THE TOXICOLOGICAL CONSTITUTION OF AMANITA PHALLOIDES.

By WILLIAM W. FORD, M. D.

(*From the Bacteriological Laboratory, Johns Hopkins University.*)

It has been pointed out in previous papers[1] that the most deadly of all poisonous fungi, and the one responsible for the majority of deaths from fungus eating, is Amanita phalloides, the hæmolytic principle of which was discovered in 1891 by Kobert,[2] who gave it the name phallin. This hæmolysin, in virtue of its acting directly upon the red blood corpuscles without the intermediation of serum, its inactivation at 65° C., and its failure when inactivated to be reactivated by substances containing complement for serum hæmolysins, should be classed provisionally with the bacterial hæmolysins.

It has also been shown that extracts of Amanita phalloides are profoundly poisonous to animals, the latter showing lesions characteristic of bacterial intoxication in general, and that non-lethal doses are capable of causing an immunity in susceptible species. The serum from immunized animals is antihæmolytic and antitoxic in character, a minimal hæmolytic quantity for one cubic centimeter of a 5 per cent. blood suspension being neutralized by certain sera in a dilution of 1:10,000, and ten times a fatal dose for rabbits being neutralized by one cubic centimeter of the same sera. Such sera can be compared with the old and weaker normal antidiphtheritic sera where one cubic centimeter neutralized ten times a fatal dose of the diphtheria toxin for a guinea-pig of 250 grams. It is now necessary to consider in greater detail the lesions produced in animals by this poison, and to determine what relation these bear to the blood-laking properties of Amanita phalloides.

According to Kobert's original publications, there is but one

[1] Ford, *Medical News*, 1905, lxxxvii, 771. *The Jonrnal of Infectious Diseases*, 1906, iii., 191.

[2] Kobert, *St. Petersburger medicinische Wochenschrift*, 1891, xvi, 463; 471.

poisonous substance in this fungus, phallin, and the changes seen in fatal intoxication in man and the lesions produced experimentally in animals by it are due entirely to blood destruction. Kobert regards phallin as a toxalbumen in virtue of its thermolability, and intravenous injections cause intravascular dissolution of the corpuscles. The specimen employed by him was of enormous hæmolytic strength, ox corpuscles being dissolved by the dried extract, in a dilution of 1:125,000.

For the purposes of our present study, the lesions produced in experimental animals may be briefly stated to be the following[3]:

I. Subcutaneous gelatinous œdema;
II. Hæmorrhagic lymphatic glands;
III. Hæmoglobinuria;
IV. Hæmorrhages in serous membranes and parenchymatous organs;
V. Necrosis and fatty degeneration of heart muscle, liver, and kidney;
VI. Pigmentation of the spleen.

The pigmentation of the spleen and the hæmoglobinuria can be referred at once to the hæmolysis produced by extracts of Amanita phalloides, since these changes are characteristic of hæmolytic intoxications in general. Can we with equal confidence, however, consider the other changes, namely, the œdema, hæmorrhages, and necrosis, as due to hæmolysis?

If we inoculate a considerable series of animals, the lesions described are seen with great regularity. There is, however, a vast difference in the amount of œdema, the extent and distribution of hæmorrhages, and the degree of tissue degeneration. As we approach the lower limits of a fatal dose, the œdema at the site of inoculation becomes proportionately less, the hæmorrhages may be lacking altogether or at least the evidence of them at autopsy is wanting, and the extent of the tissue change, especially the fatty degeneration of the liver, becomes greater and greater. This variation in the character of the lesions suggests that the disturbances produced are greater than can be explained by blood

[3] The lesions produced by Amanita phalloides will be more fully described in another paper.

destruction alone. If guinea-pigs, which are very susceptible to the toxic effects of this Amanita, be inoculated with minimal amounts of the poison they will frequently die in a short time and fail to present, at the site of inoculation, either œdema or hæmorrhage; and upon microscopical examination of the organs no pronounced lesions will be found. Nevertheless these animals develop much the same toxic symptoms as other animals which have received a larger fatal dose.

If we estimate the amount of blood destruction produced by these small lethal doses of the Amanita, an unexpected fact is brought out: the degree of blood destruction is not proportional to the profundity of intoxication. A few examples will be given.

A guinea-pig of 240 grams weight succumbed quickly to a quantity of the Amanita which outside the body is capable of dissolving about one cubic centimeter of undiluted blood; and a rabbit of 870 grams weight died in forty-eight hours from a dose of the poison which dissolves only 2½ cubic centimeters of rabbit's blood. The loss of such small quantities of blood can be borne easily by animals of these species. In estimating the hæmolytic strength of doses of the extract capable of producing either rapid or slower fatal results, the same lack of proportion between the amount of blood destroyed and the severity of the lesions was apparent.

In endeavoring to explain the facts just stated several possibilities present themselves. The toxic effects produced by the Amanita may be secondary, in that the intravascular alteration of blood corpuscles may cause changes associated with thrombosis and infarction, to which the subsequent necrosis and tissue destruction are added; or the hæmolytic principle may be less specific than is supposed and exert an injurious effect upon other cells of the body; or finally, still other poisons in addition to the one discovered by Kobert may exist in the Amanita.

As regards the first of these considerations, it may be stated that Amanita phalloides does not agglutinate blood corpuscles *in vitro*, or, so far as can be concluded from a microscopic examination of the tissue, *intra vitam*. The tissue degeneration cannot, therefore, be explained by thrombi of agglutinated erythrocytes

as some of the lesions produced by ricin and abrin have been accounted for. It is also no more easy to explain the lesions on the basis of simple destruction of a small number of red blood cells.

The second and third of these considerations can be tested experimentally. It has been pointed out that extracts of Amanita phalloides lose their hæmolytic activity entirely when heated to temperatures between 65° and 80° C., and no substance has been discovered capable of reactivating them. This inactivation may, therefore, for our present purpose be considered as destruction. Animals inoculated with extracts heated to various temperatures can be used to determine whether the entire toxicity disappears as the hæmolysins are rendered inactive. A series of rabbits and guinea-pigs was inoculated with different quantities of the Amanita extracts previously heated for one half hour to 60°, 65°, 70°, 80°, 90°, and 100° C. All the animals died after brief periods except those given the extract heated to 90° and 100° C. The extract heated to 60° C, caused, except for slighter subcutaneous œdema, lesions identical with those produced by the raw material. Hence a temperature of 60° C. fails to exert any special action upon the Amanita poison. The extract heated to 65°, to 70°, and to 80° C. is still toxic, killing animals almost as rapidly as the unheated material, but the pathological changes produced are greatly modified. Subcutaneous œdema and hæmoglobinuria are now absent; hæmorrhages are still present, but to a less degree, and the necrosis and fatty degeneration of the parenchymatous organs are diminished. Upon microscopic examination the pigmentation of the spleen is seen to be lacking, but all the other changes occur and are identical with those produced by the crude extract. With the loss of hæmolytic activity the extract has lost its power of producing subcutaneous œdema, hæmoglobinuria, and pigmentation of the spleen. The toxins are completely destroyed at a temperature between 90° and 100° C. The following protocols give the results of the heating experiments.

EXPERIMENT I. Rabbit, 1200 grams, inoculated February 18, 1905, with 1 c.c. of an extract of Amanita phalloides heated to 60° C. for ½ hour. Death Feb. 20. Autopsy: Subcutaneous œdema, hæmorrhages, and necrosis.

EXPERIMENT II. Rabbit, 1270 grams, inoculated Feb. 13, 1905, with the same, heated to 70° C. for ½ hour. Death Feb. 14. Autopsy: No exudate at site of inoculation. Hæmorrhages in lymphatic glands and adrenals. Liver congested; areas of hæmorrhage on its surface. Spleen dark and soft, bladder filled with normal looking urine. Hæmorrhagic areas in one lobe of the lung; a few minute hæmorrhages in the mucosa of the intestines. Cultures sterile.

EXPERIMENT III. Rabbit, 1445 grams, inoculated Feb. 13, 1905, with 1.2 c.c. of the same extract heated to 80° C. for ½ hour. Death Feb. 14. Autopsy: Slight congestion at site of inoculation, but no œdema. Lymphatic glands everywhere hæmorrhagic. Liver congested, but without definite areas of hæmorrhage. Spleen large, dark, and soft. Kidneys and adrenals show no definite hæmorrhages. Bladder filled with pale urine. Heart dilated; blood in heart and larger vessels clotted. Lungs, stomach, and intestines, negative. Cultures from the organs sterile.

EXPERIMENT IV. Rabbit, 1230 grams, inoculated Feb. 13, 1905, with 1 c.c. of the same extract heated to 90° C. for ½ hour. No symptoms; survived.

EXPERIMENT V. Rabbit, 1400 grams, inoculated Feb. 13, 1905, with 1.2 c.c of the same extract boiled for ½ hour. No effects.

Similar experiments which were carried out upon guinea-pigs gave the same definite results. The outcome of all these experiments shows that the extracts, when heated to 65° and 80° C., retain their toxicity, and cause death within forty-eight hours, but the lesions have entirely changed in character. In guinea-pigs a little œdema sometimes develops at the site of inoculation, but this is not of the same character as that produced by the unheated material. It probably merely indicates a great susceptibility of the blood-vessels to the poison, just as the corpuscles of the guinea-pig are so susceptible to the action of the Amanita that at times a very few corpuscles will be destroyed by the extract heated to 65° C. and even to 70° C.

Extracts of Amanita phalloides which have completely lost their hæmolytic activity still retain their toxicity. Hence after depriving these extracts of their phallin by heat there must still remain certain substances which are capable of causing rapid death, and of setting up those changes in the animal body associated with hemorrhage, necrosis, and fatty degeneration of the parenchymatous organs. To destroy completely the Amanita, temperatures of 90° to 100° C. are required. Hence I conclude that extracts of the fungus contain thermolabile and thermostable substances.

Amanita Extracts Treated with Blood.—Still another method

for determining the toxic constitution of the Amanita is to treat the extracts with various cells of the body to ascertain whether they can thus be neutralized. If the extracts owe their toxicity primarily to a blood-laking poison, then the complete saturation of this poison by blood ought theoretically to give us a neutral mixture.

A number of combinations of corpuscles and the extracts were made for this purpose and the results were uniformly negative. Thus three cubic centimeters of an extract were mixed with twelve and a half cubic centimeters of washed rabbit corpuscles; hæmolysis occurred in about twenty minutes. This proportion of blood corpuscles and extract should theoretically give a neutral mixture, assuming the poison to be primarily a hæmolytic one. About eight cubic centimeters of this mixture, representing more than a fatal dose for certain animals, were given to a rabbit. Death took place after seven days and the post-mortem appearances were those of the Amanita intoxication.

If the extracts consisted chiefly of a blood poison whose activity is inhibited but not destroyed at 80° C., then the heated material should be capable of neutralization and be rendered non-toxic by saturation with blood corpuscles. Here also the experimental results were negative. Thus three cubic centimeters of an extract were combined with twelve and a half cubic centimeters of washed rabbit blood corpuscles, and eight cubic centimeters of this mixture were given to a rabbit. Such a mixture should theoretically be neutral. The inoculated animal, however, died at the end of twenty-four hours, and showed characteristic appearances of Amanita poisoning.

From these experiments it can be concluded that blood corpuscles are in themselves unable to neutralize Amanita extracts, and that the poisons contained in this fungus are not exclusively active against these cells.

Extracts Treated with Brain Tissue.—Many of the symptoms of Amanita intoxication point to a direct action of the fungus upon the nervous tissues. The experiments of Wassermann[4] upon the neutralization of tetanus toxin by emulsions of brain tissue

[4] Wassermann and Takaki, *Berliner klinische Wochenschrift*, 1898, xxv, 5.

and those of Flexner and Noguchi[5] upon the neurotoxic constituent of cobra venom have suggested another mode of analysis of the toxic constituents of this poison. If the chief poisons of the Amanita are neurotoxic, the blood-laking constituent being an ingredient of secondary importance, the combination of the Amanita extract with brain tissue should give a mixture acting still on the blood but without action on the nerve centres. Hence animals receiving this treated poison should recover, as the blood destruction is too small to cause, by itself, fatal intoxication. The results of this series of experiments are given in the following protocols:

EXPERIMENT I. The brain of a rabbit, excised aseptically, was macerated in salt solution and mixed with 5 c.c. of Amanita extract. After several hours' contact a guinea-pig of about 300 grams weight was inoculated with 1 c.c. of the supernatant fluid. Result: death in 18 hours; usual autopsy findings.

EXPERIMENT II. The brain of a guinea-pig, excised aseptically, was macerated in salt solution and mixed with 2 c.c. of a similar extract. The mixture was kept three hours at room temperature and then injected subcutaneously into a guinea-pig of 650 grams. Death in 18 hours.

EXPERIMENT III. A whole brain of a guinea-pig, excised aseptically, was macerated in salt solution and mixed with 4 c.c. of the extract. Two and one-half c.c. of this emulsion were given to a guinea-pig of 400 grams weight Death in 24 hours.

From these experiments, it may be concluded that brain tissue has not the power to remove or neutralize the poisonous principles of Amanita phalloides.

Since, however, the experiments with heat indicate that the thermolabile substance is definitely hæmolytic, it may be that the thermostable substance is neurotoxic in character, and hence the effect of the inoculation of the crude material depends upon the presence of these two poisons. It was desirable, therefore, to destroy the hæmolytic principle by heat and to extract the heated poison with brain tissue in order to determine whether the thermostable substance is really a neurotoxin. The toxicity could not be removed in this manner as the following experiment shows:

[5] Flexner and Noguchi, *University of Pennsylvania Medical Bulletin*, 1902–03, XV, 345.

An extract of the Amanita was heated to 80° C. to destroy completely the hæmolysin, and 2 c.c. were mixed with the brain of a guinea-pig, which had been removed aseptically and emulsified in salt solution. The entire mixture was injected into a 400-gram guinea-pig, which died in two weeks and exhibited changes characteristic of Amanita poisoning.

I therefore conclude that the fungus does not contain a neurotoxin capable of being neutralized by brain tissue.

Effect of Artificial Digestion upon Extracts of the Amanita.—The fungus having been shown to contain two distinct principles—one thermolabile and the other more heat resistant—it is of interest to ascertain whether they show any differences to the action of ferments. To test this question the crude extracts, before and after heating, were digested for a number of days with pepsin and pancreatin and their hæmolytic strength and general toxicity ascertained. Table I shows that the unheated extract employed caused complete hæmolysis of 1 c.c. of a 5 per cent. suspension of rabbit corpuscles in quantities at and above 0.003 c.c., while 0.001 c.c. produced a trace of hæmolysis.

TABLE I.

Crude extract of Amanita.	5% rabbit blood.	Hæmolysis.
1.0 c.c.	1 c.c.	Complete
0.1 "	" "	"
0.05 "	" "	"
0.01 "	" "	"
0.005 "	" "	"
0.003 "	" "	"
0.001 "	" "	Trace
Control 1 c.c. Na Cl 1%	" "	None

This experiment was repeated separately with pepsin and pancreatin. In each case the crude extract was digested for eight days with pepsin and with pancreatin, and in both the hæmolysin was entirely destroyed, since when mixed in equal quantities—one cubic centimeter of the extract and one cubic centimeter of the blood suspension—no hæmolysis occurred.

The influence of the ferments on the thermostable poison was either wholly negative, or, at best, very slight.

EXPERIMENT I. Guinea-pig weighing 670 grams. Injected subcutaneously 2 c.c. extract heated to 65° C. for ½ hour and digested with pepsin for 7 days. Death in 24 hours. The autopsy showed the characteristic lesions of the heated extract including extensive hæmorrhage at the site of inoculation.

EXPERIMENT II. Guinea-pig, 440 grams, inoculated with 1 c.c. of the extract heated to 65° C. and digested with pancreatin. Dead in 24 hours. Autopsy revealed usual changes.

EXPERIMENT III. Guinea-pig, 447 grams, inoculated subcutaneously with 1 c.c. extract heated to 80° C. for ½ hour and digested with pepsin for 7 days. Dead in 24 hours. Autopsy: Hæmorrhage at site of inoculation, and dilated heart.

EXPERIMENT IV. Guinea-pig, 395 grams, inoculated with extract heated to 80° C. and then digested with pancreatin for 7 days. Death followed inoculation within a very short time, possibly less than 1 hour. The material given to this animal, owing to its evaporation, represented a considerable multiple of the fatal dose.

These experiments show that the toxic substance in the Amanita which resists heat also resists the action of pepsin and pancreatin. That the crude extract is, moreover, still toxic after artificial digestion for two weeks, is shown by the fact that a dose of two cubic centimeters killed a guinea-pig weighing 420 grams in four days.

Constitution of the Amanita.—From these experiments dealing with the action of heat and of ferments upon extracts of the Amanita, and from the attempt to neutralize this substance with blood and nerve tissue, and, finally, from a consideration of the post-mortem changes, we obtain a different idea of the constitution of the fungus from the one held by Kobert. While the hæmolytic ingredient, the phallin of Kobert, is an important constituent of the fungus and exerts a very powerful action upon all erythrocytes, it is by no means the only substance present which exerts a toxic effect on animals. In addition to phallin, which is thermolabile and susceptible to artificial digestion, there is present a powerful thermostable substance resistant to the action of ferments. To the hæmolysin are to be ascribed the subcutaneous œdema, hæmoglobinuria, and pigmentation of the spleen produced in artificial intoxication; while to the action of the thermostable substance, which is non-hæmolytic, are due the hæmorrhages, the necrosis, and fatty degeneration in various organs. Whether the hæmolytic substance can produce necrosis, fatty degeneration, and hæmorrhage cannot at the present time be stated definitely, since the different ingredients of Amanita

have been separated by chemical means, the thermolabile substance having been obtained free from the thermostable body, only within the past few weeks. It is hardly conceivable, however, that a substance acting so energetically upon blood corpuscles should not also exert a necrotizing action upon other cells of the body. The thermostable body is the most important constituent of Amanita and the one causing the most profound disturbance of the organs and tissues.

It has been pointed out in a previous paper that by the immunization of animals it is possible to obtain antibodies to extracts of Amanita phalloides which are both antihæmolytic and antitoxic in character. In view of the observations outlined in this paper the question may be asked whether this antiserum is a single body capable of neutralizing both the hæmolytic and the toxic action of the Amanita, or whether it is a combination of antibodies, each acting upon the especial substance to which the animal is immunized. To put the question in another way, it may be asked whether the particular chemical groups under whose influence the antibodies for the Amanita are produced, are identical in the thermolabile and the thermostable substances, or are quite different. If, according to Ehrlich's ideas, it be granted that every toxin is composed of two chemical complexes, one the haptophoric group uniting with susceptible cells, the other the toxophoric group causing the injury of these cells, the haptophoric group alone taking part in the processes leading to artificial immunity and the production of antibodies; then in the case of Amanita the toxophoric group of the hæmolytic or thermolabile constituent must be quite distinct from the toxophoric group of the thermostable constituent; since these groups attack different cells of the body, the one acting primarily upon the erythrocytes, the other primarily upon the cells of the parenchymatous organs. Two possibilities are, however, open in considering the haptophoric groups of these two constituents of the fungus. On the one hand, the haptophoric groups may have the same structure, in which case but one antibody should be found in the antiserum, the antithermolabile substance being identical with the antithermostable substance. On the other

hand, the haptophoric groups may be different and thus the antiserum contain two distinct antibodies, one neutralizing the hæmolysin, the other neutralizing the resistant body.

If the hæmolysin of Amanita is destroyed by heat, and an antibody be made from the residue, and if the thermolabile and the thermostable bodies are identical in haptophoric structure, the antibody produced should be antihæmolytic and at the same time antitoxic. If, on the other hand, the haptophoric groups be different, the antibody produced for the thermostable body should be merely antitoxic and have no antihæmolytic power. Such an experiment should also settle definitely the question whether there are two separate toxic substances in Amanita phalloides, in contradistinction to the notion that the action of heat and of digestion is merely to alter the arrangement of molecules in a toxic complex consisting of a single substance, in which occurs one haptophoric group attached to at least two toxophoric groups presenting differences of resistance.

Jacoby,[6] who has investigated the toxic and agglutinating action of ricin, arrived at the conclusion that this poison contains an agglutinin and a toxin, each of which possesses separate and distinct haptophoric groups. At Ehrlich's suggestion, however, Jacoby immunized a goat to ricin from which all traces of agglutinin had been removed by combination with blood corpuscles, and as the resulting antiserum was anti-agglutinating as well as antitoxic, Jacoby was led to believe that the ricin-toxin and the ricin-agglutinin possessed identical haptophoric groups.

A series of animals was immunized to extracts of Amanita heated respectively to 65° C. and to 80° C. for one half hour. It proved to be somewhat less difficult to immunize the animals to the heated than to the unheated extracts, since the local œdema and necrosis at the site of inoculation were by this means largely avoided. The immunization was pushed to the point where the animals withstood the inoculation of several multiples of the fatal dose of the heated material, after which the blood was withdrawn and tested for its antihæmolytic and its antitoxic properties.

[6] Jacoby, *Hofmeister's Beiträge*, 1902, ii, 535.

A rabbit of 1970 grams was given on successive occasions an extract of Amanita which had been previously heated to 65° C. for one-half hour. The acutely fatal dose was 1.2 cubic centimeters.

Date.	Dosage.
May 5	0.3 c.c.
" 12	0.6 "
" 19	0.9 "
" 26	1.2 "
June 10	1.4 "
" 19	1.75 "
" 28	2.00 "

Bled June 29, 20 c.c.

This serum was tested for its antihæmolytic and antitoxic effects.

TABLE II.

ACTION OF IMMUNE SERUM ON HÆMOLYSIS.

Extract.	5 % rabbit blood.	Serum.	Hæmolysis.
.005 c.c.	+ 1 c.c.	+ 0	= Complete
.005 "	+ 1 "	+ 0.1 c.c. Normal	= "
.005 "	+ 1 "	+ 0.05 " Immune	= "
.005 "	+ 1 "	+ 0.1 " "	= "

The immune serum made from the heated extract does not prevent hæmolysis. The next experiments were made in order to test the antitoxic properties of the serum.

Control: 1 c.c. of an extract heated to 65° C. for ½ hour kills a rabbit of 1500 grams in a short time.

EXPERIMENT I. Rabbit, 1050 grams, inoculated July 1 with 1 c.c. of the extract heated to 65° C. for ½ hour + 1.5 c.c. of the immune serum. Animal unaffected.

EXPERIMENT II. Rabbit, 1150 grams, inoculated July 1 with 1 c.c. of the extract heated to 65° C. for ½ hour + 1.5 c.c. of the immune serum. Animal unaffected.

The immune serum, although completely devoid of antihæmolytic action, therefore possesses definite antitoxic properties.

A second rabbit was immunized to the Amanita extract heated to 80° C. for half an hour, and its serum was tested both for antihæmolytic and for antitoxic properties. The dose of the extract which quickly caused death was 1.2 cubic centimeters.

A rabbit weighing 1860 grams was immunized as follows:

Date.	Dosage.
May 5	0.3 c.c.
" 12	0.6 "
" 19	0.9 "
" 26	1.2 "
June 6	1.4 "
" 16	1.7 "
" 26	2.0 "

July 5, 15 c.c. blood withdrawn.

The serum of this animal was wholly devoid of antihæmolytic properties, being without effect in a 1:10 dilution. Its antitoxic action was marked.

EXPERIMENT I. Rabbit, weight 1500 grams, inoculated July 6 with 2 c.c of the extract heated to 80° C. ½ hour + 2 c.c. antiserum. Recovered.

EXPERIMENT II. Rabbit, weight 1500 grams, inoculated July 6 with 2 c.c. of the extract heated to 80° C. for ½ hour + 1 c.c. antiserum. Died.

EXPERIMENT III. Rabbit, weight 1600 grams, inoculated July 6 with 2 c.c. of the extract heated to 80° C. for ½ hour + 0.5 c.c. antiserum. Recovered.

The antiserum possesses, therefore, definite antitoxic properties, two cubic centimeters completely neutralizing an excess of the toxin representing about five times the lethal dose for a rabbit of this weight. Quantities less than two cubic centimeters failed to protect regularly. The antiserum is also effective for guinea-pigs.

Control: I c.c. of the heated extract kills a guinea-pig in 24 hours.

EXPERIMENT I. Guinea-pig inoculated July 6 with 1 c.c. of the extract heated to 80° C. for ½ hour + 1 c.c. antiserum. Recovered.

EXPERIMENT II. Guinea-pig inoculated July 6 with 1 c.c. of the extract heated to 80° C. for ½ hour + ½ c.c. antiserum. Recovered.

EXPERIMENT III. Guinea-pig inoculated July 6 with 1 c.c. of the extract heated to 80° C. for ½ hour + ¼ c.c. antiserum. Recovered.

We thus see that the antiserum produced by the immunization of animals to the thermostable substance contained in the extract of Amanita phalloides heated to 65° and to 80° C. neutralizes the toxic action of this substance only, having no power to neutralize the thermolabile or hæmolytic principle. The two substances must, therefore, possess separate haptophoric and toxophoric groups. They do not, therefore, consist of a complex molecule

composed of a single haptophore group united to at least two toxophore groups.

Since the completion of this paper an investigation of the chemical constituents of Amanita phalloides has been carried on in the laboratory of Professor J. J. Abel, the report of which will appear shortly. The separation of the two principles described has now been accomplished by chemical means.

CONCLUSIONS.

From the experiments detailed in this paper it may be concluded that Amanita phalloides contains besides phallin, or the hæmolytic principle of Kobert, another body of toxic nature. Phallin is thermolabile, and is destroyed by the action of pepsin and pancreatin. The other toxic body is thermostable and is resistant to pepsin and pancreatin. The two substances, moreover, possess different toxophoric and haptophoric groups, since an antiserum produced by the immunization of animals to the thermostable body has no neutralizing effects upon phallin.

The thermolable body, phallin, produces the subcutaneous œdema and hæmoglobinuria, and, in virtue of its blood-laking properties, the pigmentation of the spleen. The thermostable body produces hæmorrhage and necrosis, and the fatty degeneration of the parenchymatous organs. The two bodies exist side by side in watery extracts of the fungus, but they cannot be considered as two constituents of a single poison exerting a variety of effects. To the hæmolysin the name phallin has already been given by its discoverer, Kobert. For the thermostable substance described now for the first time, the name Amanito-toxin is proposed provisionally.