Microbial Toxins

Edited by

Alex Ciegler

Northern Regional Research Laboratory
Agricultural Research Service
United States Department of Agriculture
Peoria, Illinois

Solomon Kadis Samuel J. Ajl

Research Laboratories
Albert Einstein Medical Center
Philadelphia, Pennsylvania

VOLUME VI

FUNGAL TOXINS

1971

ACADEMIC PRESS • NEW YORK AND LONDON

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1 7DD

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 78-84247

PRINTED IN THE UNITED STATES OF AMERICA

Preface

The capacity of fungi to produce toxic metabolites has been known since the turn of the century. As early as 1913, Alsberg and Black of the U. S. Department of Agriculture conjectured that the products of mold growth might be involved in diseases. Certainly the first described form of a mycotoxicosis, ergotism, has been known to man for much of his recorded history. In the nineteenth century, molds were recognized as somehow being responsible for outbreaks of disease such as yellow rice toxicosis in Japan and alimentary toxic aleukia in Russia. In modern times, the ability of fungi to synthesize highly toxic compounds was noted during extensive screenings of microorganisms for antibiotic production, but the significance of these findings was not fully appreciated. However, the eruption of "turkey X" disease in England in 1960, followed by the discovery of the aflatoxins, resulted in a drastic reappraisal of the mycotoxin problem. Surveys of foods and feeds revealed that the problem was worldwide, rather than confined to any geographical area. In addition to the aflatoxins, other mycotoxins, for example, ochratoxin, sporidesmins, and zearalenone, were soon discovered, and this area of research expanded at an almost explosive rate as indicated by the rapid accumulation of a massive literature. The implication of the mycotoxins, aflatoxin, sterigmatocystin, and penicillic acid, as carcinogens added another dimension to the problem. In a world already sensitive to a threatened ecology, the finding of still another menace to its food and feed supply resulted in a great outburst of research.

In contrast to mycotoxin research, investigations on toxins produced by algae have not been quite as extensive or perhaps as fruitful. Nevertheless, a number of algal toxins have been isolated, obtained in highly purified form, and adequately characterized. These toxins have practical significance insofar as many of them are toxic for gill-breathing animals and for man and experimental animals via their consumption of shellfish and other aquatic organisms. One of the most intensively studied of the algal toxins is that produced by *Prymnesium parvum*. Because of the great variety of biological activities that it exhibits, such as toxicity to gill-breathing animals, lysis of erythrocytes, cytotoxicity to various cell types (such as Ehrlich ascites cells, liver cells, and amnion cells), and pharmacological activities on muscle and nerve preparations, it has numerous potential applications.

Despite the great volume of research already reported, the field of mycotoxin research is still in its infancy. This conclusion is also applicable to the algal toxins. Consequently, the purpose of Volumes VI-VIII on

algal and fungal toxins in this multivolume treatise on microbial toxins is to review comprehensively and critically the investigations that have been carried out to date and to emphasize those areas that need additional research. It is hoped that these volumes will encourage scientists in various disciplines such as microbiology, biochemistry, epidemiology, pharmacology, toxicology, medicine, and related fields to devote themselves to some of the productive lines of research that are indicated.

Because of the extensiveness of the literature on algal and fungal toxins, it was necessary to publish three volumes in order to present, comprehensively, current and past information. Hence, the algal and fungal toxins were arbitrarily divided into three groups, but with no implication intended that the toxins in any given group are related with respect to structure, function, mode of action, or biosynthesis. This volume includes the toxins produced by the Aspergilli and Penicillia; Volume VII covers the algal toxins and the fungal toxins produced by species belonging to the genera *Fusarium, Rhizoctonia,* and *Pithomyces.* Volume VIII encompasses the toxins produced by the fungal phytopathogens, the mushrooms, and those toxins synthesized in plants in response to fungal invasion or other injury.

We have attempted to include all the known mycotoxins in these volumes. However, we recognize that the rapidity with which the field is developing will unavoidably result in omission of new toxins and information on currently known toxins that has accumulated since these volumes have gone to press.

We wish to thank the contributors for their cooperation and the staff of Academic Press for their advice and practical assistance.

ALEX CIEGLER
SOLOMON KADIS
SAMUEL J. AJL

Contents of Other Volumes

Introductory Remarks

Algal and fungal toxins comprise a wide array of naturally occurring toxic compounds in the aquatic and terrestrial environment. Their extent and the significance of their evoked toxicoses in various species have only been fully recognized within the last decade.

Some highly potent toxic compounds have been identified among these algal and fungal toxins, but the mycotoxins, perhaps due to the more intensive research on their structure, are the more completely characterized. Of the poisons produced by various species of algae thus far, only a few have been isolated in pure form and characterized. One of these is from a blue-green alga *Microcystis aeruginosa* (1) and another from the dinoflagellate *Gonyaulax catanella* (2). Structurally, the first toxin referenced above is a cyclic polypeptide while the second (saxitoxin) is a substituted purine base. The striking features of some of these toxins, for example, the one from *M. aeruginosa* called FDF (fast death factor), are their lethal effects in less than 30 minutes in experimental animals and their production of paralysis and death in man. At the present time, no effective antidote is known for these shellfish poisons. However, the toxic effect can be reduced by administration of ethanol and sodium chloride to poisoned individuals, followed by artificial respiration. Apparently these poisons are not antigenic, but it has been demonstrated that they can be used as haptens to immunize an animal against the poison (3). Ciguatera toxin challenge to man appears at times when man eats tropical fish that have consumed toxin-producing algae. Many other toxins, which become poisonous to mussels and clams and subsequently to man consuming shellfish, have been studied in their crude form only.

Since many species of algae that may represent a potential food supply produce toxins and because the toxins formed from other algae threaten the existence of edible marine organisms by massive destruction, the sporadic occurrence of these toxins causes an economic problem as well as a serious human health problem. This could become more acute as the world population increases and becomes more dependent upon food from the sea. If the processing of fish for fish protein concentrate becomes indiscriminate relevant to selectivity of fin fish types, a problem of inadvertent introduction of marine biotoxins into this important marine protein source could arise. On the other hand, as intensive pharmacological research proceeds on these marine biotoxins, it is conceivable that important biosynthetic intermediates as products in the metabolism of marine organisms may be uncovered, which will prove efficacious in drug development programs for their pharmacodynamic action and therapeutic value.

Mycotoxicoses, representing a situation more specifically associated with occasional contamination of foodstuffs and feed, have existed for many years. However, mold-induced deterioration of foods and feeds has, until recently, focused primarily upon the resultant economic losses associated with deterioration of the quality of commodities and not as a health hazard per se. The occurrence of fungal metabolites appearing in food as contaminants is to be anticipated in view of the ubiquitous distribution of fungi, the possibilities for their growth during harvest, or their development during storage and handling of food and food crops. Furthermore, we have the additional problem of the biochemical capabilities of some fungal species to produce these metabolites as toxic organic molecules. Although earlier reports in the literature recorded a mycotoxicosis such as ergotism, which has been recognized for centuries, and although there are some isolated reports associated with a variety of toxicity syndromes in animals, only in the last decade has there been an increasing awareness of the potential health significance of mycotoxins as natural chemical environmental contaminants (4).

Perhaps one of the earlier reports in this country on mycotoxicoses was that of Schofield in 1924 (5) relating to poisoning in cattle induced by fungally contaminated sweet clover. However, the first systematic studies were probably initiated in the USSR around 1940. These related to stachybotryotoxicoses, primarily affecting horses and subsequently shown to be caused by a toxin in the etiology of disease in cattle, other animals, and man (6). Later, in 1953 and 1957, there were reports of diseases in cattle and swine from feeding moldy corn. The disease was reproduced subsequently by feeding fungi cultured on various substrates (7, 8). At this time, the reports of Carll and co-workers and Forgacs *et al.* did much to renew interest in mold intoxication investigations in the United States (9, 10).

Most of the reports prior to this period were centered on livestock toxicoses of unknown etiology and only speculated about the role played by fungi, while some scientists believed fungi and their metabolites were not injurious, if not beneficial, to animal health (11, 12, 13, 14). Apparently, these diverse earlier opinions were conditioned by the absence of good methods for isolation and identification of fungal metabolites as well as a lack of good epizoology complicated by a matrix of etiological factors associated with variant symptom complexes.

More precise studies evolved in 1955-1957 involving toxic strains of *Aspergillus flavus* and *Penicillium rubrum*, which were reported to cause a toxicosis in swine. In an area where poultry hemorrhagic syndrome was extensive (15), a toxic fungus was isolated from feed and poultry litter and was identified as that of *A. flavus*. About this time, a

facial eczema in sheep and cattle in New Zealand was reported to be caused by ingestion of dried pasture grass contaminated with *Pithomyces chartarum* (16).

As previously indicated, the fact that molds could indeed produce toxic metabolites had been known for many years, but the etiology of these toxic factors was ignored, thus leading to the general reference to mycotoxicoses as the neglected disease. This neglect was soon altered drastically by outbreaks of a "turkey X" disease in poultry in England in 1960 and the concurrent development of an epizootic in trout in commercial fish hatcheries fed rations later shown to be fungally contaminated (4). The acceleration of research on this problem in several countries quickly led to the isolation and identification of the aflatoxins, one of which, aflatoxin B_1, was demonstrated to be quite toxic to many animal species and one of the most potent hepatocarcinogens for the rat and trout (4). Thus, the importance of this discovery cannot be overemphasized in terms of worldwide significance relevant to potential animal and human neoplastic disease induced by dietary contaminants.

Obviously, the latter concern intensified the interest and efforts of teams of scientists with a multidisciplinary approach. These projects soon led to an elucidation of phenomena involved in acute toxicity and provided us with a clearer understanding of the relationship of intracellular response and carcinogenic expression. It is of interest that the frequency of liver disease among trout populations investigated in the United States, at least those fed commercial rations, reached an incidence of 50 to 70%. It has been assumed that this specific disease frequency among wild trout populations would be of low order, but no definite survey data are available for this comparison (4). The synergistic role of aflatoxins with components normally present in some foods and the possible potentiation by other mycotoxins, e.g., rubratoxin, have not been overlooked. For example, the cocarcinogenic effects of gossypol and cyclopropenoid fatty acids present in cottonseed meal fats with aflatoxin B_1 in the trout have been reported (17).

Due to the brilliant and classic research on the isolation, identification, and structure determination of aflatoxins, this unique group of highly oxygenated, naturally occurring heterocyclic compounds (18) are now characterized as B_1, B_2, G_1, G_2, M_1 and M_2 (milk toxins), and B_{2a} and G_{2a}. Other fungal metabolites have been similarly isolated, identified, and their structures ascertained, and will be referenced in detail in subsequent chapters. Some of these are from the *Aspergillus* series such as ochratoxin from *A. ochraceous*, aspertoxin from *A. flavus*, sterigmatocystin from *A. versicolor* (4, 19), and kotanone from *A. glaucus-Kota* (20). A similar series from the penicillia, to name but a few, are patulin from *P. patulum*,

rubratoxin from *P. rubrum*, and some of the yellow rice toxins (islandicin, iridoskyrin, rubraskyrin, skyrin, luteoskyrin, erythroskyrin); the actinomycins have also been extensively studied (21, 22).

Perhaps less well known are the furanosesquiterpenes and related metabolites. For example, a tremorgenic-diuretic mycotoxin from *P. cyclopium* (23), and ipomeamarone, characterized in Japan (24) and causing lung edema in cattle, is elaborated by the sweet potato only when fungus grows on the sweet potato and will, therefore, be classified as a microbial toxin. There may be other similar situations so classified.

Mycotoxicosis assumes worldwide significance with respect to population groups depleted in sources of protein (kwashiorkor), in which additional liver pathology may develop from dietary insults from mycotoxins. Similarly, corrective measures for malnutrition in which protein sources are used that may be fungally contaminated (legumes and cereals) could add a new dimension in geographic pathology from such a stress. Certainly, controls are required in agricultural technology and commercial processing to eliminate unfavorable conditions of storage. These include factors relevant to temperature, humidity, and the destructive processes in harvesting of any such toxic fungal products that may been produced.

Although there is a plethora of information and data on the toxicological and biochemical aspects of mycotoxin action in experimental animals, including ultrastructural effects, changes in RNA/DNA ratio, mitochondria, and template activity, there is only suggestive evidence from such investigations that may or may not provide a strong case for causal relationship between these environmental toxicants and incidence of disease in man. Epidemiological studies, supported in the sub-Sahara region of Africa and certain regions of Asia, could, for example, delineate the pathogenesis of neoplasia and other associated diseases induced by the mycotoxins. There are pieces of information that provide some support as to the implication of these agents in human health, such as alimentary toxic aleukia in Russia and yellow rice as an etiologic factor in human disease in Japan. In Africa, there is presumptive evidence that the etiology of aflatoxicoses and hepatomagenesis in the Bantu and isolated cases of disease in infants in Senegal allegedly caused by ingestion of contaminated peanut products offer no assurance that man is not equally susceptible to these hepatotoxin, as observed in other species (25).

It is hoped that the compilation of information within a single series of volumes on these natural toxins and the evaluation of the chemical, biological, and biomedical aspects of the algal and fungal toxins will increase the awareness of the problem of these environmental stresses relevant to public health. Furthermore, such a compendium provides an

excellent reference source for the future to those interested in the chemistry and toxicology of these specific natural toxic compounds not hitherto available from a single reference source.

HERMAN F. KRAYBILL
Food and Drug Administration
Department of Health, Education, and Welfare
Washington, D.C.

REFERENCES

1. Bishop, C. T., Amet, E. F. L. J., and Gorham, P. R. (1959). *Can. J. Biochem. Physiol.* **37**, 453.
2. Schantz, E. J., Lynch, J. M., Voyvada, G., Matsumoto, K., and Rappoport, H. (1966). *Biochemistry* **5**, 1191.
3. Johnson, H. M., Frey, P. A., Angelotti, R., Campbell, J. E., and Lewis, K. H. (1964). *Proc. Soc. Exptl. Biol. Med.* **117**, 423.
4. Kraybill, H. F., and Shimkin, M. B. (1964). *Advan. Cancer Res.* **8**, 191-248.
5. Schofield, F. W. (1924). *J. Am. Vet. Med. Assoc.* **64**, 553-575.
6. Sarkisov, A. (1947). *Veterinariya* **24**, 25-27.
7. Sippel, W. L., Barnside, J. E., and Atwood, M. B. (1953). *Proc. 90th Ann. Meeting Am. Vet. Med. Assoc. Toronto, Canada* 171-181.
8. Burnside, J. E., Sippel, W. L., Forgacs, J., Carll, W. T., Atwood, M. B., and Doll, E. R. (1957). *Am. J. Vet. Res.* **18**, 817-824.
9. Carll, W. T., Forgacs, J., and Herring, A. (1954). *Am. J. Hyg.* **60**, 8-14.
10. Forgacs, J., Carll, W. T., Herring, A. S., and Mahlandt, B. (1954). *Am. J. Hyg.* **60**, 15-26.
11. Biester, H. E., Schwarte, L. H., and Reddy, C. H. (1940). *Vet. Med.* **35**, 636-639.
12. Eckles, C. H., Fitch, C. P., and Seal, J. H. (1924). *J. Am. Vet. Med. Assoc.* 716-722.
13. Gorcica, H. J., Peterson, W. H., and Steenbock, H. (1935). *J. Nutr.* **9**, 691-714.
14. Petty, M. A., and Quigley, C. D. (1947). *Poultry Sci.* **26**, 7-13.
15. Forgacs, J., and Carll, W. T. (1955). *Vet. Med.* **50**, 172.
16. Clare, N. T. (1955). *Advan. Vet. Sci.* **2**, 182-211.
17. Lee, D. J., Wales, J. H., Sinnhuber, R. O., Ayres, J. L., and Roehm, J. N. (1967). *Federation Proc.* **26**, 322.
18. Buchi, G., and Rae, I. D. (1969). *In* "Aflatoxin" (L. A. Goldblatt, ed.), pp. 55-75. Academic Press, New York.
19. Rodricks, J. F., Henery-Logan, K. R., Campbell, A. D., Stoloff, L., and Verrett, M. J. (1968). *Nature* **217**, 668.
20. Buchi, G. (1970). Personal communication.
21. Perone, V. P., Scheel, L. D., and Meltus, R. J. (1963). *Am. Chem. Soc. Div. Biol. Chem. Abstr.* **22**, 14A.
22. Dickens, F., and Jones, H. E. H. (1961). *Brit. J. Cancer* **15**, 85-100.
23. Wilson, B. J., Wilson, C. H., and Hayes, A. W. (1968). *Nature* **220**, 77.
24. Kubota, T., and Matsuura T. (1958). *J. Chem. Soc.*, p. 3667.
25. Kraybill, H. F., and Shapiro, R. E. (1969). *In* "Aflatoxin" (L. A. Goldblatt, ed.), pp. 401-441. Academic Press, New York.

Contents

Section B *Penicillium* Toxins