CHEMISTRY AND TOXICOLOGY OF THE TOXINS OF *AMANITA PHALLOIDES*

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Recorded history, from its very beginnings, tells of lethal poisonings from mushrooms. Even today, death from such poisonings continues to claim its victims all over the globe in an estimated annual number of several hundreds. It is now known that the responsibility rests solely on mushrooms from the genus *Amanita* which includes a few species of severe toxicity. In Central Europe, the predominant culprit is the greenish *A. phalloides Fr.*, but there are also reports of poisoning by the white *A. verna*. In the United States, it is this mushroom, called "destroying angel," which along with another poisonous species, *A. tenuifolia*, appears to play the major part in lethal mushroom poisonings (10, 11). The yellow *A. mappa Batsch*, widely distributed throughout Europe and readily distinguishable from *A. phalloides* by the color of its flesh and its characteristic potato germ odor, is devoid of any trace of the lethal poisons.

I. CHEMISTRY OF THE TOXIC COMPONENTS

A. Isolation of the toxic ingredients

Attempts to obtain the lethal poison of *A. phalloides* in a pure state started at the beginning of the last century. The following survey up to 1932, taken from Raab (53), gives the historically interested reader the chronological sequence of the major developments.

1793–1808 Paulet (51): The toxin of *A. phalloides* is soluble in water and alcohol and is not destroyed by drying or by heating in boiling water. Animal experimentation with variously treated extracts from the mushroom.

1811 Vauquelin (70): The toxic substance is a "fatty matter."

1826 Letellier (41): The amanita poison is identical with that of the toadstool and has the properties of an alkaloid ("amanitine").

1866 Boudier (12): First systematic study of botanically well determined mushrooms, establishing that the toadstool poison is definitely different from that of amanita.

1877 Oré (49, 50): The toxic principle (called "phalloidine") is said to be very similar, or even identical, to strychnine.

1891 Kobert (37): Freshly expressed juices from *A. phalloides* possess strong hemolytic activity. The unknown hemolysin is called "phallin." It is highly sensitive to heat, alkali and acids and insoluble in organic solvents. It is said to be the active principle in human poisonings.

1893 Seibert (63): Mushrooms definitely toxic for various animals may lack hemolysin which, accordingly, is not the active principle in the typical poisonings.

1909 Kobert (38): The hemolysin is present in *A. phalloides* in greatly varying amounts and may even be completely absent. As many as three toxins are supposed to occur in *A. phalloides*.

1906–1913 Ford *et al.* (23): *A. phalloides* contains two toxic substances: a thermolabile hemolysin identical with Kobert's phallin, said to be a sulfur-containing glucoside, and another toxin not an alkaloid in nature and called "amanitatoxin." Neither can be obtained in pure form.

1932 Raab (53): Introduction of the guinea pig test. Approximately 100-fold concentration of "amanitatoxin," principally by precipitation with heavy metal salts. Impurities removed by precipitation with lead acetate. Poisoned animals lose weight rapidly.

1934–1935 Renz (62), with Raab (54) and Kimmig (36): Use of chromatographic adsorption. The toxin is difficult to dialyze and can be extracted from water with butanol.

1937 Lynen and U. Wieland (42): The poison is precipitable from aqueous solution by ammonium sulfate. It consists of two components. The more rapidly acting one is obtained in crystalline form and designated as phalloidin and data are given on its chemical nature. The other component, slower acting but more toxic, is brought to 50% purity. Indications for a third component are reported.

1941 H. Wieland and Hallermayer (76): The highly potent component is obtained in crystalline form and called amanitin.

When work was resumed following World War II, new methods of fractionation such as countercurrent distribution and paper chromatography and the paper electrophoresis elaborated by T. Wieland were available. Hence it was soon possible to demonstrate that amanitin consists of two crystalline components, a neutral α- and an acid β-component. The β-substance also proved to be toxic. On paper electrophoresis it travels to the anode (92). Subsequently, one of a great number of combinations of solvents tried (the upper phase of a system of 20 volumes of methyl ethyl ketone, 2 volumes of acetone and 5 volumes of water) was found to allow paper-chromatographic separation of the phytotoxins and thus rapid analysis of mushroom juices (89). The toxins give, with cinnamic aldehyde in an atmosphere rich in hydrochloric acid vapors, blue and bluish-purple colors (see Table 1). Still another compound, γ-amanitin, present in very small amounts, was detected with the aid of this procedure; it was recently isolated and also proved to be toxic (86). This new compound persistently accompanies phalloidin in regular paper chromatograms, but ultimately its separation and micropreparation became possible by impregnating the paper with borate buffer. Finally, a fifth toxin, phalloin, was crystallized from *A. phalloides*; in its rapid action it is comparable with phalloidin (87).

TABLE 1

Main characteristics of the amanita toxins

	Phalloidin	Phalloin	α- and β-Amanitin	γ-Amanitin
Cinnamic aldehyde-HCl	Light blue, fleeting; threshold 10–20 μg	Light blue, fleeting; threshold 10–20 μg	Deep purple; threshold 10–20 μg	Deep purple; threshold 10–20 μg
Fe^{3+}-containing conc. H_2SO_4	Bright blue	Bright blue	Olive green, fades toward yellow	Olive green, fades toward yellow
Folin-Denis	Blue	Blue	Blue	Blue
Millon's reagent	Brown	Brown	Brown	Brown
Diazot. sulfanilic acid (Pauly)	Yellow	Yellow	Red	Red
Hopkins-Cole	Negative	Negative	Blue	Blue
Ammoniacal silver sol.	Negative		At once reduced	Rapidly reduced at 70°C
Maximum of UV absorption	290 mμ	290 mμ	305 mμ	305 mμ
R_f value*	0.5	0.6–0.65	0.41 (α); 0.24 (β)	0.5
Toxicity†	2 mg	ca. 1 mg	0.1 mg (α); 0.4 mg (β)	0.8 mg

* Solvent: 2-butanone-acetone-water (20:2:5), upper phase.

† The toxicity (LD50) was tested in the albino mouse on intraperitoneal injection. Doses are calculated per kg body weight.

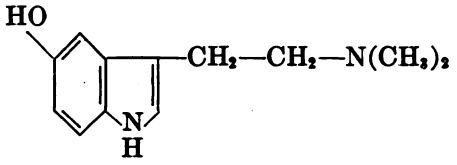
For isolation of the various toxic components of *A. phalloides*, the following general procedure is now being employed. Fresh mushrooms are placed in methanol and expressed after a few days. The extracts are filtered and evaporated to a syrup *in vacuo*. On addition of 5 to 10 volumes of methanol much potassium chloride precipitates and is removed by filtration. Methanol is again evaporated, the residue is taken up with water, lead acetate is added until marked precipitation no longer occurs and the filtrate, freed of lead, is saturated with ammonium sulfate. This precipitates the bulk of the toxins; the rest is extracted with *iso*-propanol. In this manner almost all the toxin is obtained as so-called "primary material" containing 10 to 20% toxins along with predominantly acid, but also some basic, impurities. They are removed by treatment with anion and cation exchangers whereby the weight is reduced by 50% without loss in activity (43). By partition and adsorption chromatography on alumina phalloidin, α-amanitin and β-amanitin are then separated from each other and crystallized from methanol. γ-Amanitin remains in the mother liquors of the phalloidin crystallization. Phalloin, which travels faster than phalloidin, is obtained in the chromatograms in such purity that it crystallizes at once from water (87).

The amanita toxins are readily soluble in water, methanol, liquid ammonia and pyridine; moderately soluble in ethanol; dissolve in higher alcohols and phenol only on addition of water; and are insoluble in weakly polar organic solvents. Some of them differ markedly in toxicity and in their identification criteria (Table 1).

In regard to the amounts of the individual toxins contained in amanita, only tentative conclusions can be drawn from the yields of isolated substances or

from toxicological data. More reliable is the spectrophotometric assay after paper-chromatographic separation and elution of the compounds, particularly when these procedures are applied to the greatly pre-purified and quantitatively prepared "primary material" (43). Such assays yield 10 mg phalloidin, 8 mg α-amanitin, 5 mg β-amanitin, 0.5 mg γ-amanitin and traces of phalloin per 100 g of fresh mushrooms. In a mixture of this ratio, the α-amanitin is largely responsible for the toxic effect; since its lethal dose is about 0.1 mg/kg, it is conceivable that the toxin content of a mushroom weighing 50 g may be sufficient to kill a human being.

As mentioned above, the typical combination of toxins has hitherto been determined in only a few amanita species. In a comparative study of the toxin content of various amanita species by American authors (10, 11), phalloidin is conspicuous by its absence. Since the cinnamic aldehyde-HCl color reaction is about 10 times less sensitive for phalloidin than for the amanitins and the extracts employed were not concentrated, it would appear that phalloidin, as well as the closely related phalloin, was overlooked in the paper-chromatographic analyses. As indicated already, the congeneric *A. mappa* does not contain any of the toxins but instead an indole derivative which proved to be bufotenine (5-hydroxy-N-dimethyl-tryptamine (I)) (91), a base occurring in the toad (*Bufo vulgaris*) and also recently isolated from *Piptadenia peregrina* (24).



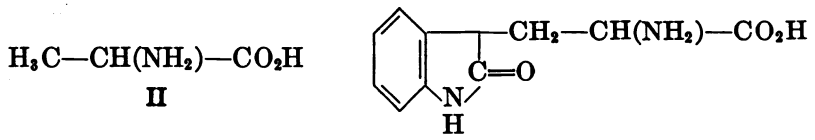
I

B. Chemical structure of the toxic ingredients

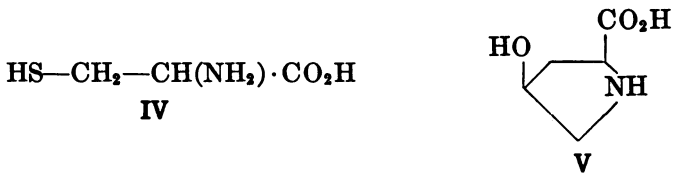
Chemically the five toxins have certain characteristics in common. They are all cyclopeptides composed of only a few amino acids (molecular weight ca. 1000), some of which do not occur in proteins. Each contains one S atom per molecule, which belongs to a cysteine residue but is present neither as —SH nor as —S—S—. In the ultraviolet they exhibit an intensive absorption maximum near 300 mμ. This suggests the presence of an indole chromophor, which is in accord with certain color reactions, particularly a positive pine splint reaction after alkaline fusion.

1. Phalloidin. Phalloidin is the best known of all the toxins. By hydrolyzing the crystalline toxin with 20% hydrochloric acid or 25% sulfuric acid at 100°C, as is customary for cleavage of polypeptides, Lynen and U. Wieland (42) obtained alanine (II), and H. Wieland and Witkop (77) obtained L-α-oxytryptophane (III); more correctly termed β-oxindolyl-α-alanine because of its lactam structure) and L-cysteine (IV). They also found L-*allo*-hydroxyproline (V), the diastereoisomer of the hydroxyproline usually occurring in nature, in which car-

boxyl and hydroxyl groups are situated on opposite sides of the plane of the 5-membered ring. The alanine was identified as the L-form.



II III



IV V

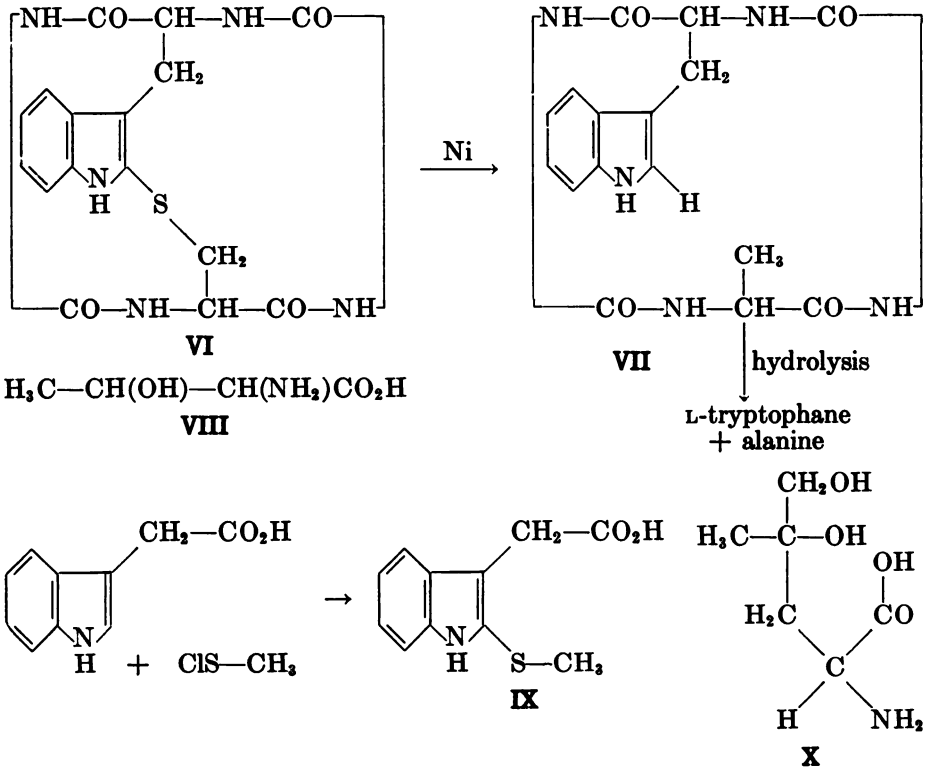
Thus the toxin is a rather unusual peptide. Moreover, it has no terminal amino or carboxyl groups and hence was considered to be a cyclopeptide. In later studies, T. Wieland and Schmidt (89) demonstrated the peculiar nature of the linkage between cysteine and tryptophane, previously suggested by Neuberger *et al.* (15) on the basis of spectroscopic observations. The oxindolylalanine (III) building block, when isolated, exhibits maximum absorption at 250 mμ (indicating a N-acyl-aniline rather than an indole spectrum) whereas in phalloidin the greater wave length maximum (290 mμ) points to an indole conjugation. On the basis of these observations and of the absence of the nitroprusside reaction even after reduction, a linkage of the cysteine sulfur to position 2 of the tryptophane ring was postulated. Indeed, reductive desulfuration by boiling with Raney nickel converted phalloidin to a non-toxic, sulfur-free cyclopeptide, desthiophalloidin (VII), whose hydrolyzate yielded tryptophane instead of oxytryptophane and had a higher alanine content (89). These findings tended to substantiate a thio-amide structure (VI). In the same investigations, threonine (VIII), which had not been isolated by earlier investigators, was disclosed by paper chromatography as another building block of the phalloidin hydrolyzate. This constituent belongs to the D-series (unpublished work of A. Schöpf). Simultaneous investigations were carried out by Šorm *et al.* (64, 65).

Further evidence for the presence of a thio-amide structure came from synthesis of model substances. In various indole derivatives, including tryptophane itself, it is possible to introduce sulfur into position 2 by reaction with disulfurdichloride, ClSSCl, and sulfenic acid chlorides, RSCl (93). Such thio-amides, *e.g.*, 2-S-methylindolyl acetic acid (IX), are very similar to phalloidin in their absorption characteristics. By comparing the extinction of the model compound (IX) (ϵ = 12270, molecular weight 221.3) with that of phalloidin, a molecular weight of 890 could be calculated for phalloidin.

In further studies of phalloidin hydrolyzates paper chromatography proved inadequate, whereas cooled paper electrophoresis at elevated voltages was extremely helpful (88). With this method, a sixth hitherto unknown peptide

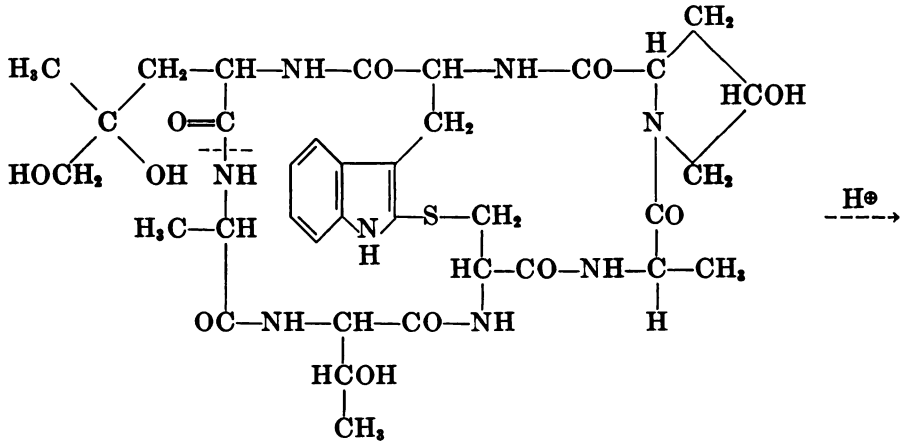
building block was discovered (90). In the freshly prepared acid hydrolyzate a ninhydrine-positive cationic compound was demonstrable which is rapidly converted to a neutral α-amino acid by dilute alkali and reconverted by mineral acids. This suggested an α-amino-γ- (or δ-) hydroxy acid structure traveling in an electric field to the cathode as an amino lactone. The compound was eluted as the lactone hydrochloride, crystallized and identified as α-amino-γ-hydroxy-γ-hydroxymethyl *n*-valeric acid (X).[1] It was called δ-oxyleucenine hydrate. By an enzymatic method, it has been shown that the aspartic acid, obtained by oxidative degradation of the new amino acid, belongs to the L-series (A. Schöpf, unpublished).

With complete information on the component amino acid building blocks and their molar ratios at hand, it became possible to establish the formula of phalloidin as follows: 1 cysteine, 2 alanines, 1 *allo*-hydroxyproline 1 threonine, 1 oxindolylalanine, 1 δ-oxyleucenine,[2] less 7 H_2O (seven peptide linkages), less 1 H_2O (one thio ether linkage), corresponding to the molecular formula $C_{35}H_{48}O_{10}N_8S \cdot 6\ H_2O$.

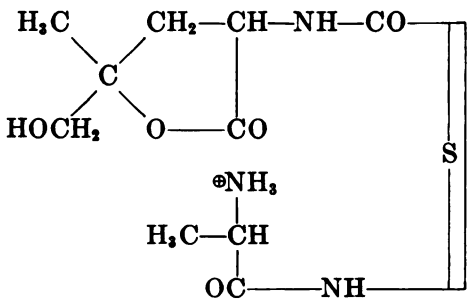


[1]Recently, A. Schöpf showed that in phalloidin the branched amino acid occurs in the γ-hydroxylated state (X), and not, as we reported earlier (90), as an unsaturated compound.

[2]See footnote 1, above.



XI



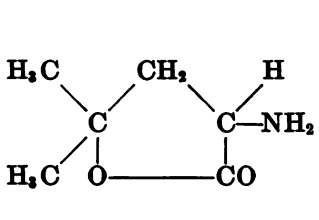
XII

Since the air-dry peptide contains 6 moles of water rather stably bound, the molecular weight must be augmented to 898 (determined from spectroscopic evidence: 890). On this basis the analytical values for all elements agree well with the calculated values.

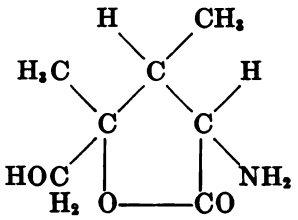
In order to determine the arrangement of the building blocks the cyclic molecule was opened hydrolytically in order to provide a starting point for Edman's cleavage procedure with phenyl mustard oil. Selective opening at the broken line (in XI) by heating for 30 min at 100°C with 0.2 N sulfuric acid yielded a "half-open" phalloidin (XII) cyclized only by the S-bridge, which, like the other monocyclic derivative, the desulfuration product desthiophalloidin (VII), is devoid of toxicity and cannot be hydrolyzed by peptidases. By stepwise cleavage it was finally possible to establish the structural formula of phalloidin (XI) (90).

2. Phalloin. Phalloin, the last one of the amanita toxins to be found, is chemically closely related to phalloidin. Both give the same characteristic color reactions and have the same ultraviolet spectrum (see Table 1) (87). The close relationship of the two peptides is further indicated by their amino acid composition. The two-dimensional paper chromatogram of a phalloin hydrolyzate also shows

alanine, threonine, cysteine, *allo*-hydroxyproline and oxindolylalanine (87). In addition to the amino acids enumerated above, the sulfuric acid hydrolyzate of phalloin contains a ninhydrine-positive amino lactone which, on electrophoresis, travels to the cathode somewhat faster than the analogous building block of phalloidin. It turned out to be the γ-lactone (XIII) of γ-hydroxyleucine. The only difference between phalloidin and phalloin, therefore, consists in the lack of one O-atom in the δ-position of the branched amino acid (XIV) in the latter (87a).



XIII



XIV

3. The amanitins. Our knowledge of the chemistry of the more toxic amanitins is less complete. Chemically, they differ from the toxins already discussed principally in giving a more intensive color reaction with cinnamic aldehyde-HCl and in reducing ammoniated silver solution. In all of them, the reducing activity is ascribed to an O-atom in the *para*-position to the indole-N. Such a *p*-aminophenol grouping is suggested by the absorption maximum at the greater wavelength of about 300 mμ, which, for instance, is also observed in 2,5-methoxytryptophane carrying sulfur in position 2 (75). In this connection, the occurrence of bufotenine (I) in the congeneric *A. mappa* (91) is not without significance.

The relation of α- to β-amanitin is that of an acid amide to the respective carboxylic acid (89). The α-compound, which does not travel in the paper chromatogram, contains in the hydrolyzate the same building blocks as β-amanitin, including aspartic acid, but contains in addition one molecule of ammonia. Thus it appears that the neutral cyclopeptide differs from the acid one only in the amido group of asparagine. As further building blocks, cysteine, glycine, hydroxyproline (not in *allo* form), isoleucine and a lactonizing amino acid could be demonstrated with certainty in the sulfuric acid hydrolyzate of α-amanitin (28). Recently, we could elucidate its structure as the γ-lactone of α-amino-β-methyl-γ-hyroxy-γ-hydroxymethyl-n-valeric acid (86a). The most interesting section of the molecule, the reducing building block, which is also responsible for the absorption in the ultraviolet, has as yet resisted isolation because of its great instability. It has recently become possible to work out conditions of hydrolysis which may permit its isolation. For this purpose, the phenolic hydroxyl group of α-amanitin must be converted to a methyl ether. Contrary to earlier reports (76) it has been observed that the methylamanitin formed reduces ammoniated silver solution only at elevated temperature (70°C), as is the case with *p*-aminophenol ethers. Methylamanitin is largely split with 2N H_2SO_4 at 100°C in 1 to 2 hours and then shows in the paper chromatogram the

spot of an amino acid giving a red color with diazotized sulfanilic acid and separable from the hydrolyzate by precipitation with mercury (28).

In regard to the binding of the cysteine in the amanitins, a situation similar to the case of phalloidin was disclosed by desulfuration with Raney nickel (89). Desthio-α-amanitin, in paper chromatograms of the hydrolyzate, exhibits the new spot of alanine rather than that of cysteine. A binding of the cysteine sulfur to the 2-position of the indole ring is contradicted by the positive Hopkins-Cole reaction of amanitin, which is said to be given only by indole compounds with a free 2-position. However, as yet too little is known about the behavior of 5-hydroxylated indole derivatives to justify definitive structural conclusions merely from the positive color reaction.

By a specific enzymatic determination (52) of the aspartic acid content of the hydrolyzate (12.34%) the molecular weight of α-amanitin, on the assumption of one aspartic acid rest per molecule, was ascertained to be 1072.

Hitherto γ-amanitin, an accessory toxin of *A. phalloides*, has been obtained only by tedious purification procedures and in small amounts (86). Its congenerity with the major toxins is indicated by various properties, chiefly by almost identical ultraviolet absorption. However, unlike the other compounds, it reduced diamino silver rapidly at only 70°C, suggesting a *p*-aminophenol ether since methylamanitin shares this property. γ-Amanitin also contains cysteine in a linkage still undetermined as well as (*allo*)-hydroxyproline, isoleucine and aspartic acid, probably as β-amide. Also detected in the hydrolyzate was the basic lactone of a hydroxyamino acid apparently different from those characteristic of either phalloidin, phalloin or α-amanitin. A definite difference from the other amanitins consists in the presence of alanine instead of glycine among the building blocks.

II. BIOCHEMISTRY AND TOXICOLOGY

Efforts to unravel the mechanism of action of the amanita poisons have been no less intensive, though less successful, than the chemical investigations. In addition to the alimentary poisonings periodically reported, which have offered an opportunity for clinical studies of the effects of mushroom poisoning upon the human organism, there is also an impressive number of experimental investigations in animals, conducted originally with the entire mushroom, later on with crude mushroom extracts and recently also with the isolated toxins α-amanitin and phalloidin. The literature on this subject to 1943 is reviewed in a comprehensive paper of Neuhann (48), to which the student of the problem may be referred for details exceeding the scope of the present review.

A. Symptomatology and pathological anatomy of amanita poisoning

In the cases of poisoning in man, a prominent feature is always the long latent period between the ingestion of the fateful mushroom dish and the appearance of the first signs. It is usually not until 10 to 20 hours have elapsed that, abruptly, violent emesis and diarrhea begin. These gastrointestinal signs do not occur after experimental parenteral administration of mushroom extracts. They may

completely dominate the clinical picture and result in rapid death with cholera-like manifestations. If this phase is overcome, possibly with the aid of appropriate therapy such as fluids and sodium chloride, a transient and false remission takes place followed gradually by more pronounced signs of injury to parenchymatous organs, chiefly the liver, culminating in edema and tenderness of the organ and often accompanied by icterus and urinary excretion of biliary pigments. Involvement of the kidney is manifested by the appearance of albumen, cylinders and erythrocytes in the urine. The terminal phase is initiated by rapid general decay and fall in blood pressure followed by signs of central nervous system disturbance, *e.g.*, general excitation and tonic-clonic convulsions, and finally lethal coma. In some instances there is a striking anomaly of blood glucose levels, with a short rise at the onset of the poisoning and greatly lowered values toward the end.

According to some descriptions, the capillaries appear to be subject to the toxin action, as evidenced by retinal and conjunctival bleedings, cutaneous petechiae and positive Rumpel-Leede phenomenon. Hypoplastic anemia following a severe amanita poisoning (59) also deserves mention.

The post-mortem findings are of remarkable uniformity. Roughly summarized [for details see (48)] they consist of fatty degeneration of heart, liver, kidney and skeletal muscles, in extravasates at the serous and mucous membranes and frequently in swelling of the lymphatic tissues of the intestine. Inflammatory processes of the gastrointestinal tract are also observed in varying intensity.

Most important are the alterations in the large parenchymatous organs, especially the fatty degeneration and destruction of the liver. The pattern is quite similar to that in other hepatopathies, *e.g.*, in phosphorus poisoning and acute yellow liver atrophy: massive fatty degeneration of the liver cells with all signs of incipient or progressed lysis of cell body and nucleus. There are also predominantly hemorrhagic forms with capillary stasis and extravasates (39) that may even assume hematome-like character.

Not only the liver but also the kidney proves, on histological examination, to be extensively involved. Here it is not so much the glomeruli but rather the tubular parts, the various sections of which exhibit the picture of nephrosis with cloudy swelling, fatty degeneration, necrosis and nuclear atrophy. The injury favors the sections most important for excretion, *i.e.*, the convolute tubules and the ascending limb of the loop of Henle. Often the adrenals also participate in the general fatty degeneration.

According to various observers, heart and skeletal muscle are further sites of predilection of the poison-induced degeneration and fat infiltrations. In severe cases they may lead to destruction of the muscle fibers.

B. Experimental studies with mushrooms and extracts

Concurrently with the clinical and anatomical investigations reported above, elucidation of the toxin problem was also attempted by animal experimentation. For this purpose poisoning was induced either by feeding appropriate preparations of whole mushrooms or by injection of mushroom extracts, usually alcoholic.

The following animals were found to be highly sensitive to the toxins: guinea pig, rabbit, rat, mouse, horse, goat, sheep, monkey and pigeon (19). Poikilothermic animals, having a more sluggish metabolism, are much less sensitive to the poison. In frogs and toads, for instance, the lethal dose is as high as 1.5 mg/kg and causes death only after 5 to 10 days (83). In invertebrates (*Helix pomatia; Limax arborum*) toxic effects were observed only with α-amanitin, 100 mg/kg of which was lethal, whereas phalloidin was well tolerated. Probably the great difference in lethal dose is directly related to the metabolic rate of the various animals (83). Heart fibroblasts in tissue culture as well as monocellular organisms such as yeast and lactobacilli are not killed by the toxins (83).

As far as histological effects upon the various organs were concerned, these experiments resulted essentially in findings already known from poisonings in man. In the case of parenteral administration of extracts, subserous hemorrhages and fatty infiltration of muscle were absent. There was also an essential difference in that the gastrointestinal manifestations, *i.e.*, emesis and diarrhea, were missing (14, 74, 78). A further outcome of these animal experiments was the conclusion that, by repeated injection of subthreshold doses of extract, liver cirrhosis could not be induced (69).

C. Earlier metabolic experiments

Whereas this work was mainly directed by morphological considerations, the change to animal experimentation brought more and more into the foreground the problem of the interference of the toxins with metabolic processes. As these studies have major importance from a biochemical and toxicological viewpoint, they will be dealt with here in somewhat greater detail.

The blood sugar drop to hypoglycemic values in the terminal stage of amanita poisonings invited further experimental analysis of this phenomenon. The numerous studies established, in complete agreement, that the blood glucose concentration progressively decreases, with pronounced hypoglycemia appearing shortly before death (4, 5, 7, 17, 18, 34, 35, 46, 47, 67, 68, 69). In the opinion of several authors this explains the convulsive seizures frequently occurring in the final phase (4, 68). However, as will be discussed later, this interpretation does not invariably hold true for poisonings with pure phalloidin. In addition to the hypoglycemia, a brief initial rise in blood sugar was repeatedly noted (46, 69).

Animal experiments also confirmed the clinical observation of a rise in the serum lactic acid level (5, 6, 34). Even in perfusion experiments the poisoned dog liver is reported to form larger amounts of lactic acid and to be incapable of synthetizing glucose from lactate (33). Urea formation was not impaired in the same preparation (33); however, in more recent studies with crystalline α-amanitin (85) a decrease in the urea content of the mouse liver during poisoning was observed (from 35 to 45 mg/100 g to 20 mg/100 g tissue).

Post-mortem analysis of the liver of lethally poisoned rabbits demonstrated, in conformity with histological findings of other authors, a decrease of glycogen to amounts below the sensitivity of the analytical method (8, 17).

Apart from the fatty infiltration of various organs, consistently described by the histologist, the disturbance of fat metabolism in experiments with *A. phal-*

loides extracts was further evidenced by an increase in serum lipids, involving at first chiefly the non-saponifiable fraction but later more and more the other lipid components including cholesterol and particularly the free fatty acids (9, 17). In the dog there was a rise of β-hydroxybutyric acid and acetone blood levels (17) and in the rat an increase in acetoacetic acid (44). These findings as well as the lowered alkali reserve in rabbits (3, 40) are readily explained as signs of starvation acidosis.

Other characteristic humoral alterations are the rise in blood urea and glutathione together with a slow drop of the chloride concentration (3, 19, 40). The rise of blood glutathione is accompanied by decrease of liver and muscle glutathione (3, 40), a concomitance likewise encountered after poisoning with pure γ-amanitin (85). According to French investigators, the picture of lethal amanita poisoning also includes impairment of blood coagulation (9).

In the histological field, too, there have been interesting reports, according to which amanita extracts induced pancreatic β-cell hyperplasia and simultaneous α-cell atrophy in the rat (71). On the basis of these alterations, which other investigators were unable to produce with crystallized α-amanitin (81), the hypoglycemia could possibly be interpreted as a sequel of hyperinsulinism. This concept is supported by reports (73) that rats previously made diabetic by alloxan survived doses of toxin lethal to normal animals. Alloxan experiments in rats treated with phalloidin (80), which essentially confirm these results, will be discussed below.

D. Therapeutic investigations

Clinical considerations, important in view of the annual number of human poisonings, also called for animal experiments as a means of obtaining more reliable information on the effectiveness of therapeutic measures. Thus the question of whether any decisive effect can be achieved with glucose administration was studied from various aspects. The results were in part positive (4) and in part negative (19, 74). However, when preference is given to experiments conducted with the isolated toxins under exactly reproducible conditions of dosage, the glucose treatment must be denied any specific therapeutic value beyond merely symptomatic effects (81).

Animals receiving lethal doses of α-amanitin or phalloidin succumb within the usual period of time despite continuous administration of adequate amounts of glucose. The same holds true for treatment with 17-oxy-11-dehydrocorticosterone (79), desoxycorticosterone (14, 81) and choline (61), although specific protective action against the toxins has been ascribed to the latter as well as to methionine (45, 72). Successful therapy has also been reported with adrenal extracts, as well as with hypertonic sodium chloride solution which, on intraperitoneal injection, kept alive 70% of the animals receiving lethal doses (14). These results have not as yet been confirmed with isolated toxins.

In this context, the interesting problem of immunization must briefly be discussed. This procedure, suggested by French investigators and promising possible specificity, has repeatedly been put to test. Critical evaluation of the

existing literature appears to justify the conclusion that in all cases exhibiting increased tolerance after repeated subthreshold doses of toxin (1, 2, 13, 20, 21, 22, 74) there was increased resistance rather than genuine immunity. In no instance, for example, could specific antibodies be demonstrated convincingly (19, 55, 66, 69), least of all in the French "sérum antiphallinique" (66) allegedly found effective in some cases of poisoning in man (56).

E. Investigations with pure amanita toxins

Isolation and crystallization of the individual components of *Amanita phalloides* allowed studies of their mechanism of action under incomparably more exact conditions.

1. Phalloidin. Phalloidin, the first of the toxins to be crystallized, was subjected to closer pharmacological investigation in 1938 (74). Extended toxicity determinations yielded an intramuscular LD50 of 3.3 μg/g in the 15 g albino mouse, and an LD100 of 6.7 μg/g. With multiples of the LD100 the time until death could be shortened to 3 to 4 hours, but no further. The pharmacological picture disclosed all the symptoms produced by the whole mushroom: initial rise of blood glucose with drop to subnormal levels shortly before death, adynamia, icterus in some cases, and also, histologically, fatty degeneration of the liver. In these experiments glucose administration yielded no detectable effect. Under "immunizing" pretreatment, tolerance in mice could be increased to 4 times the LD100 of phalloidin.

In continued attempts at elucidation of the site of action, attention was directed mainly to certain metabolic rather than morphological changes. A primary result of these studies was the finding that, measured by the Warburg method, respiration of liver slices of normal as well as poisoned animals was not impaired by addition of phalloidin. Nor was the utilization of various substrates such as lactate and pyruvate modified by the toxin (78). Thus, blockage of the enzyme systems of biological oxidation by phalloidin appeared to be excluded in advance. Later, the suggestive concept that phalloidin exerts its poisonous effect by interference with energy-yielding processes, instigated measurements of the phosphorylation processes coupled with tissue respiration. Phalloidin in a concentration of 5.7×10^{-5} M did not influence the P:O ratio of succinate respiration of mouse liver mitochondria. Likewise, liver mitochondria of animals poisoned with 3 to 4 times the LD100 1½ hours prior to death showed no reduction in phosphorylation with either succinate or glutaminate (79). The same is true of isotonic liver homogenates and suspensions of mitochondria after poisoning with amanitin (85) (see also below). On the other hand, in recent experiments phalloidin, added *in vitro* to digitonin-treated rat liver mitochondria, caused an inhibition of the phosphorylation connected with the last step of the respiratory chain, *i.e.*, the reaction ascorbic acid-cytochrome C-oxygen. This decrease of the P:O ratio, as compared to the control values, was 55% at a final concentration of 10^{-5} M phalloidin and 99% at a final concentration of 10^{-4} M (27). The meaning of these findings will be discussed later.

With adequate amounts of crystallized phalloidin available, it recently became

possible to attack the problem anew from various aspects. It turned out quite generally that the sensitivity of mice and rats to the toxin varies so markedly with the strain that statements on uniform dosage are impossible. The course of the poisoning also appeared different from earlier findings. For instance in a virus-free mouse strain, a single subcutaneous injection of 4 μg/g of phalloidin causes death after an average interval of 1 hour whereas after a dose of 3 μg/g the usual symptoms such as adynamia and convulsions are rapidly manifested, but all animals recover completely. In Sprague-Dawley rats the time until death cannot be reduced below 2 hours; after intraperitoneal doses of 2 μg/g it amounts to 2 to 3 hours. Invariably animals killed by decapitation failed to bleed from their wound. An impressive picture is offered by the liver, which is greatly enlarged (its weight in mice one hour after poisoning being increased by 63%), uniformly dark-red in color and very brittle (44). This led to the expression "exsanguination into the liver." Usually even the liver of surviving animals shows circumscribed hemorrhagic areas. Accurate information is obtained from determination (according to 31, 32) of the blood content of the organs which in mice is increased approximately tenfold (44). Fatty infiltration was never noticed in these experiments. Sometimes the brain also exhibited excessive blood content (in one instance there occurred an unequivocal monoplegia of the hind leg) suggestive of a central origin of the convulsive and paralytic phenomena, especially since a hypoglycemic genesis is out of the question, the blood sugar levels usually being elevated at this time. After only 2 hours the glycogen content of the liver of phalloidin-poisoned rats had decreased to less than 1 % of the initial value (44).

The hemorrhagic changes in the liver suggest a primary attack of phalloidin on the structure of the blood capillaries. Since such an effect cannot be explained quantitatively by the isolated impairment of phosphorylation at only one of the links in the respiratory chain (*cf.* below), one would have to consider a hitherto unknown specific inter-relation between energy yield from that last process in the respiratory chain and integrity of capillary structure.

Further interest turned to the behavior of some liver enzymes in phalloidin poisoning. The activities of the following enzymes were measured in crude liver extracts by optical methods: glucose-6-phosphate dehydrogenase, (glucose) hexokinase, lactic acid dehydrogenase, glutamate-oxalacetate transaminase, condensing enzyme (citrate), β-hydroxyacyl dehydrogenase and β-ketoacyl thiolase. Whereas the activities of the first three enzymes were not conclusively altered, the activities of condensing enzyme, β-ketoacyl thiolase and transaminase, were considerably diminished in phalloidin-poisoned animals (44, 80). This phenomenon is remarkable inasmuch as it is manifest as early as 60 minutes after toxin injection, representing a relatively early metabolic change. The fact that the enzymes involved are those anchored to cell mitochondria appears to be another indication of a particular affinity of the toxin to structural elements. The breakdown of the entire metabolism, which ultimately results in death, can readily be traced back to the inactivation of these enzymes, each of which occupies a key position in its field.

In this context, it is of interest that in the course of experimental phalloidin poisoning the ratio of the serum proteins in mice is found to be altered. The shift resembles that produced by amanitin (84) and results in an evident decrease in the content of albumins along with an increase of the α-globulins (44).

Finally, the influence of alloxan diabetes on the course of experimental phalloidin poisoning was also re-examined. In confirmation of the findings of French authors cited above (73) it was found that it is indeed possible to protect rats against the LD100 of phalloidin by previously producing an alloxan diabetes. In a representative experiment, all 5 diabetic rats survived, whereas all 4 control animals succumbed to the same toxin dose within an average of 132 minutes (80). The peculiar protective effect of alloxan diabetes is also evidenced by other investigations. For instance, at the time of death of the phalloidin controls the liver of the alloxan-phalloidin animal has an average glycogen content of 1.55% as compared with less than $\frac{1}{100}$ that value (0.012%) in the controls (80). Likewise the extremely high blood content of 42.5% in the liver after phalloidin poisoning is not observed in alloxan-pretreated animals, whose hepatic blood content of 7.8% is within the normal range (80).

The first observers of the unique protective influence of alloxan interpreted it as a direct "contra-insulary" action upon the pancreatic β-cells (73). Recent investigations allow still another interpretation: they disclosed a considerable inhibition of the insulinase activity of the liver by the amanita toxins (16). Naturally, in such a situation alloxan would also be expected to have a favorable influence upon the hyperinsulinism.

2. α-Amanitin. a. Toxicology. α-Amanitin is about 20 times more toxic than phalloidin; it invariably causes death in a dose of 0.2 μg/g mouse (81). Moreover, the two substances differ greatly in the onset of their effects: the time until death is about 20 times greater with amanitin than with phalloidin. For amanitin, too, it holds true that different strains of the same species vary in sensitivity to poison.

b. Metabolic studies. Pharmacological and biochemical studies with crystalline α-amanitin, aiming at elucidation of the mechanism of action, are no less numerous than those performed with phalloidin. They started with re-examination of the disturbances of carbohydrate metabolism frequently observed in earlier investigations [(7); see above]. In extensive experiments in mice it was found that after 1 μg/g α-amanitin, liver glycogen decreased to less than 10% of control value within 8 hours after injection; during the same period, the short initial hyperglycemia passed its peak value of approximately 16% above normal (81). The serious drop in blood sugar level to 20% of normal took place gradually between 20 and 40 hours after toxin administration.[3] Progressive hypoglycemia

[3] The amanita peptides are no longer unique in regard to hypoglycemic effect; recently two substances, Hypoglycine A and B, inducing considerable hypoglycemia and loss of liver glycogen were isolated from a West Indian plant, *Blighia sapida* (25, 26). Elucidation of the chemical structure showed Hypoglycine A to be an amino acid, β-methylenecyclopropylalanine, and Hypoglycine B to be a peptide of this amino acid and of glutamic acid (30, 30a).

following experimental poisoning with crystalline α-amanitin has also been described by others (7). As mentioned before, administration of large amounts of glucose completely failed to modify the course of the poisoning in these experiments (81). In cases of longer survival in rats, urinary excretion of glucose (81) and albumen (84) increased from the 48th hour on, indicating that kidney function was also severely impaired. The renal origin of the glucosuria was evidenced by concomitant low blood glucose levels. By these findings the results of earlier experiments with *A. phalloides* extracts and other preparations of whole mushrooms were shown to be valid in all major respects for crystalline α-amanitin as well.

Simultaneous with the glycogen loss, the pyruvic and lactic acid content of the liver also decreases (85)—a finding which is contrary to earlier observations with respect to lactic acid (compare above). Reports agree that the glutathion content of the liver also diminishes (3, 85) while the blood glutathion level simultaneously rises (3). The same holds true for the urea concentrations in liver and blood (3, 85). Considerable decrease of liver ATP and an approximately equivalent increase of inorganic phosphate were found 24 to 48 hours after amanitin administration. With phalloidin, the same changes were attained after 6 hours (85). Here, moreover, DPN was found decreased to about 50% of control value.

Obviously, the disappearance of ATP is not caused by inhibition of oxidative phosphorylation since studies with liver homogenates and isolated liver mitochondria, employing customary methods, did not reveal changes in the P:O ratio (57, 85). It should be mentioned, however, that amanitin has not yet been studied under the same conditions (see above) which disclosed interference with the oxidative phosphorylation at the cytochrome C-oxygen stage. The intensity of aerobic phosphorus uptake decreased only after prolonged amanitin poisoning, which was taken as evidence that the mitochondria were now critically deprived of ADP (85). On the basis of these results, stimulation of excessive hepatic breakdown of ATP, and probably ADP as well, rather than inhibition of oxidative phosphorylation, must be contemplated as the primary action of amanitin. Thus, the pool of energy-rich phosphates would be diminished as a result of prevalence of the cleavage over the synthetic processes. In simultaneous experiments in poisoned mice, there occurred, in addition, a 30 to 40% increase in gaseous metabolism without a change in RQ. With phalloidin poisoning this increase lasted 10 to 15 hours and with amanitin poisoning about 50 hours. It was considered to indicate an attempt by the poisoned organism to compensate for the increasingly greater loss of ATP by increased combustion of food-stuffs and reserve substances (85).

c. Cause of the glycogen depletion in amanitin poisoning. Numerous investigations were undertaken to elucidate the factors primarily responsible for the glycogen depletion. Interesting information was obtained by experiments revealing complete inhibition of the conversion of glucose into liver glycogen both in intact animals (81) and in isolated liver slices of amanitin-poisoned mice (58), which also explains the failure of glucose therapy. In a search for the cause of this

inhibition, the activity of some of the enzymes essential in the process of glycogen formation was studied.

First, glucose was found to be equally utilized by brain homogenates from normal and poisoned mice. Under the assumption of equal conditions in liver and brain, this was taken to evidence unimpaired hexokinase activity (57). This interpretation agrees with the behavior of liver hexokinase in phalloidin-treated mice whose activity proved to be undiminished (44). It was further established that in non-starved mice 7 hours after 2 μg/g of amanitin, the phosphorylase activity of the liver was undiminished; hence, interference with this function can also be excluded as causing the inhibition, then beginning, of hepatic neo-formation of glycogen from glucose (60).

In addition to an inhibition of synthesis the possibility of a precipitate glycogen mobilization by amylase, comparable to that encountered in the liver after administration of diphtheria toxin (29), was taken into consideration. Experimentally, however, such a mechanism could not be ascertained conclusively (57). Nevertheless, in view of the clearly demonstrable initial blood sugar rise, an in creased glycogenolysis must also be contemplated as a factor.

After these failures to attribute the action of amanitin to interference with certain enzymes of sugar and glycogen metabolism, the ionic environment of the liver was examined. The fact that glycogen synthesis depends on an optimal K^+ concentration whereas its cleavage is enhanced by Na^+ excess invited a study of these ions in experimental amanitin poisoning. It was possible to demonstrate a gradual diminution of the K^+/Na^+ ratio in the liver of amanitin-treated mice (57), which suggests that this change in intracellular ionic equilibrium may be one of the factors favoring a shift toward glycogen cleavage in the enzymatic reaction involved in glycogen metabolism (57).

Synthesis of protein is also decisively interfered with by amanitin; electrophoretic serum protein investigations disclose that it is chiefly the albumin fraction that is involved. As was found with phalloidin serum albumin decreases transiently to one half its normal concentration around the 96th hour, which may be interpreted as evidence of a disturbance of hepatic protein synthesis (84).

III. CONCLUDING CONSIDERATIONS

Notwithstanding the abundance of data now available, the question of the site of action of the amanita toxins still cannot be answered unequivocally. In the multiplicity of effects observed either in intact animals or in isolated preparations, it is hardly possible to differentiate between primary and secondary deficiencies. This holds true particularly for experiments with the slow-acting amanitin.

Both in phalloidin and in amanitin poisoning, hepatic glycogen mobilization associated with blood glucose elevation must be considered the first demonstrable change. Whether the glycogen cleavage is effected by direct influence upon the respective enzymes, *i.e.*, glycogen phosphorylase or amylase, or is due to a primary impairment of the ionic composition of the cell with decrease of the ratio between potassium and sodium, must remain undecided at the present time.

Neither is the cause of the subsequent inhibition of liver glycogen synthesis clearly comprehensible. Inhibition of the synthetizing enzymes has not thus far been demonstrated. Lack of ATP energy cannot be responsible since, after administration of amanitin, the drop in the hepatic ATP content occurs only at a much later stage of the poisoning.

The inhibition of some important enzymes of carbohydrate, fat and protein metabolism, already observable one hour after phalloidin administration, must be counted among the disturbances beginning at an early stage of the poisoning. Although it is still undetermined whether these enzymatic deficiencies are due to direct toxin action, major features of the picture later developing can be traced back to them. For instance, deficiencies such as the inhibition of protein synthesis could readily be understood as a consequence of the breakdown of metabolic energetics caused by inactivation of both the citric acid-synthesizing enzyme and the glutamate-oxalacetate transaminase of the liver.

In this context, the strange protective effect of alloxan deserves attention. Besides the interpretations discussed earlier there comes now into view another antagonism on the enzymatic level: phalloidin induces inhibition of β-ketoacyl-thiolase whereas in alloxan diabetes this enzyme, as well as other enzymes of fat metabolism, displays increased activity (82). However, for the time being the question of a direct relationship between these conditions and the "therapeutic" effects of diabetes in amanita poisoning must remain unanswered.

So far as the fatty degeneration of the liver and other organs is concerned, it is undoubtedly a secondary, non-specific process such as may be caused by a great variety of noxious influences.

On the other hand, the strikingly severe liver hemorrhages so regularly observed in phalloidin-poisoned rats and mice are probably more directly related to the toxin action (44). This type of injury points to a selective attack of the toxin upon the structure of the hepatic blood capillaries and perhaps of the cell interfaces in general. In this connection, one should once more call to mind the amanitin-produced disturbances of ionic equilibrium in which the progressive loss of intracellular potassium testifies to an injury to the liver cell membrane (57).

Another observation of interest is the *in vitro* inhibition by phalloidin of the formation of energy-rich phosphate, which is coupled to the last link in the respiratory chain (27). It is difficult to make the failure of only one of the three steps of phosphorylation quantitatively responsible for the catastrophic consequences of the poisoning. However, it may be that the ATP energy produced in the respiratory chain process is not of uniform biological usefulness but is fed into different channels of utilization according to the stage at which it is liberated. Such a concept of qualitatively varying energetics may permit establishing a relation between the ATP energy obtained at the cytochrome C-oxygen step and the endergonic processes which maintain the structure of the capillary and other cell surfaces; interference with this interdependency might then lead to devastating sequelae. Unfortunately, all our attempts at an understanding of these problems are as yet of entirely speculative character.

Likewise the chemical mechanism of the toxin action is still completely obscure. The fact (demonstrated in the case of phalloidin) that these cyclopeptides lose all their activity when the S-bridge of the molecule is abolished and the compound has thereby lost its bicyclic structure indicates that peculiar specific reaction at the site of action is involved. On the other hand, the nature of this reaction is difficult to understand, since the molecule lacks reactive groups available for genuine chemical transformations, such as $-NH_2$ or $-COOH$. This structural peculiarity is also the basis for the outstanding stability of these peptides to enzyme actions. No enzyme capable of degrading amanitin or phalloidin has as yet been encountered.

All available data indicate that this class of phytotoxins does not display its action directly upon the "soluble" catalytic systems of cell metabolism, the enzymatic proteins. Rather, one has to search for their lethal activity in those areas of the cell where biological function depends on the integrity of microstructure, *i.e.*, in the hitherto still largely unexplored field of living membranes. Possibly these ring-shaped molecules possess particular affinity to certain structural cell elements and, at these sites, induce disturbances of vital processes. Further research on the amanita toxins is of wider importance than might have been attributed to it prior to the chemical characterization of these substances.

REFERENCES

1. Abel, J. J. and Ford, W. W.: On the poisons of Amanita phalloides. J. biol. Chem. 2: 273–288, 1907.
2. Abel, J. J. and Ford, W. W.: Further observations on the poisons of Amanita phalloides. Arch. exp. Path. Pharmak. Suppl., 8–15, 1908.
3. Binet, L. and Leblanc, M.: Intoxication par l'amanite phalloïde. Réactions humorales. Ann. Inst. Pasteur 85: 139–147, 1953.
4. Binet, L. and Marek, J.: La thérapeutique sucrée dans l'intoxication par les champignons. Pr. méd. 44: 1417–1418, 1936.
5. Binet, L. and Marek, J.: Hypoglycémie au cours de l'intoxication par les champignons (Amanita phalloïdes). C. R. Acad. Sci., Paris 202: 1219–1220, 1936.
6. Binet, L. and Marek, J.: Les troubles du metabolisme hydrocarboné dans l'intoxication par les champignons (Amanite phalloïde). C.R. Soc. Biol., Paris 124: 13–14, 1937.
7. Binet, L., Fabre, R. and Marquis, M.: L'intoxication par les champignons: L'hypoglycémie amanitinique. C. R. Acad. Sci., Paris 219: 376–377, 1944.
8. Binet, L., Leblanc, M. and Wellers, G.: Recherches nouvelles sur l'intoxication par champignons. Pr. méd. 58: 938–939, 1950.
9. Binet, L., Hrachovec, J., Leblanc, M. and Quivy, D.: Nouvelles recherches sur l'intoxication par l'amanite phalloïde. Pr. méd. 64: 1453–1464, 1956.
10. Block, S. S., Stephens, R. L. and Murill, W. A.: The amanita toxins in mushrooms. Agric. Food Chem. 3: 584–587, 1955.
11. Block, S. S., Stephens, R. L., Murill, W. A., and Baretto, A.: Chemical identification of the amanita toxin in mushrooms. Science 121: 505–507, 1955.
12. Boudier, E.: Des champignons, au point de vue de leurs caractères usuels chimiques et toxicologiques. 1867.
13. Calmette, A.: Sur le mécanisme de l'immunisation contre les venins. IX. Congreso internac. Higiene y Demografia, Madrid 1899. Actas Mem. 1: 102–106, 1900.
14. Cheymol, J. and Pfeiffer, A.: Atteinte de la surrénale au cours de l'intoxication phalloïdienne. Essai de traitment par les hormones cortico-surrénales. Arch. int. Pharmacodyn. 79: 273–281, 1949.
15. Cornforth, T. W., Dalgliesh, C. E. and Neuberger, A.: β-3-Oxindolyl-alanine (Hydroxytryptophan). 2. Spectroscopic and chromatographic properties. Biochem. J. 48: 598–603, 1951.
16. Cova, N.: Contributo allo studio dell'attività insulinasica di fegati profondamente lesi (fegati steatonecrotici). Farmaco 12: 357–361, 1957.
17. Cova, N., Polli, E. and Ratti, G.: La intossicasione sperimentale da amanitatossina (Amanita phalloides Fr.). Quad. sci. Lo Smeraldo No. 1, 1948.
18. Cova, N., Polli, E. and Ratti, G.: La steatosi epatica da amanitatossina. Sperimentale 99: 3–28, 1949.
19. Dessy, G. and Francioli, M.: Le amanite falloide e verna ed i loro velini. Boll. Ist. sieroter. Milano 17: 779–812, 1938.
20. Dujarric de la Riviere, R.: Essai de sérothérapie contre les empoisonnements par champignons vénéneux. Bull. Acad. Méd., Paris 94: 1000–1002, 1925.

21. Dujarric de la Rivière, R.: Étude physiologique d'un extrait d'Amanite phalloïde. Thesis Univ. Paris 1929.
22. Dujarric de la Rivière, R.: Le poison des amanites mortelles. Masson, Paris 1933.
23. Ford, W. W.: As mentioned by Raab (53).
24. Fish, M. S., Johnson, N. M. and Horning, E. C.: Piptadenia alkaloids. Indole bases of *P. peregrina* (L.) Benth. and related species. J. Amer. chem. Soc. 77: 5892–5895, 1955.
25. Hassall, C. H., Reyle, K. and Feng, P.: Hypoglycin A, B: Biologically active polypeptides from *Blighia sapida*. Nature, Lond. 173: 356–357, 1954.
26. Hassall, C. H. and Reyle, K.: Hypoglycin A and B, two biologically active polypeptides from *Blighia sapida*. Biochem. J. 60: 334–339, 1955.
27. Hess, B.: Über die Hemmung der oxydativen Phosphorylierung durch Phalloidin auf der Cytochrom-Stufe. Biochem. Z. 328: 325–327, 1956.
28. Hofer, A.: Zur Konstitution des Amanitins. Diplomarbeit, Frankfurt am Main 1955.
29. Holmes, E. C.: Effect of toxaemia on metabolism. Physiol. Rev. 19: 439–471, 1939.
30. Holt, C. von and Leppla, W.: Die Konstitution von Hypoglycin A. Angew. Chem. 70: 25, 1958.
30a. Holt, C. von, Leppla, W., Kröner, B. and v. Holt, L.: Zur chemischen Kennzeichnung der Hypoglycine. Naturwissenschaften 43: 279, 1956.
31. Holzer, H.: Nachtrag zur Arbeit: Bestimmung des Blutgehaltes von Leberproben zur Korrektur biochemischer Analysen. Biochem. Z. 329: 117–118, 1957.
32. Holzer, H., Sedelmayr, G. and Kiese, M.: Bestimmung des Blutgehaltes von Leberproben zur Korrektur biochemischer Analysen. Biochem. Z. 328: 176–186, 1956.
33. Imhäuser, K.: Die Harnstoffsynthese in der Fettleber. (Untersuchungen bei der Knollenblätterschwammvergiftung). Arch. exp. Path. Pharmak. 145: 120–130, 1929.
34. Imhäuser, K.: Über den Kohlehydratstoffwechsel der Fettleber bei Amanitavergiftung. Arch. exp. Path. Parmak. 162: 506–514, 1931.
35. Jagic, N. V. and Lipiner, J.: Zur Symptomatologie der Pilzvergiftungen. Wien. klin. Wschr. 31: 1029–1032, 1918.
36. Kimmig, J.: Beiträge zur Kenntnis des Vomicins. Versuche zur Isolierung von Amanitatoxin. Thesis, Univ. München 1935.
37. Kobert, R.: Über Pilzvergiftung. St. Petersb. med. Wschr. 16: 463–466; 471–474, 1891.
38. Kobert, R.: Lehrbuch der Intoxikationen, II, 624, 763, Enke, Stuttgart 1909.
39. Laux, F. J.: Ein Beitrag zur Pathogenese der Knollenblätterschwammvergiftung. Virchows Arch. 264: 11–18, 1927.
40. Leblanc, M.: Recherches sur l'intoxication par l'amanite phalloïde. Ann. pharm. franç. 10: 615–628, 1952.
41. Letellier, J. B.: Sur les propriétés alimentaires médicales et vénéneuses des champignons qui croissent environs de Paris. Thesis, Univ. Paris 1826.
42. Lynen, F. and Wieland, U.: Über die Giftstoffe des Knollenblätterpilzes IV. Liebigs Ann. 533: 93–117, 1937.
43. Mannes, K.: Über die Verwendung von Ionenaustauschern bei der Isolierung der giftigen Inhaltsstoffe des grünen Knollenblätterpilzes. Diplomarbeit, Univ. Frankfurt am Main 1956.
44. Matschinsky, F.: Über den Wirkungsmechanismus des Knollenblätterpilzgiftes Phalloidin. Thesis, Univ. München 1959.
45. Mensel, W. and Orzechowsky, G.: Über die Behandlung der Knollenblätterschwamm-Vergiftung mit Cholin. Ärztl. Wschr. 1: 961–963, 1947.
46. Moretti, P.: La glicosio-terapia negli avvelenamenti da funghi. Rif. med. 13: 9–14, 1937.
47. Münch, H.: Über Knollenblätterschwammvergiftung. Klin. Wschr. 3: 1695–1696, 1924.
48. Neuhann, W.: Über Pilz-Vergiftungen. Samml. Vergiftungsf. 12: 59–118, 1941–1943.
49. Orfi: Recherches expérimentales sur l'empoisonnement par l'agaric bulbeux. Bull. Acad. Méd. Paris sér. 2, 6: 350–351; 877–886, 1877.
50. Orfi: Recherches expérimentales sur l'empoisonnement par l'agaric bulbeux. Arch. Physiol. norm. path. sér. 2, 4: 274–309, 1877.
51. Paulet, G.: Traité des champignons, vol. II. Paris 1808.
52. Pfleiderer, G., Gruber, W. and Wieland, T.: Eine enzymatische Bestimmung der L-Asparaginsäure. Biochem. Z. 326: 446–450, 1955.
53. Raab, H. A.: Beiträge zur Kenntnis der Giftstoffe der Amanitaarten. Hoppe-Seyl. Z. 216: 224–228, 1933.
54. Raab, H. A. and Renz, J.: Beiträge zur Kenntnis der Giftstoffe der Amanitaarten. Hoppe-Seyl. Z. 216: 224–228, 1933.
55. Radais, M. and Sartory, A.: Sur l'immunisation du lapin contre le poison des Amanites à phalline. C.R. Acad. Sci., Paris 151: 156–158, 1910.
56. Rayel: Empoisonnement par l'"amanite phalloïde" traité au moyen du sérum antiphallinique. Pr. méd. 33: 1373–1374, 1925.
57. Reiter, M.: Über die Ursachen des Glykogenschwundes der Leber bei der experimentellen Amanitinvergiftung. Habilitationsschrift, Univ. München 1954.
58. Reiter, M.: Glykogensynthese in Leberschnitten amanitinvergifteter Mäuse. Biochem. Z. 328: 408–410, 1957.
59. Reiter, M. and Borchers, H. G.: Hypoplastische Anämie nach Knollenblätterpilzvergiftung. Med. Klinik 43: 1–10, 1948.
60. Reiter, M. and Noé, J.: Die Phosphorylaseaktivität der Leber unter dem Einfluss von Hunger und bei Vergiftung mit Amanitin. Biochem. Z. 328: 454–457, 1957.
61. Reiter, M. and Wieland, O.: Zur Cholintherapie toxischer Lebererkrankungen. Klin. Wschr. 28: 615–617, 1950.
62. Renz, J.: Über das Amanitatoxin. Hoppe-Seyl. Z. 230: 245–258, 1934.

63. Seibert, J.: Beiträge zur Toxikologie der Amanita phalloides. Thesis, Univ. München 1893.
64. Šorm, F. and Keil, B.: Über Proteine und Aminosäuren, 9. Mitt. Über die Konstitution des Peptides Phalloidin. Chem. Listy 45: 278–283, 1951 (Czech.); Collect. Czechoslov. chem. Commun. 16: 366–379, 1951 (Engl.); ref. Chem. Zbl., 5090, 1955.
65. Šorm, F., Keil, B. and Meloun, B.: Über Proteine und Aminosäuren. 9. Mitt. Über die Konstitution des Peptides Phalloidin. 2. Mitt. Chem. Listy 47: 1504–1510, 1953 (Czech.); Collect. Czechoslov. Chem. Commun. 19: 153–161, 1954 (German); ref. Chem. Zbl. 5090, 1955.
66. Schloessberger, H. and Menk, W.: Zur Frage der Serumbehandlung von Knollenblätterschwammvergiftungen. Klin. Wschr. 10: 1346–1350, 1931.
67. Stark, Th.: Ein Fall von Pilz-Vergiftung (Knollenblätterschwamm ?). Samml. Vergiftungsf. 11: 29–30, 1940.
68. Steinbrink, W.: Über klinische und experimentelle Beobachtung der hypoglykämischen Reaktion bei Leberparenchymschädigungen. Klin. Wschr. 3: 1029–1030, 1924.
69. Steinbrink, W. and Münch, H.: Über Knollenblätterschwammvergiftung. Z. klin. Med. 103: 108–146, 1926.
70. Vauquelin, M.: Expériments sur les champignons. Ann. Chim. 85: 5, 1811.
71. Verne, J.: Formule cellulaire des îlots de Langerhans et stéatose hépatique au cours de l'intoxication par l'Amanite phalloïde. C.R. Soc. Biol., Paris 143: 668–669, 1949.
72. Verne, J. and Hébert, S.: Action de la choline sur le rat intoxiqué par l'amanite. C.R. Soc. Biol., Paris 144: 741–743, 1950.
73. Verne, J., Ceccaldi, P. F. and Hébert, S.: Action de l'amanite sur le rat à diabète alloxanique. C.R. Soc. Biol., Paris 144: 645–647, 1950.
74. Vogt, M.: Pharmakologische Untersuchung des kristallisierten Giftes "Phalloidin" des Knollenblätterschwammes. Arch. exp. Path. Pharmak. 190: 406–416, 1938.
75. Weissmann, H.: Studien an 5-Oxytryptophanderivaten. Thesis, Univ. Frankfurt am Main 1956.
76. Wieland, H. and Hallermayer, R.: Über die Giftstoffe des Knollenblätterpilzes VI. Amanitin, das Hauptgift des Knollenblätterpilzes. Liebigs Ann. 548: 1–18, 1941.
77. Wieland, H. and Witkop, B.: Über die Giftstoffe des Knollenblätterpilzes V. Zur Konstitution des Phalloidins. Liebigs Ann. 543: 171–183, 1940.
78. Wieland, O.: Zum Wirkungsmechanismus der Knollenblätterschwammgifte. Habilitationsschrift, Univ. München 1951.
79. Wieland, O.: Unpublished.
80. Wieland, O. and Matschinsky, F.: Unpublished.
81. Wieland, O., Fischer, H. E. and Reiter, M.: Über den Wirkungsmechanismus des Knollenblätterpilzgiftes α-Amanitin. Arch. exp. Path. Pharmak. 215: 75–84, 1952.
82. Wieland, O., Reinwein, D. and Lynen, F.: Die Verteilung der Enzyme des Fettsäurecyclus im tierischen und menschlichen Organismus. In: Biochemical Problems of Lipids, ed. by G. Popják and E. le Breton, 155–161. Butterworth's Scientific Publ., London 1956.
83. Wieland, T.: Die Giftstoffe des grünen Knollenblätterpilzes (*Amanita phalloides*). Angew. Chem. 69: 44–50, 1957.
84. Wieland, T. and Dose, K.: Veränderungen der Proteinverteilung im Blutserum bei der Amanitinvergiftung. Biochem. Z. 325: 439–447, 1954.
85. Wieland, T. and Dose, K.: Zur Biochemie der Knollenblätterpilsvergiftung. Biochem. Z. 327: 345–353, 1956.
86. Wieland, T. and Dudensing, Ch.: Über die Giftstoffe des grünen Knollenblätterpilzes XI. γ-Amanitin, eine weitere Giftkomponente. Liebigs Ann. 600: 156–160, 1956.
86a. Wieland, T. and Höfer, A.: Über die Giftstoffe des grünen Knollenblätterpilzes XVI. Die Bausteine des α-Amanitins. Liebigs Ann. 617: in press.
87. Wieland, T. and Manns, K.: Über die Giftstoffe des grünen Knollenblätterpilzes XIII. Phalloin, ein weiteres Toxin. Angew. Chem. 69: 389–390, 1957.
87a. Wieland, T. and Manns, K.: Über die Giftstoffe des grünen Knollenblätterpilzes XV. Die Konstitution des Phalloins. Liebigs Ann. 617: 152–163, 1958.
88. Wieland, T. and Pfleiderer, G.: Analytische und mikropräparative Trägerelektrophorese mit höheren Spannungen. Angew. Chem. 67: 257–260, 1955.
89. Wieland, T. and Schmidt, G.: Über die Giftstoffe des Knollenblätterpilzes VIII. Liebigs Ann. 577: 215–233, 1952.
90. Wieland, T. and Schön, W.: Über die Giftstoffe des grünen Knollenblätterpilzes X. Die Konstitution des Phalloidins. Liebigs Ann. 593: 157–178, 1955.
91. Wieland, T., Motzel, W. and Merz, H.: Über das Vorkommen von Bufotenin im gelben Knollenblätterpilz. Liebigs Ann. 581: 10–16, 1953.
92. Wieland, T., Wirth, L. and Fischer, E.: Über die Giftstoffe des Knollenblätterpilzes VII. β-Amanitin, eine dritte Komponente des Knollenblätterpilzgiftes. Liebigs Ann. 564: 152–160, 1949.
93. Wieland, T., Weiberg, O., Fischer, E. and Hörlein, G.: Darstellung schwefelhaltiger Indol-Derivate. Liebigs Ann. 567: 146–161, 1952.