ON THE POISONS OF AMANITA PHALLOIDES.

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It has recently been shown by one of us (W. W. Ford1) that the poisons of the fungus *Amanita phalloides*, the variety responsible for the great majority of deaths from mushroom intoxication, belong to the group of bacterial toxins. They were thus classified in virtue of their causing characteristic lesions in animals after a definite latent period, and because an immunity may be established toward them in susceptible animals after the administration of non-lethal doses. The serum obtained by Ford from his immunized animals was antihæmolytic and antitoxic in character; it prevented the solution of blood corpuscles *in vitro* and neutralized the poisonous action of the fungus on animals.

Ford2 also proved that the hæmolytic principle first described by Kobert under the name phallin, as the only poison present in the fungus, is in fact accompanied by a highly toxic, thermostabile substance devoid of hæmolytic properties, whose presence can be demonstrated by biological experiments. For this thermostabile substance an antitoxin3 can be produced devoid of any antihæmolytic action and Ford has proposed for it the provisional name Amanita Toxin.

In view of the importance that attaches to the physical and chemical properties of toxins in elucidating questions of immunity it was determined to make a chemical study of the poisons of *Amanita phalloides*.4

1 *Journ. of Infect. Dis.*, iii, pp. 192-224, 1906.
4 It was only after this paper was partly written that we learned of Kobert's announcement in 1899 that he had discovered a second poison with alkaloidal properties in the fungus in question. That this paper
The earliest investigation of a chemical nature on the active principles of poisonous fungi is probably that of Letellier, who in 1826 obtained from a considerable variety of fungi (A. muscaria, A. phalloides, and A. verna) a highly resistant body which he called Amanitin. Subsequently Letellier and Speneux obtained two poisons from a fungus found in the vicinity of Paris and known as Hypophyllum crux miliente (Paulet), probably a variety of Amanita. One of these poisons, which was obtained from both alcoholic and aqueous extracts, caused in cats a violent inflammation of the mucous membrane of the alimentary canal, with vomiting and diarrhoea; the other poison was very resistant, withstanding boiling and treatment with acids; it also had the capacity after boiling with weak sulphuric acid of reducing copper solutions. This latter substance is characterized as a glucosidal alkaloid, identical with the Amanitin originally described by Letellier. The action of this substance is described as being purely narcotic.

Boudier in 1866 attempted to give a methodical analysis of the constituents of Amanita phalloides. As present in the fresh juice he names glucose, albumin, a mucilaginous substance, viscosine, a hygroscopic viscous substance, mycetide, tannin, citric acid, malic acid, and their salts, fats and oils and inorganic salts, as potassium chloride and calcium phosphate. None of these substances are described as being toxic, and, indeed, his methods of treatment were such that his products could not have retained their toxicity. At one stage of his operations he ob-

1 Théa de Paris, 1826.
2 Annales d'hyg. pub. et de méd. leg., p. 71, 1867.
3 Des champignons au point de vue de leurs caracteres usuels, chimiques et toxicologiques, 1866.
tained a small quantity of an impure residue which was supposed to contain the neutral salt of an alkaloid. He reports that experiments with this material on mice were inconclusive in their results. Boudier named this assumed alkaloid *bulbosine*, but he was unable on account of lack of material to isolate it or even to establish its presence to the satisfaction of subsequent workers.

Oré\(^1\) in 1877 experimented with extracts made from both fresh and dried fungi and concluded on biological grounds alone that the toxic principle of the fungus is an alkaloid "qui offre avec la strychnine une parenté physiologique incontestable." To this hypothetical substance Oré gave the name *phalloidin*.

From this time on, little of importance was accomplished until Kobert\(^2\) in 1891 made the important observation that aqueous and saline extracts of dried *Amanita phalloides* contain a substance which rapidly dissolves the blood corpuscles of a variety of animals even in dilution of 1:125,000 of the dried material. This substance, which Kobert named phallin, he regarded in his earlier communication as the sole toxic constituent of the fungus in question. As contained in simple extracts, Kobert found phallin to be a highly unstable substance, easily rendered inactive by contact with acids, alcohol, and other agents, and by exposure to a temperature of 70° C. His extracts contained a little coagulable proteid and the similarity of this unstable hæmolytic substance to a hæmolytic poison secreted by certain spiders (*Spinnengift*), which Kobert also believed to be proteid in character, led him to assert that the blood-laking poison of *Amanita phalloides* is a toxalbumin.

It must be clearly pointed out that phallin was not isolated by Kobert and that his statements in regard to its chemical nature rest on inference alone. Our own observations lead us to believe that Kobert's characterization of this hæmolytic substance as a toxalbumin is entirely erroneous.

A few years later Seibert\(^3\), working in Kunkel's laboratory with *Amanita citrina*, a variety of *A. phalloides*, was unable to demonstrate any hæmolytic substance in this fungus although it was a definitely toxic to certain animals.

\(^1\) Arch. de physiol. norm. et path. (II), xl, p. 274, 1877.


\(^3\) Inaug. Dissert., München, 1893.
Kunkel\textsuperscript{1} concludes that the phallin of Kobert, while very interesting from the pharmacological point of view, plays no role in recorded cases of poisoning, since on the basis of his pupil Seibert's work he believes that this principle is not present in the juice of the fresh fungus (\textit{A. citrina}), and furthermore, even if present, it would be rendered inert by a temperature far below that at which mushrooms are cooked. Finally, neither clinical symptoms nor the pathological lesions in recorded cases of poisoning can be brought into agreement with the action of any known hæmolytic substance.

Bourquelot,\textsuperscript{2} while accepting phallin as being the hæmolytic and poisonous principle of the fungus, is not convinced that it is a toxalbumin since the fungi remain poisonous after cooking while toxalbumins lose their activity at 70\textdegree C.

Finally, in 1899, Kobert\textsuperscript{3} in his second communication upon this subject noted that after precipitating aqueous extracts of the fungus with alcohol the clear filtrate contained a highly active poison without hæmolytic properties. This he stated to be an alkaloid.

This poison also was not isolated by Kobert. He limits himself to the statements that it is insoluble in ether, soluble in alcohol, and "gives precipitates with certain alkaloidal reagents." As these statements apply equally well to many plant extracts which contain non-nitrogenous glucosides, citrates, chlorides, pigmentary and resinous substances, but no alkaloid, it cannot be claimed that they throw much light on the chemical nature of this second poison.

\textbf{OUR METHOD OF PREPARING A HÆMOLYTIC EXTRACT.}

About 50 grams of dried \textit{A. phalloides},\textsuperscript{4} which had been accurately identified as such in the fresh state, were crushed in a mortar and then mixed with 300 c.c. of distilled water. After

\textsuperscript{1} Handbuch der Toxikologie, ii, p. 1048, 1901.
\textsuperscript{2} Richet, \textit{Dict. de physiol.}, iii, 1898, Art., Champignons.
\textsuperscript{3} Journal cited in footnote to p. 273.
\textsuperscript{4} In all cases the fungi employed were identified by the help of standard publications on mycology, especially those of Atkinson, Farlow, and Peck. Only the pure white \textit{A. phalloides} or \textit{A. verna} was used, the yellow variety, frequently called \textit{A. citrina}, and specimens with a brownish or grayish pileus being reserved for special investigation.
standing on ice for two days the mixture was strained through linen and the fluid thus obtained passed through filter paper and then through a Berkefeld candle. This process was repeated in order to remove completely the poisons from the dried material.

The combined filtrates from the Berkefeld candles, usually amounting to between 600 and 700 c.c., were concentrated under reduced pressure at a temperature not exceeding 35° C., and to the concentrated extract absolute alcohol was added in small quantities at a time till no further precipitate was found. This precipitation occurs rapidly and the supernatant alcohol should be poured off quickly to avoid any but the briefest contact. The precipitate thus formed contains the hæmolytic substance while the straw-colored alcoholic filtrate contains the deadly Amanita toxin. This filtrate was concentrated immediately under reduced pressure until freed from alcohol.

FURTHER TREATMENT OF THE IMPURE HÆMOLYSIN.

The precipitate containing the hæmolytin can be further purified by solution in a small quantity of water and by precipitation with "Columbian spirits."\(^1\) This precipitation must be effected as quickly as possible and the alcohol then removed, as experience has shown that prolonged contact with methyl alcohol is decidedly injurious to the hæmolytic activity of our substance. The flocculent or granular precipitate is dried in vacuo over sulphuric acid. When dry it consists of brown scales and lumps so hygroscopic in character that exposure to air soon causes them to assume a tarry consistency. This material is powerfully hæmolytic. It contains, so far as our observations show, a very small amount of proteid, a pigmentary substance, inorganic salts, and a glucoside which constitutes by far the greater bulk of the precipitate.

The presence of proteid can best be demonstrated as follows. A considerable quantity of the precipitate just described is dissolved in a small quantity of water, thymol is added, and the solution is dialysed in a collodion sac for forty-eight hours. The sac is renewed three or four times

\(^1\)It seemed to us that this commercial preparation of methyl alcohol which contains a little free acid of organic nature is more effective as a precipitant than pure methyl alcohol of the same strength (93 to 97 per cent.).
during the progress of the dialysis. The pigmentary substances which obscure proteid reactions are thus largely removed. Trichloracetic acid in substance is now added to the contents of the sac till no further precipitation occurs. The turbid mixture is transferred to a separatory funnel and shaken with an equal volume of ether. As a result the precipitated proteid collects at the boundary between the ether and the water and may easily be obtained upon a filter. It is then found to be so far free from pigment that it responds in the most satisfactory manner to the biuret reaction and to Millon's test. The color obtained in this biuret reaction was the fine violet characteristic of native proteids. The dialysates were found to be free from proteids both of the coagulable and non-coagulable class.

ON THE REMOVAL OF PROTEID FROM THE METHYL ALCOHOL PRECIPITATE WITHOUT LOSS OF HÆMOLYTIC ACTIVITY.

Having thus shown that proteid is present in the methyl alcohol precipitate, it remains to determine whether it can be removed from this precipitate without materially lessening its hæmolytic activity. We have found that this may be accomplished by at least two methods, involving the use of metaphosphoric acid and of uranyl acetate.

METHOD I. METAPHOSPHORIC ACID. This acid must be freshly prepared and used at once to ensure satisfactory results. Phosphorus pentoxide is spread out in a thin layer on the bottom of a wide-mouthed Erlenmeyer flask, and a plug of moistened filter paper is then suspended in the mouth of the flask. In half an hour or less the metaphosphoric acid is ready for use. By means of a glass rod a little of the thick viscous acid is added to an aqueous solution of the methyl alcohol precipitate until the proteid is completely coagulated. The proteid material is removed by means of a small filter charged with absorbent cotton, and the filtrate is again tested with metaphosphoric acid. After this treatment our solution gives no turbidity when poured upon crystals of trichloracetic acid.

This proteid-free solution after neutralization with sodium bicarbonate to cochineal as an indicator is found to have retained its hæmolytic activity practically unaltered. Control experiments with similar quantities of metaphosphoric acid, also

1 Neither here nor when uranyl acetate was used did we wash the precipitate.
neutralized with sodium bicarbonate, had absolutely no injurious effect on red corpuscles.

In proof of the above statement that our hæmolytic solution is now entirely free from proteid material capable of giving the biuret reaction we may cite the following experiment.

After treatment with metaphosphoric acid as above described, the neutralized solution was treated with lead acetate, until precipitation was almost complete, was filtered, and the filtrate completely precipitated with solution of basic lead acetate. The two precipitates were then separately suspended in water and each decomposed with hydrogen sulphide. After filtering off the lead sulphide, the filtrates were separately concentrated to a small volume. With neither solution could the biuret reaction be obtained. We thus see that freshly prepared metaphosphoric acid is a complete precipitant for native proteids.

METHOD II. URANYL ACETATE. The methyl alcohol precipitate containing the hæmolysin was dissolved in water made faintly alkaline with sodium carbonate and then treated with a saturated solution of uranyl acetate until no further precipitate was obtainable. During the precipitation care must be taken that this mixture remains nearly neutral in its reaction toward litmus, as more than a trace of free acid is decidedly injurious to the hæmolysin.

After removing the precipitated proteid by filtration, the excess of uranyl was removed from the filtrate by treating it with a solution of disodium hydrogen phosphate. The filtrate from the uranyl phosphate showed no diminution in hæmolytic activity as compared with the original solution. Our second method leads us to the same conclusion that the small amount of proteid originally present in our methyl alcohol precipitate has nothing to do with its hæmolytic activity.

It may be stated here that Kowalewsky\(^1\) has shown that uranyl acetate will completely remove from various albuminous fluids every trace of proteid giving a biuret reaction, and that Jacoby\(^2\) and others have used this reagent for the removal of proteids from faintly alkaline solutions.

We have thus been able to show that proteid may be precipi-

\(^1\) Zeitschr. f. anal. Chem., xxiv, p. 551, 1885.
tated from our hæmolytic solutions without appreciable loss of their power to dissolve red blood corpuscles. We feel justified in asserting, therefore, that this hæmolytic principle is not a toxalbumin and that Kobert was in error when he characterized it as such.

We do not forget that there are substances found among the end products of digestion (peptoids, peptids) and in the urine (oxyproteic and uroproteic acid) which no longer give the biuret reaction, but which on chemical grounds are to be regarded as closely allied to the true proteids. Substances of the first class which might possibly be formed by autolysis in drying fungi or even in the regressive metabolism of these plants may be eliminated on the ground that they are more resistant in their behavior toward chemical reagents (acids) and heat, more soluble in weak alcohol, and diffuse more rapidly through a collodion sac than does our hæmolyein. Substances of the second class (uroproteic acid, oxyproteic acid) are precipitated by cupric acetate, which reagent fails to precipitate the hæmolysein. Furthermore, both Kobert and Ford have shown that the hæmolysein loses its activity on exposure to those digestive ferments which would have no further action on substances of the peptoid class.

Criticisms of the above nature, however, lose their force in view of the facts presently to be adduced, which show that the hæmolytic substance is a glucoside. This new point of view, again, is confirmed by the results of the experiments with ferments. We have found that the hydrochloric acid alone of a pepsin-hydrochloric mixture will deprive the hæmolysein of its blood-laking power, so that it becomes unnecessary now to attribute any action to pepsin itself. In reference to the action of pancreatin, it must be borne in mind that this product constitutes a mixture of ferments which is known, for example, to be capable of liberating glucose¹ from triacetyl glucose, and it is not surprising that it should also effect the decomposition of our unstable glucoside. Kolliker and Müller² have found that pancreatic juice has the power of decomposing the glucoside amygdalin, and Fischer and his pupils have shown that it can effect the hydrolysis of various synthetically prepared esters. It will later be shown that pancreatic juice also deprives our hæmolytic glucoside of its blood-laking power.

² Cited from Green, The Soluble Ferments and Fermentation, 1899, p. 147.
It has already been stated that Seibert, working in Kunkel's laboratory, was unable to demonstrate any haemolytic activity in poisonous extracts of freshly gathered *A. citrina* and that Kunkel therefore concluded that the haemolytic substance described by Kobert is not the active agent to which recorded cases of Amanita poisoning can be referred.

In view of this difference of opinion among authors we have recently made some observations on perfectly fresh specimens of *A. phalloides* (white variety). Aqueous extracts were made within a few hours after gathering the fungi. Three small plants were crushed in a mortar with 15 c.c. of distilled water. The perfectly colorless but slightly turbid filtrate was powerfully haemolytic, one drop of the fluid when diluted one hundred times sufficing to lysis completely within an hour 1 c.c. of a 5 per cent. suspension of rabbit's blood. Haemolysis occurs very rapidly with large quantities of the undiluted juice, but here secondary changes occur due to the acidity of the extract. If, however, the extract be first neutralized, the lysis of the blood occurs in as perfect a manner as with the diluted but non-neutralized extract. Similar observations were made with the fungi from various localities and we feel justified, therefore, in asserting that the Amanita-haemolysin is present in large amount in the freshly gathered fungi.

From this extract of the fresh fungi the proteids were also removed by precipitation with freshly prepared metaphosphoric acid and with uranyl acetate, the necessary precautions in the use of these reagents being duly observed. The resulting proteid-free solutions were perfectly colorless, as clear as water, and powerfully haemolytic, showing no noticeable loss of activity.

To some of the undiluted fresh extract hydrochloric acid to the extent of three tenths of one per cent. was added and the mixture allowed to stand in the thermostat for two hours at 37.5° C. At the end of this time the mixture was practically devoid of haemolytic activity. A second experiment in which pepsin was used in addition to the hydrochloric acid led to the same result. These experiments show how extremely susceptible the haemolysin is to the action of free acids even in great dilution.
The hæmolyisin is also injured by contact with weak alkalies but more slowly than with acids. To some of the undiluted fresh extract sodium carbonate was added until it had attained the strength of an $\frac{1}{2}$ Na$_2$CO$_3$ solution. Protected with thymol, the extract was then placed in a thermostat ($37.5^\circ$ C.) together with a control tube containing only extract and thymol. After remaining for seventy-two hours in the thermostat, the extract containing the sodium carbonate showed a decided diminution in hæmolytic power. Three drops added to 5 c.c. of a 5 per cent. suspension of rabbit's blood showed no indication of hæmolysis until ten minutes had elapsed, while the same quantity of the native extract from the control tube effected the laking of blood almost instantly.

To 2 c.c. of the fresh extract containing thymol and the above-named amount of sodium carbonate 0.5 c.c. of pancreatic juice obtained from the dog after the injection of secretin was added and the mixture placed in the thermostat. After twenty-four hours the hæmolytic power of the extract was greatly diminished and after forty hours it was found to have entirely lost its blood-laking power. We have shown that the hæmolyisin is not an albuminous substance and we see in this experiment a confirmation of our opinion that it is, in fact, a glucoside and one which is capable of being decomposed by one of the ferments of pancreatic juice. We hope later to throw some light on the true nature of this ferment action.

While the foregoing experiments with fresh fungi were all made with A. phalloides, we may add that we have lately found that the juice of fresh specimens of A. citrina, as growing in the vicinity of Baltimore, is also powerfully hæmolytic.

It seems hardly possible that Seibert could have failed to demonstrate this property in his fresh extracts of A. citrina had it been present, and we can only conclude, in view of his negative results, that specimens of this fungus growing in different parts of the world may differ widely as to hæmolytic activity.

We have thus shown that the Amanita-hæmolyisin is present in fresh specimens both of the white and of the yellow variety of the fungus and we are of the opinion that, in consequence of its great susceptibility to acids, it is under all ordinary conditions destroyed in the stomach if it had not already become
innocuous through cooking and plays no rôle whatsoever in mushroom poisoning. This opinion finds support in the absence of hæmoglobinuria and of pigmentation of the spleen, two of the characteristic signs of intoxication from hæmolytic agents.

The Amanita-toxin, on the other hand, is far less susceptible to the action of weak acids and heat. Ore found that weak solutions of acetic acid will extract a deadly poison from both dried and fresh specimens of Amanita, so that the residue becomes edible and harmless. The acetic extract, however, contains the poison with its virulence apparently unaltered. Ford has shown that this toxin will withstand prolonged heating and digestion with pepsin-hydrochloric acid and pancreatin. We are therefore forced to conclude that our second poison, the Amanita-toxin, is from a practical point of view, the poisonous principle of the fungus, in a word, the only poison which is operative in man after the ingestion of this deadly plant.

**Nature of the Hæmolytic Substance.**

The chemical instability of this substance renders its isolation and purification a matter of great difficulty, and we do not claim that we have yet obtained a product pure enough for elementary analysis. Nevertheless, we believe that the following experiments throw much light upon the nature of the hæmolysin and indicate that its identification is now possible.

*Treatment with Basic Lead Acetate.* Although Kobert states that the hæmolysin will not endure treatment with lead acetate and subsequent removal of lead with hydrogen sulphide, nevertheless we have met with partial success in applying this method of isolation. The methyl alcohol precipitate from 25 grams of dried fungi was precipitated with basic lead acetate, the precipitate was thoroughly washed with a dilute solution of the acetate and then suspended in water and treated at room temperature with a solution of sodium sulphate. After the precipitate had been repeatedly shaken with this solution it was found that little, if any, decomposition had taken place. The precipitate was

We wish again to emphasize the point that in thus speaking of the Amanita-toxin as a single poison we are guided by the biological experiments of Ford; we are well aware that further chemical investigations may show that this toxin consists of more than one poison.
then freed from sodium sulphate, suspended in much cold water, and decomposed with hydrogen sulphide. After removal of the lead sulphide, the excess of hydrogen sulphide was driven off by means of a current of hydrogen and the fluid neutralized with sodium carbonate to cochineal as an indicator. The solution was then concentrated to a small volume at a temperature not exceeding 35°C. The concentrated solution was definitely haemolytic, although much reduced in activity. From this solution the haemolysin was precipitated with ethyl alcohol, immediately freed from alcohol, and dried in vacuo over sulphuric acid. When dry it consisted of hard, gray lumps which rapidly absorbed moisture on exposure to air and which in smallest quantity gave all the glucosidal reactions to be later described. This material also exhibited hemolytic activity, though not to the extent that would be demanded if the large amount of originally active extract be taken into account. Lack of material prevented us from making a more careful study of this method at the time, especially with regard to the influence of temperature and the maximum strength of free acetic acid that can be tolerated by the haemolysin. The result of the experiment nevertheless points unmistakably to the conclusion that our haemolytic principle must be classed with the glucosides. This inference is further strengthened by the following experiment.

**Dialysis and Removal of Proteid.** A quantity of the highly active methyl alcohol precipitate obtained from 20 grams of the dried fungus was dialysed in the presence of thymol in a collodion sac against distilled water for three days, the sac being renewed every twelve hours. Although the dialysing solution remained free from bacteria its haemolytic power slowly diminished. Both pigment and glucoside appeared in the dialysate. After the dialysis had continued uninterruptedly for three days the contents of the sac were reduced to a small volume at a low temperature and freed from proteid with freshly prepared metaphosphoric acid. On neutralizing the proteid-free solution it was found to be actively haemolytic. It was now evaporated to dryness and the small residue thus obtained was subjected to qualitative analysis. Its chief constituent was a glucoside requiring previous hydrolysis with an acid for the full development
of its reducing power for Fehling's solution or ammoniacal silver nitrate. With this glucoside were associated a little pigmentary matter and the salts due to the use of metaphosphoric acid in the removal of proteids. The same results were obtained in a number of experiments with collodion sacs and we therefore believe that dialysis leads again to the conclusion that our hæmolytic principle is a glucoside.

The Dialysates. All portions of the dialysate, even when tested after being concentrated at a low temperature, were found to be quite devoid of hæmolytic activity. Chemical tests showed that the dialysate contained a glucoside which behaved toward reagents in every way like that which still remained in the dialysing sac and like that which was obtained by the use of basic lead acetate. The later portions of the dialysate contained this glucoside in relative excess as compared with other constituents. The small amount of active glucoside left behind after dialysing for three days cannot represent the total amount of hæmolytic substance with which we began the experiment, and we are forced to conclude that the active glucoside is so altered in its passage through the wall of the sac as to be deprived of its hæmolytic properties. Kobert found that the hæmolysin will not pass through parchment; we have not yet had the opportunity of trying this method.

REACTIONS AND PROPERTIES OF THE HÆMOLYTIC GLUCOSIDE.

We have thus been able to show that Amanita-hæmolysin cannot be classed with proteids or toxalbumins but must be considered a glucoside. We do not claim that we have freed this glucoside from all traces of foreign matter. We hope in the near future to obtain it in a state sufficiently pure for elementary analysis, without loss of its hæmolytic properties, but to secure both of these conditions at the same time may be found to be as difficult in this case as with certain other glucosides, such as the saponins.

In its present state of purity this hæmolytic glucoside shows the following reactions:

1. It reduces Fehling's solution and ammoniacal silver nitrate only very slightly without previous hydrolysis with a mineral acid, but very powerfully after such hydrolysis.
It gives an abundant precipitate with neutral or basic
lead acetate and a precipitate with tannic acid soluble in water
and in excess of tannic acid.

3. With phosphotungstic and phosphomolybdic acids it
gives a faint turbidity suggesting the presence of an impurity.
4. Cupric acetate fails to precipitate the glucoside but reveals
the presence of retained phosphates.

5. It does not ferment with brewer's yeast either before or
after hydrolysis with acids. The small amount of fermentation
gas obtained on a few occasions after eighteen hours in the
thermostat was referred by us to bacterial action rather than
to fermentation by yeast. A larger quantity of gas obtained
after four days in the thermostat was found to consist largely
(33 per cent.) of hydrogen and combustible gases, thus verifying
our conclusion in regard to bacterial action in the exceptional
cases above mentioned.

6. The glucoside gives the following tests for pentoses in the
most satisfactory manner:

a. A fine purple violet color on heating gently with α-napthol
and sulphuric acid.

b. A fine cherry red color after similar treatment with
phloroglucinol and hydrochloric acid.

c. A deep green color on heating gently with orcinol, hydro-
chloric acid, and a drop of ferric chloride solution. Like the
red pigment formed in the preceding reaction, this green pigment
is easily soluble in amyl alcohol.

d. It decolorizes an alkaline solution of potassium permaman-
ganate at room temperature.

e. It gives a yellow color on boiling with sodium hydrate
if it be first carefully hydrolyzed with hydrochloric acid.

These tests when considered in connection with the behavior of
our glucoside toward brewer's yeast, Fehling's solution, and
ammoniacal silver nitrate show conclusively that the sugar
contained in the molecule is a pentose. It remains for future
experiments to determine which pentose or methyl pentose is
present.

7. All specimens of our hämolytic glucoside after having
been freed from proteid and otherwise purified as above stated,

either by dialysis or by treatment with basic lead acetate, still contain nitrogen as is proved by positive results obtained by the Laissaigne test. Such specimens also evolve alkaline vapors when heated with sodium hydrate. These vapors have an odor that is suggestive of methylamine. The fact that all specimens of our hæmolysin contain nitrogen, whether obtained by dialysis or by the use of basic lead acetate, leads us to infer that this element is an integral constituent of its molecule. The experiments that have been cited lead us to believe that our hæmolytic substance is a nitrogenous glucoside which is very sensitive toward the action of heat, acids, and certain glucoside-splitting ferments and which is easily decomposed by acids so as to yield a pentose and a volatile base or bases, such as ammonia and methylamine.

We hope in the near future to give the results of further work in this field, especially as to the nature of the thermostable poison (or poisons), which we have called the Amanita-toxin, with which Ford has immunized animals and for which he has produced an antitoxic serum. We have not yet brought our study of this substance to a satisfactory conclusion. It is our purpose to discover if possible whether this poison bears any chemical relation to the hæmolytic glucoside above described or to the alkaloid which Kobert declares to be present in the fungus.

**SUMMARY.**

1. The fact that an immunity can be established toward the two poisonous principles of *Amanita phalloides* and that the serum of immunized animals is antihæmolytic and antitoxic in character gives renewed interest to a chemical study of these poisons.

2. The hæmolytic principle contained in the Amanitas and first detected by Kobert is not a toxalbumin as this author supposed, but a nitrogenous glucoside which is very sensitive toward the action of heat and acids, less so toward the action of alkalies, and is easily decomposed by acids so as to yield a pentose and a volatile base or bases, such as ammonia and methylamine.

3. This glucoside, which we designate Amanita-hæmolysin,
is present in fresh specimens of *A. phalloides* and *A. citrina*. Seibert failed to detect its presence in fresh extracts of *A. citrina* and we therefore conclude that specimens of this fungus growing in different parts of the world may or may not contain this glucoside.

4. The properties of the Amanita-hæmolysin are such as to preclude it from playing any role as a *blood poison* in case of poisoning by the Amanitas.