CLITOCYBE TOXICA

A NEW SPECIES

N. SAPEIKA AND E. L. STEPHENS, *Department of Pharmacology, University of Cape Town*

The agaric genus *Clitocybe* includes a very large number of named species. About 200 to 300 species can be considered to belong to this genus.[1] Some of them are very closely related and could possibly be united. Quite a number are rare. A description of many of the phenotypes is available in a number of books.[2-6]

The majority of *Clitocybe* species of fungi have been regarded as generally non-toxic and even as good eating. There is scant information on this subject in the scientific literature. There are a few species that are reported as being poisonous to man ; unpleasant symptoms of transient nature may occur, but at least 2 species are described as being deadly poisonous. The features are stated to be identical with those produced by *Inocybe* and *Amanita muscaria* which are rare causes of death. Although all white species of *Clitocybe* are not poisonous, they may contain toxic substances and should be avoided.[7,8] One species *C. gigantea f. candida* has been shown to produce an antibiotic named clitocybin which causes lysis of tubercle bacilli.[7]

Specimens of a *Clitocybe* fungus collected at different times during September, October and November 1964 from the same local area (Monterey Estate) in the Cape Peninsula have been identified as a new species, *Clitocybe toxica*.[9] An experimental investigation of this fungus is reported in this paper.

METHODS AND RESULTS

Several batches of *C. toxica* were studied at different times.

Oral Administration

White mice each weighing approximately 25 G were given the fresh fungus to eat after being deprived of food overnight. Groups of animals were studied. Each animal was given 1, 2, or 4 G of the material. Of a total of 34 mice that ate the fungus 23 died in 12 - 72 hours. The larger doses did not necessarily kill a larger number of animals. Albino rats of the Wistar strain each weighing 200 - 300 G and starved overnight, were given 1, 2, 4, or 5 G of the fungus; no ill-effects were observed in 24 animals. Of 3 guinea-pigs each given 9 G one died in 3 hours.

Aqueous extracts were prepared by grinding portions of fungus caps with fine sand and water. The fluid obtained after centrifugation was opalescent and brownish-green in colour; 1 ml. of final extract represented 0·3 G of the original fungus. Such extracts, prepared at different times and first tested for potency by subcutaneous injection in mice, were used in the following experiments.

By Injection

Administration of 1 ml. of extract into the ventral lymph sac of each of 4 frogs (*Xenopus laevis*) caused death in 4 - 8 hours. In 2 adult male rabbits the intravenous administration of 1 ml. per kg. and 0·2 ml. per kg. body weight caused violent clonic convulsions and death in a few minutes.

Subcutaneous injection of 1 ml. in each of 16 mice produced apathy, closing of the eyes, and death in 2 - 12 hours. Heating the extract at boiling point for 5 minutes rendered it non-toxic.

Subcutaneous injection of 2 ml. in each of 4 rats caused death in 24 - 48 hours. Intraperitoneal injection of 2 ml. in each of 6 rats caused death in 2 - 4 hours; not unexpectedly the extract produced marked congestion and haemorrhagic exudate in the peritoneal cavity.

In 4 cats anaesthetized with pentobarbitone the extract, unboiled and boiled, injected intravenously in doses of 0·25 ml. per kg. body weight, produced a fall in blood pressure. However, after the administration of atropine no fall in blood pressure followed injection of the extracts, but a rise in blood pressure occurred indicating the presence in the extract of a pressor substance which is not destroyed by boiling. Some increase in the respiration occurred associated with the fall in blood pressure produced by the extract, but after atropine there was little change produced by the extract. Even with a marked fall in blood pressure the respiration continued virtually unchanged, indicating a primary direct effect of the injected extract on the cardiovascular system (Fig. 1).

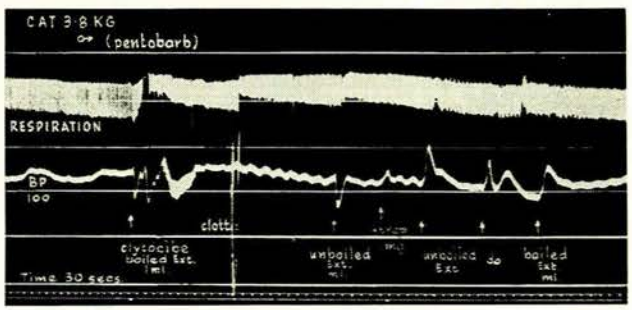

Fig. 1. See text.

Isolated tissues: The normal contractions of segments of small intestine of the cat and guinea-pig were inhibited by the extract; the concentration used represented 1 in 200 of the original fungus (Fig. 2). Also, the rhythmical contractions of rat non-pregnant uterus were temporarily delayed by this concentration of fungus extract.

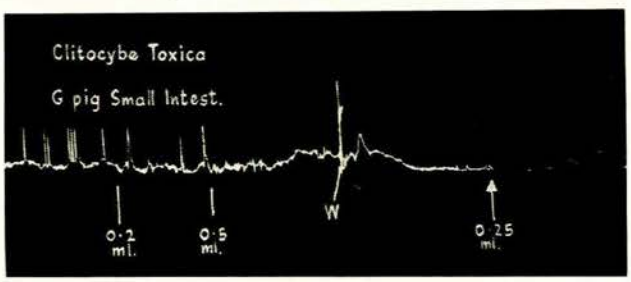

Fig. 2. See text.

Cytochrome oxidase: The method used[10] showed that unboiled extract oxidizes *p*-phenylenediamine; it presumably therefore contains cytochrome oxidase. After boiling the extract for 2 minutes an inhibitor of the enzyme was released, but boiling for 5 minutes destroyed or removed the inhibitor.

Ascorbic acid: The content of this reducing substance in the freshly excised liver and adrenal glands of albino rats that received injections of extract was estimated by the method of Kennaway and Tipler;[11] the amounts of ascorbic acid are expressed as mg. per 100 G of tissue in the accompanying Table I. No depletion of ascorbic acid occurred in the liver, but a decrease was observed in the adrenal glands.

Histological examination of the liver obtained from these injected rats revealed no significant change.

DISCUSSION

Clitocybe dealbata and *C. rivulosa* are known to be toxic and have caused death which has been attributed to the

action of a muscarine-like substance;[3] the symptoms included sweating, miosis, slow pulse rate, vomiting and diarrhoea.[3] The transient symptoms caused by *C. olearia* (*illudens*), the Copper Trumpet or Jack-o'-lantern, are

TABLE I. INJECTIONS OF EXTRACT

Dose of extract	*Liver (control 28)*	*Adrenals (control 340)*	*No. of rats*	*Hours after injection*
0·5 ml. per 100 G by intraperitoneal injection	26	203	4	2
	24·5	200	2	2½
0·25 ml. per 100 G by subcutaneous injection	32	315	2	6
	25	252	4	24

attributed to muscarine-like substances since they include nausea, vomiting, diarrhoea, abdominal pain, headache, variable pulse and laboured breathing.[12] The profuse sweating produced by *C. sudorifica* has also been attributed to the action of muscarine-like substances.[2] Whether these species really owe their toxicity to muscarine remains to be established by chemical investigation of the fungi. Muscarine has long been suspected in *C. rivulosa*, but thus far this has not been established by chemical isolation of the alkaloid.[13]

Certain plants contain cyanogenetic glycosides, and certain *Clitocybe* species are stated to contain cyanide, for example a small amount of hydrocyanic acid producing the smell of bitter almonds is found in *C. infundibuliformis*. *C. toxica* causes inhibition of cytochrome oxidase, but a cyanide-containing complex has not been demonstrated; no reaction was obtained with the benzidine-copper acetate and the alkaline picrate tests for hydrocyanic acid. The pharmacological effects of the fresh fungus and of its aqueous extracts indicate the presence of a lethal substance that has an action unlike that produced by muscarine. After boiling for 5 minutes the extract is unable to inhibit cytochrome oxidase, and such an extract is no longer toxic when injected into mice. When injected intravenously in anaesthetized cats, unboiled and boiled extracts produce a fall in blood pressure, which is blocked by atropine; after atropine only a rise in blood pressure is produced. This effect of *C. toxica* is unlike that produced by muscarine which does not cause a rise in blood pressure in atropinized animals since it has little or no nicotinic action; also muscarine stimulates cat and guinea-pig small intestine and the rat uterus, whereas *C. toxica* inhibits these structures. The changes produced by *C. toxica* in the blood pressure, with little change in the respiration, differ from that produced by cyanide which causes death from respiratory arrest; cardiac irregularities are commonly observed with cyanide, but the heart beat invariably outlasts breathing movements which become very rapid and then slow and irregular.

A very small amount of *C. toxica* causes death when given by injection and much larger quantities are less toxic or non-toxic when given by mouth. This has also been noted in the case of hepatotoxic fungi.[14]

No significant decrease in the concentration of ascorbic acid of the liver was produced in rats by *C. toxica*, although some decrease was produced in the adrenal glands; this is different from the effect of *Amanita capensis*, a hepatotoxic phenotype of *A. phalloides*, which produces a decrease in the ascorbic acid concentration in rat liver.[14] *C. toxica* produced no significant histological change in the liver.

The following *Clitocybe* species are stated by various authors to be toxic to man:

C. dealbata: poisonous, sometimes deadly.
C. rivulosa: poisonous, sometimes deadly.
C. sudorifica: profuse sweating.
C. olearia (*illudens*): vomiting, diarrhoea, prostration.
C. cerussata, C. nebularis, C. albissima, C. pithyophylla and *C. phyllophila*: possibly poisonous.

The following species are specifically mentioned in the literature as being edible:

C. adirondackensis, C. clavipes, C. cyathiformis, C. flaccida, C. geotropa, C. gigantea, C. infundibuliformis, C. maxima, C. monadelpha, C. multiceps, C. nebularis, C. odora. The manner in which these fungi are prepared for eating may determine whether certain potentially toxic constituents are destroyed.

Fatal poisoning from the ingestion of mushrooms has occurred in man all over the world. Severe poisoning is mainly caused by mushrooms belonging to the genus *Amanita* which includes a few species of great toxicity,[14-17] but a variety of other types of poisoning has been described caused by toxins, some of which are known, others as yet not properly determined.[16,17]

CONCLUSIONS

Although a large number of *Clitocybe* species have been described, little is known about the chemical and pharmacological features of these fungi. Further investigation is desirable since some species are edible, some contain active principles toxic to man and animals, and at least one has been shown to be bacteriostatic.

The toxic effects of a new species named *Clitocybe toxica* are described in this paper. They do not appear to be due to a muscarinic or cyanide action but to a potentially lethal factor that is destroyed by heat; among other actions it inhibits cytochrome oxidase. The reversal of the depressor action of (injected) fungus by atropine suggests the latter as a useful agent in the event of poisoning.

REFERENCES

1. Singer, R. (1949): *Lilloa (Revista de Botanica)*, vol. 22. Argentine: Tucuman.
2. Krieger, L. C. C. (1947): *The Mushroom Handbook*. New York: Macmillan.
3. Lange, M. and Bayard Hora, F. (1963): *Mushrooms and Toadstools*. London: Collins.
4. Wakefield, E. M. and Dennis, R. W. G.: *Common British Fungi*. London: Gawthorn.
5. Lange, J. E. (1935): *Flora Agaricina Danica*, vol. 1. Copenhagen: Recato A/S.
6. Saccardo, P. A. (1887): *Sylloge Fungorum*, vol. V, pp. 141-200. Michigan, USA: Edwards.
7. Ramsbottom, J. (1953): *Mushrooms and Toadstools*. London: Collins.
8. De la Riviere, R. D. and Heim, R. (1938): *Les Champignons Toxiques*. Paris: Encycl. Medico-Chirurg.
9. Stephens, E. L. (1965): S. Afr. J. Bot. (in the press).
10. Zwarenstein, H. and Van der Schyff, V. E. (1963): *Practical Biochemistry*. Edinburgh: E. & S. Livingstone.
11. Kennaway, E. L. and Tipler, M. M. (1947): Brit. J. Exp. Path., **28**, 351.
12. Stephens, E. L. (1953): *Some South African Poisonous and Inedible Fungi*. Cape Town: Longmans, Green & Co.
13. Waser, P. G. (1961): Pharmacol. Rev., **13**, 465.
14. Sapeika, N., Uys, C. J. and McKenzie, D. (1960): S. Afr. J. Lab. Clin. Med., **6**, 12.
15. Wieland, T. and Wieland, O. (1959): Pharmacol. Rev., **11**, 87.
16. Steyn, D. G., Steyn, D. W., Van der Westhuizen, G. C. A. and Louwrens, B. A. (1956): S. Afr. Med. J., **30**, 885.
17. Buck, R. W. (1961): New Engl. J. Med., **265**, 681.