# Technical Papers

## Chemical Identification of the Amanita Toxin in Mushrooms

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Folklore has provided many fanciful but dangerous procedures for distinguishing poisonous from nonpoisonous mushrooms: "A poisonous mushroom has a peculiar color or odor." "It will turn a silver coin black." "It has a devil's cup." Although these fallacious concepts have general popular acceptance, the mycologist depends upon complete botanical identification. This, however, is sometimes difficult or impossible. For example, there is the case in which a child has eaten a wild mushroom, and the worried mother can produce no specimen or perhaps only a small fragment of the mushroom.

It is reported (*1*) that fully 90 percent of the deaths caused by poisonous mushrooms—about 50 each year in the United States (*2*)—result from the closely related *Amanita phalloides, A. verna,* and *A. virosa.* These mushrooms contain the amanita toxin, which does not induce its toxic manifestations for 10 to 15 hr after ingestion, thus permitting thorough absorption of the toxin (*3*). This toxin does not have an available antidote (*4*) although the symptoms are relieved with injections of glucose and amino acids (*5*). This toxin is therefore unlike muscarine, from *A. muscaria,* which produces symptoms quickly and is specifically antidoted by atropine (*3*). Thanks to the works of Kobert, Ford, and the Wielands, with their associates, we know that the toxin of *A. phalloides* is made up of four components: a hemolytic glucoside, phallin; and three peptides, phalloidine, α-amanitine, and β-amanitine, which affect the liver, kidney, and heart. Since the amanitines are not destroyed when the mushrooms are cooked, these toxins are of primary importance in cases of poisoning. They represent 0.005 percent of the fresh *A. phalloides* (*6*) and have a minimum lethal dose of 0.1 to 0.4 mg/kg to dogs (*7*) and mice (*8*). Human beings and guinea pigs are even more susceptible (*9*).

A simple rapid method for chemically identifying the amanita toxins in mushrooms has been developed and tested in our laboratories. It will permit the identification of the toxin in as little as 0.1 g of fresh mushroom tissue. This method can be run by any technician and takes 1 hour, provided that the reagents and equipment are at hand. It is based upon a sensitive color test giving a violet color with the amanitines and a bright blue color with phalloidine (*6*). A survey of more than 50 mushrooms, representing 46 species (including 13 amanitas, but not *A. muscaria*) was made employing this procedure. Each mushroom extract was also tested for toxicity to mice by intravenous injection. Only the mushrooms that gave a positive color test for the amanitines [*A. verna* (the Destroying Angel) and *A. tenuifolia* (*10*)] were lethal to the mice. All the other fungi, except one, gave negative tests and were nontoxic. The exception, *Lepiota cretacea,* gave a weak violet color but no toxicity. Since the specimen employed was very small, it remains to be determined whether, in this exceptional case, there was an interfering substance or whether a small quantity of amanitine was actually present.

The procedure is as follows. The mushroom is minced but preferably not mashed and covered with several volumes of methanol in a beaker. Extract the tissue by heating the mixture to boiling and keeping it hot for 2 min or longer; during the heating stir the mixture and press the tissue with a stirring rod. (Do not inhale vapors of methanol.) Separate the extracted tissue by filtration or centrifuging, pressing it to squeeze out the liquid. The tissue is discarded, and the methanol extract is evaporated to dryness on a steam bath. If only a small quantity of tissue is available for extraction, the container in which the extract is evaporated should be correspondingly small. A centrifuge tube is desirable so that the residue after evaporation will be concentrated at one point.

This residue may then be dissolved in a few drops or more of methanol, depending upon the quantity of tissue employed. The more concentrated the solution, the better. The residue that does not dissolve in methanol may be compacted and separated from the liquid by centrifuging.

The concentrated methanol solution is then used to run a chromatogram. A strip of filter paper (Whatman No. 1 or S & S 2043b), measuring 1 by 10 in., is marked with a pencil dot centered 1 in. from the bottom of the paper. With a glass capillary tube, apply the solution to the filter paper directly on the dot. After each application, permit the methanol to evaporate and repeat the process several times so that the applied material will be concentrated in a circle around the dot not larger than 1/16 in. in diameter. The filter paper is immersed up to 0.5 in. from the dot in a chromatographic solvent made up of 20 : 6 : 5 : 1 parts methyl ethyl ketone, acetone, water, and butanol, respectively. The paper strip, immersed in the solvent, is suspended free of the sides of the tube or cylinder container, which is then stoppered.

After 40 min the strip is removed and hung by the top to dry. It is then sprayed lightly with a solution of 1-percent cinnamaldehyde in methanol, allowed to dry, and suspended in a stoppered tube above concentrated hydrochloric acid. As soon as the color develops, the strip may be removed and examined. The appearance of one or more violet- or blue-colored spots on the paper indicates the presence of the amanita toxin. Orange, yellow, brown, or pink spots are not significant.

If this procedure is to be used for research rather than for simple detection of the toxin, the following three modifications are recommended. Extract the mushroom tissue in methanol for 1 hr or longer. Evaporate the methanol extract and redissolve the residue three times to coagulate the polypeptides. Employ a 14-in. filter-paper strip and run the chromatogram for 2 hr. These modifications assist in the extraction and separation of the individual toxins.

### References and Notes


1. R. T. Rolfe and F. W. Rolfe, *The Romance of the Fungus World* (Lippincott, Philadelphia, 1926), p. 232.
2. *New York Times*, 22 Oct. 1949, p. 19.
3. W. W. Ford, *J. Pharmacol.* **29**, 305 (1926).
4. An antiphalloidin serum that is claimed to be effective has been produced, but it is available only at the Pasteur Institute, Paris.
5. *Merck Manual* (Merck & Co., Rahway, N.J., ed. 8, 1950).
6. T. Wieland, G. Schmidt, L. Wirth, *Ann.* **577**, 215 (1952).
7. L. Binet, R. Fabre, M. Marquis, *Compt. rend.* **219**, 376 (1944).
8. T. Wieland, L. Wirth, E. Fischer, *Ann.* **564**, 152 (1949).
9. S. S. Block, R. L. Stephens, W. A. Murrill, paper submitted elsewhere for publication; it contains a table of lethal doses for the amanita toxin.
10. W. A. Murrill, *Mycol.* **37**, 270 (1945).


20 December 1954.

## Effect of Hypothermia on 17-Hydroxycorticosteroid Secretion in Adrenal Venous Blood in the Dog


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This paper summarizes our studies on adrenal vein 17-hydroxycorticosteroid secretion in acutely traumatized dogs during the induction of and rewarming from hypothermia. Adrenal venous blood samples were obtained by a technique (*1*) that consists of placing a cannula in the lumbar portion of the lumboadrenal vein, and intermittently occluding the adrenal vein-caval junction by means of a polyethylene choker for periods of 1 min. 17-Hydroxycorticosteroids were determined by the method of Nelson and Samuels (*2*). Hypothermia was induced in 20 mongrel male dogs either by cooling an external vascular shunt or by ice water immersion. In three of these experiments the animals were given a continuous intravenous infusion of ACTH, 40 U/1000 ml of 0.99-percent NaCl at a rate of 40 drops/min.

Table 1. Experiments in which hypothermia was carried to 25 percent or less of normothermic 17-hydroxycorticosteroid minute output in adrenal venous blood.

| No. of dogs | Temperature (°C) at which 25% of normothermic corticoid output was reached |
|---|---|
| 6 | 27.0–28.0 |
| 5 | 26.0–26.9 |
| 4 | 25.0–25.9 |
| 1 | 23.0 |
| 1 | 22.0 |

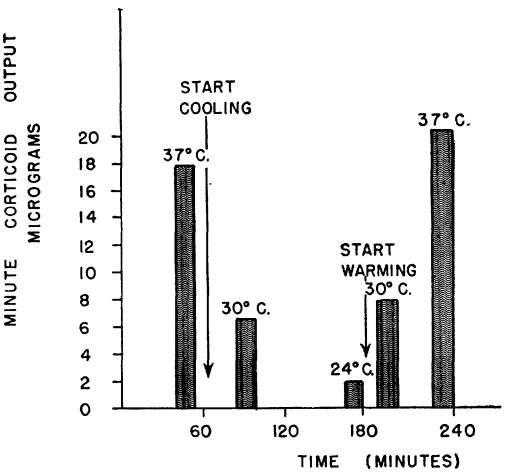


Fig. 1. Typical hypothermia experiment.

Table 1 illustrates that in most animals hypothermia to between 25° and 28°C is accompanied by a 75-percent decrease in adrenal venous blood minute corticoid output, but that an occasional animal may be cooled to lower temperatures before such a decrease is observed. Figure 1 is an example of a typical experiment demonstrating a marked fall in minute corticoid output from the adrenal gland during hypothermia, near-zero corticoid values at 24°C, and return to normal values upon rewarming. In Fig. 2, a similar sequence of events, with near-zero corticoid values at 21°C, is shown in an animal receiving an ACTH infusion during the procedure. In general, adrenal venous blood flow decreased with progressive hypothermia, as did the minute corticoid output. There were occasional exceptions, however, in which a relatively high adrenal blood flow was associated with a low minute corticoid output. Maintenance of the normal systemic blood pressure by the infusion of norepinephrine did not alter the minute corticoid output

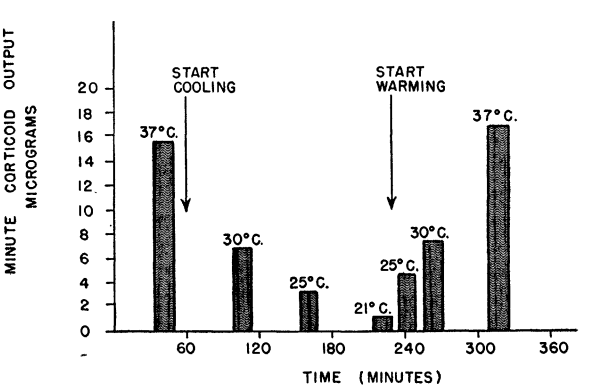


Fig. 2. Hypothermia experiment during which the animal received ACTH infusion.