\text{m}$ (200 measurements, spores taken from dried material).

Habitat: Moist mixed forest with *Picea abies* as dominant tree and *Vaccinium myrtillus* as dominant dwarf-shrub. Moss cover mainly formed by *Pleurozium schreberi* (also *Hylocomium splendens* and *Dicranum scoparium* abundant). Other fungal species occurring in the same habitat were e.g. several *Lactarius* species (*L. trivialis*, *L. rufus*, *L. camphoratus*, *L. vietus*), *Tricholoma inamoenum* and *Leccinum* spp.

Occurrence: At the time of the first collection (Aug. 20) *C. speciosissimus* was quite abundant: 30 fruit-bodies were found in an area of ca. 10 m^2 . At the second time (Sept. 10) the usable material was scantier; many overaged and infected fruit-bodies were seen.

Cortinarius gentilis Fr.

The material was collected by Mrs. Lahja Hakala in Sb, Vehmersalmi, on the southern slope of Puutosmäki hill, 23. IX. 1973.

Cap: width 3–5 cm, ferruginous yellow to orange brown, bell-shaped, expanding with age, retaining a small acute umbo even when mature, hygrophanous.

Gills: broad, distant, colour of the cap or somewhat deeper reddish brown with age.

Stem: more slender than in the previous species: length 8–10 cm, thickness 0.3–0.6 cm, base often attenuated into a root-like extension, orange brown with a few fibrillose, easily disappearing yellow zones.

Spores: subglobose to ellipsoid, minutely warty, smaller than in *C. speciosissimus*: $7 - 8.5 \times 5 - 6.5 \mu\text{m}$ (100 measurements, spores taken from dried material).

Habitat: coniferous forest with *Picea abies* as dominant tree; ground covered by moss (mainly *Pleurozium schreberi*). Nearest accompanying fungal species was *Lactarius rufus*.

Occurrence: The fungus was quite abundant, though there had already been frosts.

All the *C. gentilis* material and the first *C. speciosissimus* collection (HH. No. 72) were originally collected as herbarium specimens, and thus dried and thereafter frozen for a week in -20°C in order to kill eventual harmful organisms. In addition, the *C. gentilis* material had been stored in room temperature for a year. — The second *C. speciosissimus* collection (HH. No. 75) was merely dried and immediately thereafter sent to be used in experiments.

Methods

24 two month old, male, Sprague-Dawley strain rats were used in the experiment. Their weights ranged from 130 to 188 g. The animals were divided into four groups, of six each. The rats were fasted for 18 hours prior to the administration of the mushroom, but received water *ad libitum*. Group I served as a control group, receiving only water. Group II received *C. speciosissimus*, which had been first dried and then frozen. Group III received *C. speciosissimus* which had only been dried, and group IV received *C. gentilis*. A mature mushroom, including the stem, was selected from each group of specimens (dry weight 500–650 mg). It was homogenized to a water suspension, using the Potter-Elvehjem glass homogenizer. 10 ml/kg of the suspension thus obtained was administered orally to the rats by stomach tubing. The rats in each group received a dose of 500 mg dried mushroom/kg body weight. The rats were subsequently weighed daily.

Three rats from each group were killed after nine days: group I/rats 4–6, II/4–6, III/4–6 and IV/4–6. At the end of 13 days one rat from each group was killed: group I/rat 3, II/3, III/3 and IV/3. The remaining rats (group I/rats 1–2, II/1–2, III/1–2 and IV/1–2) were killed 17 days following the administration of the mushroom. The rats were not fasted prior to the killing, which was carried out by cutting the aorta under light ether anesthesia. Immediately prior to the killing a blood sample was obtained by cardiac puncture. The thymus, heart, liver, spleen, kidneys, adrenal glands and testes were weighed. Samples obtained from the above organs were embedded in paraffin, sliced into $7 \mu\text{m}$ sections and stained with the hematoxylin-eosin technique. Serum urea was determined by splitting urea into ammonia and carbon dioxide using urease, and the determination performed employing Berthelot's phenol-hypochlorite reaction.

Results

A slight delay in weight gain was found in rats II/3 and III/3. Rat III/5 clearly began to lose weight after three days. The weight gain and behavior of the other animals were normal.

From Table I we see that the largest relative kidney weights were found in test group III, which received dried *Cortinarius speciosissimus*. The weights were greatest for animals 1, 3, 5 and 6. Table II shows that the same animals also had the highest serum urea values (mmol/l). In test group

Table I. Relative weights of the kidneys of the animals employed in the experiment.

Animal	Groups			
	I	II	III	IV
/1	67.6	75.8	104.1	76.8
/2	78.5	68.8	78.9	67.9
/3	74.9	103.4	139.4	76.3
/4	73.3	89.4	71.4	78.2
/5	74.5	77.1	187.1	81.1
/6	71.1	83.8	104.0	81.5

II the kidneys of animal 3 had a high relative weight (received *C. speciosissimus* which was first dried and then frozen), but the serum urea was not elevated.

Table II. Serum urea values, mmol/l, of the animals employed in the experiment.

Animal	Groups			
	I	II	III	IV
/1	9.8	8.1	14.6	8.1
/2	10.1	9.7	8.7	8.3
/3	8.3	8.8	13.3	8.1
/4	5.8	8.1	7.7	6.2
/5	6.8	7.4	13.0	5.8
/6	10.2	10.8	14.1	7.2

In microscopic studies * changes were demonstrated only in the kidneys, the rest of the organs being normal (thymus, heart, liver, spleen, adrenal glands and testes). Table III shows that the renal lesions considered to be the most severe were found in the

Table III. Degree of the microscopically demonstrated renal lesions. N = no lesions, M = mild lesions, S = severe lesions.

Animal	Groups			
	I	II	III	IV
/1	N	M	S	N
/2	N	N	M	N
/3	N	S	S	N
/4	N	M	M	M
/5	N	M	S	N
/6	N	N	S	N

same test animals which showed increased relative renal weight and serum urea values. Upon microscopic examination the renal lesions were considered severe when round, inflammatory focuses on the medullar-cortical border were found almost diffusely throughout the section as a belt, and when tubular dilatation was demonstrated quite diffusely

throughout the cortex, extending to the capsule. Mild lesions signified cases in which the same lesions as mentioned above were seen scattered along the medullar-cortical border, as well as isolated lesions were found in the region of the cortex. The microscopic lesions were similar in quality, but with an increase in the period of time, the maturity and amount of scar tissue increased, and the number of inflammatory cells correspondingly decreased.

The histopathological changes corresponded to those of tubulo-interstitial nephritis. The size, shape and structure of the glomeruli were normal in all specimens. Roundish, inflammatory focuses, in which polymorphonuclear leucocytes, lymphocytes and fibroblasts were seen in various amounts, were found in the interstitial tissue along the medullar border. Very often the lesion was clearly peritubular, in which case the centrally located tubulus was dilated, the epithelium either regenerating by mitosis or atrophic. Detached epithelial cell masses, polymorphonuclear leucocytes and mononuclear inflammatory cells were often found in the tubuli. Tissue edema was not demonstrated. The changes in the convoluted tubules were quite apparently secondary. Only dilatation, without indication of inflammation, was seen. The epithelium was at times somewhat atrophic, but on the whole, however, normal. Cell elements were rarely encountered within the tubular lumen, and when found consisted of epithelial origin.

Discussion

This study shows that both of the mushroom species collected from near Kuopio, which were under investigation, *Cortinarius speciosissimus* and *C. gentilis*, caused renal damage in rats. A few cases of mushroom poisoning, occurring in Finland in the past few years, have similarly caused only renal damage¹. In these cases it is suspected that the poisoning was caused by *C. speciosissimus*, which is known to contain toxins similar to those of *C. orellanus* which has caused similar poisonings in Poland⁴. On the basis of the results of this study it is possible that the poisonings could as well have been caused by *C. gentilis* or perhaps by some other species of *Cortinarius*. For this reason it would be worthwhile to study systematically the toxicity of as many species of *Cortinarius* as possible. Exact information about the possible toxic effects of the mushrooms would thus be obtained. This is parti-

* Figs 1–6 see Table on page 670 a.

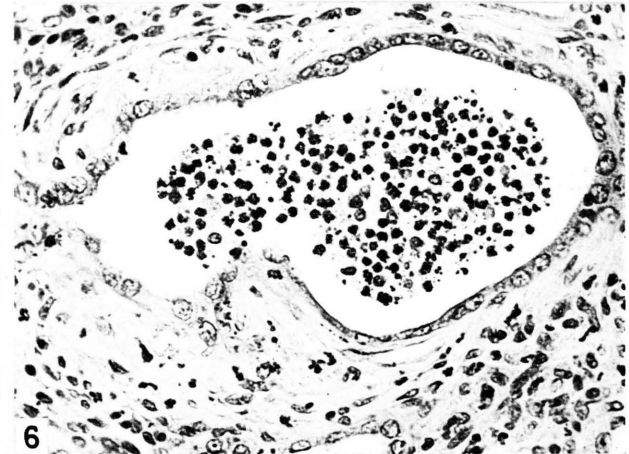
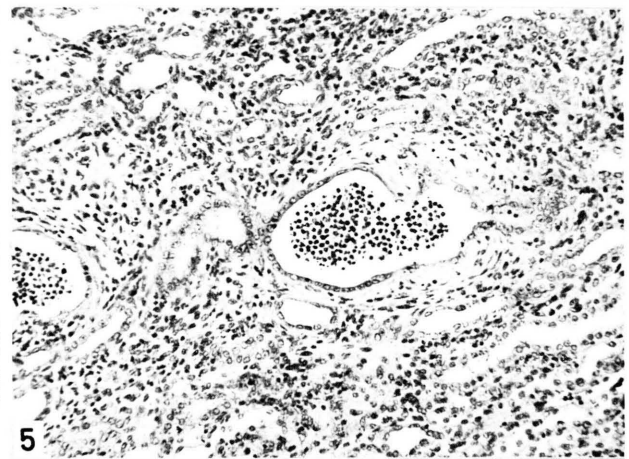
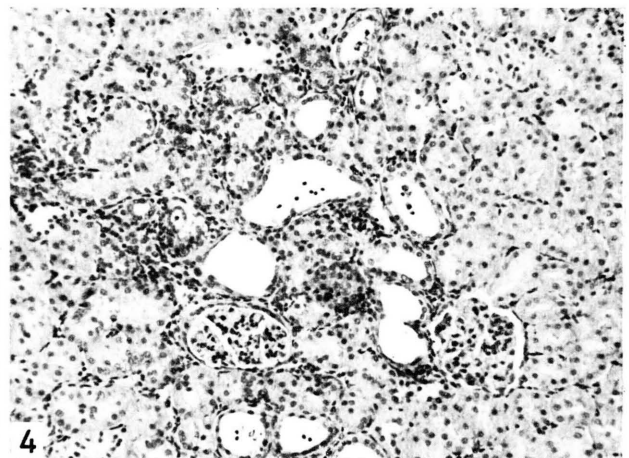
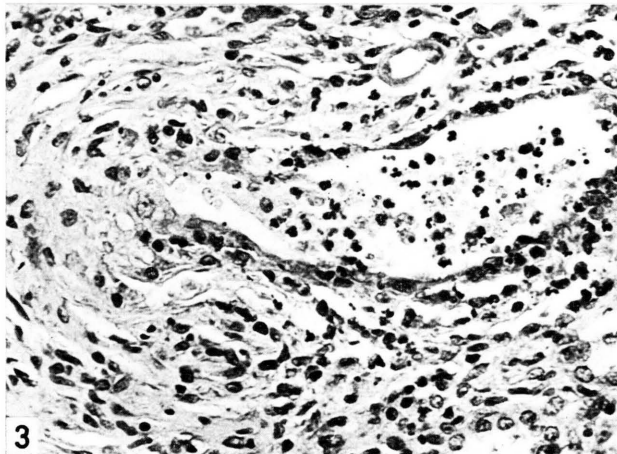
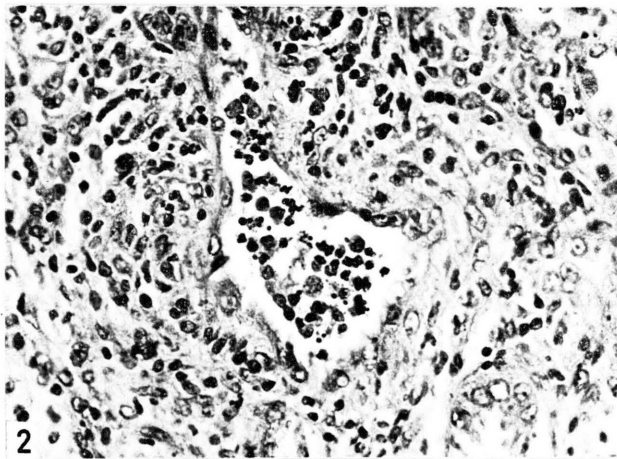
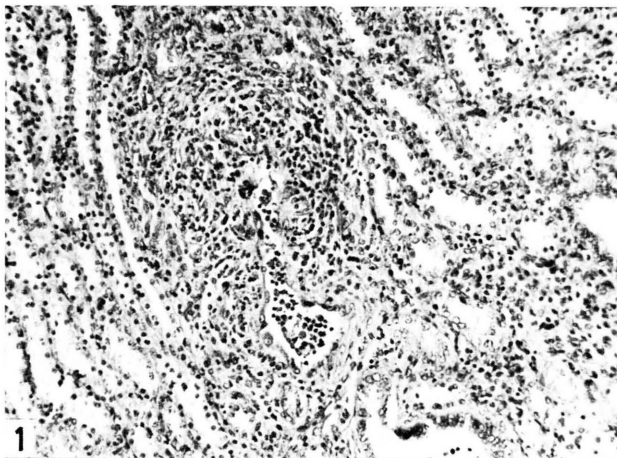


Fig. 1. Peritubular inflammatory focus on the medullar-cortical border. Specimen from a rat which received dried *C. speciosissimus*, group III, 17 days post-administration. $\times 75$.

Fig. 2. Greater magnification of Fig. 1. Dilated tubulus, the epithelium of which is in regenerative mitosis. The tubular lumen contains epithelial cell masses, polymorphonuclear leucocytes and a few lymphocytes. Fibroblasts and scar tissue surround the tubulus. $\times 250$.

Fig. 3. Dilated tubulus. Regenerating epithelium. The lumen contains deteriorating epithelial and inflammatory cells. Surrounding the tubulus are a few leucocytes and fibroblasts. Specimen from a rat which received dried *C. speciosissimus*, group III. Nine days post-administration. $\times 200$.

Fig. 4. Dilated convoluted tubuli, in which the epithelium is either normal or slightly atrophic. The lumen contains a few cells. The glomeruli and interstitium are normal. Specimen from a rat which received *C. gentilis*, group IV. Nine days post-administration. $\times 75$.

Fig. 5. Inflammatory focus on the medullar-cortical border, located peritubularly. Dilated tubulus, contains cell mass. Specimen from a rat which received *C. gentilis*, group IV. Nine days post-administration. $\times 75$.

Fig. 6. Greater magnification of Fig. 5. Tubulus cut diagonally, epithelial nuclei enlarged. Fibroblasts and a few inflammatory cells surround the tubulus. The lumen contains epithelial cells, of which a part are necrotic, and inflammatory cells. $\times 200$.

cularly important in light of the prevailing world food shortage. Mushrooms comprise a large, so far little tapped, source of energy.

Generally the sensitivity of fungal toxins varies greatly between individuals⁵. This was also observed in this study. The same dose of fungus caused relatively serious renal changes in some rats, but left the kidneys of some individuals, in the same group, completely normal. Serum urea was shown to be elevated above control levels only in those rats whose kidneys were relatively seriously damaged. For this reason it can be assumed that minor renal damage caused by species of *Cortinarius* remains hidden. Because several cases of serious renal damage, apparently caused by *Cortinarius* species, have occurred in Finland, it is possible that there are hundreds of thousands of cases of minor renal damage.

In this study group II received *C. speciosissimus* which had been dried and frozen and group III received *C. speciosissimus* which had only been dried. Both groups of mushrooms caused renal damage.

Generally, however, it can be demonstrated that in the rat group (III) which received mushroom that had only been dried, the renal damage was more serious. Whether this difference is a result of the freezing, of differences in the individual reactions of the rats, or of differences in the toxicity of the individual mushroom specimens remains to be clarified by future experiments.

Mushroom poisonings are divided into two groups according to whether symptoms begin rapidly, within six hours, or are delayed⁶. *Cortinarius* species belong to the latter group¹⁻³. In this study the first rats were not killed until nine days after the administration of the mushroom. At that time the renal damage was already fully developed. In many respects it would be the most interesting to find out what degree of renal damage occurs immediately after the administration of the mushroom. A complete understanding of the process of poisoning is essential to the development of effective methods of therapy in cases of mushroom poisoning.

¹ S. Hulmi, P. Sipponen, J. Forsström, and J. Vilska, *Duodecim* **90**, 1044 [1974].

² S. Grzymala, *Bull. Méd. Lég. Toxicol.* **3**, 60 [1965].

³ S. Grzymala, *Bull. Méd. Lég. Toxicol.* **3**, 73 [1965].

⁴ H. Harmaja, *Sienilehti* No. 2, p. 6 (1973).

⁵ R. K. Myler, J. C. Lee, and J. Hopper, Jr., *Arch. Int. Med.* **114**, 196 [1964].

⁶ K. F. Lampe, *Clin. Toxicol.* **7**, 115 [1974].