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THE ANTIGENIC PROPERTIES OF PROTEOSES

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BY

EMANUEL BERNARD FINK

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THE ANTIGENIC PROPERTIES OF PROTEOSES

EMANUEL B. FINK

From the Department of Pathology, University of Chicago, and the Otho S. A. Sprague Memorial Institute, Chicago

INTRODUCTION

Of fundamental importance in immunity is the question whether only proteins are antigenic. Wells[1] says, "as yet, it has not been finally established that any colloids other than proteins can act as antigens." Assuming then, that only proteins possess antigenic properties, it becomes of interest to inquire what there is in the nature or constitution of proteins to which this is due. Two characteristics of proteins have received the greatest attention in this connection; their colloid nature and their huge molecular weight. Attempts to explain the antigenic properties of proteins on the basis of their colloid nature have been largely speculative and consist of the application of the principles of colloid chemistry to theories of immune reactions. The efforts to determine the influence of the size of the molecule on the antigenic properties of proteins have led to numerous attempts to produce antibodies for the products of protein hydrolysis and digestion.

Our understanding of the chemistry of immune reactions is limited by our knowledge of the chemistry of proteins. Each new insight into the nature and constitution of proteins is followed by a readjustment of our theories of immuno-chemistry. Underhill and Hendrix[2] place the discovery of the phenomenon of anaphylaxis among the more important recent advances in the physiology of proteins. It has also furnished considerable impetus to the study of protein chemistry.

For a satisfactory explanation of the anaphylaxis reaction, it became necessary to determine by what changes proteins were rendered toxic, since it was well known that native proteins were of themselves nontoxic. This led to the theories of Vaughan, Friedmann and Friedberger that anaphylaxis is due to parenteral digestion of proteins, and that the symptoms are due to intoxication with the resulting products. To obtain evidence in support of these theories the products of protein

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[1] Chemical Pathology, 1914, p. 156.
[2] Jour. Biol. Chem., 1915, 22, p. 443.

digestion and cleavage have been injected into animals in attempts to produce the symptoms of anaphylactic shock.

Considerable work has been done by many investigators in an effort to demonstrate the formation of antibodies against incomplete protein molecules. Most of the attempts have been made with the higher cleavage products of protein digestion.

HISTORICAL

(*a*) *Anaphylaxis and "Peptone Shock."*—Evidence is not lacking that the higher products of protein disintegration may act as antigens. The most successful results have been reported with the anaphylaxis reaction.

Rosenau and Anderson[3] were among the first to ascribe anaphylactogenic properties to "peptone." They state that "peptone" seems to have slight sensitizing and intoxicating properties but the table showing their results indicates that 0.004 gm. was injected subcutaneously into a guina-pig, followed 31 days later by a like amount which produced no symptoms of anaphylactic shock. They fail to mention the source or kind of "peptone" used.

Arthus[4] studied the anaphylaxis reactions of proteoses as represented by commercial Witte peptone. His first experiments were on dogs. He noted a striking resemblance between the reaction of sensitized animals to a second injection of serum and the reaction of normal dogs to an injection of "proteoses." He was able to increase the toxicity of Witte peptone by repeated subcutaneous injections; this he interprets as a hypersusceptibility to proteoses. Experiments with rabbits yielded practically the same results. In a sensitized rabbit (to horse serum) a dose of "peptone" which produced no effect in a normal animal caused a marked fall in blood pressure, dyspnea and loss of sphincter control.

It is doubtful if any of the reactions described by Arthus following the injection of Witte peptone represent true anaphylaxis reactions. Zunz[5] has pointed out that it is not at all surprising that conflicting results have been obtained by the use of Witte peptone, since its composition is extremely variable, especially the proteoses contained in a given sample. Much emphasis was laid on changes in respiratory rate in "sensitized" rabbits, whereas mere handling of these animals is often sufficient to produce marked dyspnea and tachycardia.

Pick and Yamanuchi,[6] using a 10% solution of Witte peptone from which all coagulable material had been removed by boiling, produced symptoms of anaphylaxis and death in a rabbit sensitized to beef serum. The dose of peptone was 4 c c intravenously. Two young rabbits passively sensitized against Witte peptone died on intravenous injection of 6 c c of 10% heated peptone. Control animals showed no symptoms. In these experiments young rabbits were used, and sensitization was passive.

After reviewing the symptoms of anaphylaxis in the different laboratory animals, Biedl and Kraus[7] called attention to the fact that the symptom complex as described in the literature up to that time was not at all definite.

[3] Hyg. Lab. Bull. 36, U. S. Pub. Health Serv., 1907.
[4] Arch. Internat. de Physiol., 1909, 7, p. 471; 1910, 9, p. 157 and p. 179.
[5] Ztschr. f. Immunitätsforsch., 1913, 16, p. 580.
[6] Ibid., 1909, 1, p. 676.
[7] Wien. klin. Wchnschr., 1909, 22, p. 363.

They therefore made a careful study of anaphylaxis in the dog because they considered this animal most suitable for the study of physiologic phenomena. All of the dogs used did not respond to a second injection of horse serum. The characteristic symptoms in those that did react was a fall in blood pressure due to peripheral vasodilatation, a loss of coagulability of the blood and a primary leukopenia followed after several hours by a leukocytosis. None of their dogs died of an acute anaphylactic reaction. In comparing these symptoms with the action of Witte peptone, they noted a close similarity between the two. They concluded from their experiments and from review of the literature that the effect of peptone is in all respects similar to the reinjection of horse or beef serum even in minutest detail. They do not say that anaphylaxis is produced by peptones or proteoses but believe that Witte peptone contains the active principle which is responsible for the symptoms of anaphylaxis. As additional evidence they state that dogs were rendered antianaphylactic against serum by the injection of Witte peptone, and conversely animals which had recovered from the effects of a second injection of serum could withstand larger doses of peptone.

In a subsequent paper Biedl and Kraus[8] state that they were unable to confirm the observations of Arthus[4] that the symptoms of anaphylaxis in the rabbit are similar to those in the dog. They emphasize the fact that their experiments apply only to dogs. By the injection of cultures of various bacteria they were able to partly reproduce the symptoms of anaphylactic shock in normal dogs. These failed to appear when the cultures were grown on peptone-free medium. They therefore conclude that the symptoms were due to the small amounts of peptone present in the medium. Later the same authors[9] report the results of experiments on rabbits and guinea-pigs. In their opinion the mechanism of anaphylaxis is different in the dog and in the guinea-pig. The effect in the former is on the smooth muscle of the peripheral vessels leading to vasodilatation, while in the guinea-pig the reaction consists of a tetanic contraction of the smooth muscle of the bronchioles. Rabbits were found unsuited to anaphylaxis experiments because they died from blood pressure changes. Peptone proved to be very toxic for the guinea-pig, but the symptoms were not like those in the dog. After intravenous injection of 0.25 to 0.30 gm. Witte peptone into guinea-pigs they found that the interference with respiration and the physiologic and anatomic changes in the lungs were exactly like those in anaphylactic shock. From these results they conclude that in the guinea-pig as well as in the dog, Witte peptone has the same action as the toxic principle responsible for the symptoms of anaphylactic shock.

In discussing the results of Biedl and Kraus it may be said that altho they selected the dog as being most suitable for physiologic experimentation, anaphylaxis represents a special manifestation of immune phenomena, and it does not follow that an animal especially suited to work in the one field will also prove satisfactory in the other. As a matter of fact, the symptoms which they found characteristic of anaphylaxis in the dog do not support their contention. Changes in blood pressure may be produced by a number of chemical substances. The same is true of delayed coagulability of the blood. As Wells[10] points out, "the results obtained by observing changes in blood pressure in dogs are by no means comparable with results obtained

[8] Cent. f. Bakteriol., 1909, 44, Ref. Beiheft, p. 68.
[9] Ztschr. f. Immunitätsforsch., 1910, 7, p. 205.
[10] Jour. Infect. Dis., 1909, 6, p. 506.

with guinea-pigs, on which most of the work so far reported has been done, since in these animals the symptoms are entirely different from the symptoms in the dog, and much more closely resemble the effects seen in man."

It is not the purpose to review in detail the physiologic properties of proteoses. This has been done by Chittenden, Mendel and Henderson.[11] They used purified albumoses obtained by acid hydrolysis and digestion of coagulated egg-albumin. All proteoses of whatever source, produced a fall in blood pressure which varied only in degree, and blood withdrawn even 1 or 2 hours after the injection failed to coagulate for at least 24 hours. To produce the characteristic effect 3 to 5 cg. per kg. of body weight are necessary and the intensity of the reaction depends much more on the rapidity of injection than on the dosage. An animal which has recovered from an injection of proteoses may fail to respond to a second injection. This has been termed "immunity" to proteoses, but might better be spoken of as a refractory state, the explanation for which is unknown.

Biedl and Kraus cite the experiments of Popielski and Pick and Spiro on the physiologic action of Witte peptone. The former ascribes the toxicity of the commercial mixture to a substance precipitable by hot absolute alcohol, containing no cholin. This substance he called "vasodilatin." The latter believed the toxic symptoms due to a hypothetical contaminating substance "peptozyme." Underhill[12] after purifying samples of Witte peptone according to the directions of Pick and Spiro, was unable to detect any loss in toxicity. Using purified proteoses obtained by acid hydrolysis of proteins of both animal and vegetable origin he was able to obtain the typical physiologic reactions of proteoses described by Chittenden, Mendel and Henderson. He concludes that "at present there is no occasion for attributing the physiological effects following the injection of proteoses into the circulation to the presence of contaminating substances derived from animal tissue or elsewhere. . . . No method of 'purification' has been found which will deprive proteoses of their characteristic physiological behavior in the circulation; when the chemical make-up of the proteoses is profoundly altered and they lose their chemical identity, the typical physiological action may also be lost."

It seems probable, therefore, that the symptoms of intoxication following intravenous injection of Witte peptone into dogs are due to proteoses contained in the preparation, and is a physiologic reaction differing fundamentally from anaphylaxis in the absence of the phenomenon of sensitization following a suitable incubation period.

The work of Biedl and Kraus stimulated a great deal of investigation and discussion. Richet[13] believes that it is scientifically impossible to compare the physiologic action of peptones with the action of anaphylactic substances. Fall in blood pressure is not sufficient to explain all the symptoms (vomiting, profuse diarrhea, agitation, paraplegia, intoxication, psychic blindness, coma, all phenomena coming on with extreme rapidity) in a dog rendered anaphylactic with actino-congestine. Amyl nitrite, which in small doses produces a fall in blood pressure, does not result in any such grave symptoms. Regarding incoagulability of the blood, he finds that in anaphylaxis produced by mytilo-congestine or actino-congestine, there is no appreciable diminution in the coagulability of the blood. The congestines have the general characters of peptones.

[11] Am. Jour. Physiol., 1898-9, 2, p. 142.
[12] Ibid., 1903, 9, p. 345.
[13] Presse Medicale, 1909, 17, p. 249.

Each of the symptoms described by Biedl and Kraus as characteristic of anaphylaxis was studied separately by Salus.[14] He injected intravenously into guinea-pigs a sufficient quantity of magnesium sulphate, sodium citrate or hirudin to produce incoagulability of the blood equal to that in anaphylactic shock without finding any of the lung changes at necropsy. Pure preparations of pepsin were nontoxic for guinea-pigs, only those preparations being toxic which contained albumoses as impurities. The albumoses themselves varied in toxicity. Later he[15] attempted to determine whether the principle in anaphylatoxin is a peptone-like, dialyzable ninhydrin-reacting substance. By dialyzing anaphylatoxin he was never able to obtain a toxic substance in the dialysate. According to this anaphylatoxin does not seem to be a peptone-like substance. Horse serum did not sensitize guinea-pigs to peptone, nor did an injection of peptone render an animal refractory to a second injection of horse serum.

According to Doerr and Muldovan[16] the changes in the lungs in anaphylaxis are not specific for this condition but may be produced by such substances as peptone and saponin. Furthermore, they consider it unsafe to speculate on the identity of the toxin in anaphylaxis and a hypothetical substance in Witte peptone which is supposed to produce the symptoms of "peptone intoxication" until the two have been isolated and identified. Witte peptone is a mixture of substances and the toxic principle contained in it has not yet been identified. They evidently were not familiar with the work of Underhill on proteoses.

DeWaele and Vandevelde[17] observed no symptoms of anaphylaxis following repeated subcutaneous injections of Witte peptone into rabbits. The dosage varied from 0.2-1 gm.

Werbitzky[18] sensitized guinea-pigs with 0.01 gm. horse serum and then injected peptone by various means without producing symptoms except in one animal. The same animals showed the usual reactions of sensitized guinea-pigs when injected with a second dose of horse serum. Peptone did not increase their susceptibility. Doses of peptone which were said to have produced symptoms of anaphylactic shock in dogs (Biedl and Kraus) were found to have absolutely no effect on guinea-pigs. The conclusion is therefore drawn that "peptone intoxication" and protein sensitization are two separate and unrelated phenomena.

The constancy of temperature changes in the guinea-pig during anaphylactic shock is emphasized by Pfeiffer and Mita.[19] There is a fall in temperature often as much as 4 C. They noted a similar drop in temperature after intraperitoneal injection of Witte peptone. By subcutaneous injections of peptone they obtained the Arthus phenomenon or local tissue necrosis at the site of injection. The injection of peptone caused the symptoms to become pronounced in an anaphylactic animal, but peptone was unable to sensitize to itself. The differences between anaphylactic shock and "peptone intoxication" were sufficient to lead them to believe that the two are not identical.

St. Bächer and Wakushima[20] made determinations of the opsonic index in the dog during anaphylactic shock and found that there was a marked drop

[14] Med. Klinik, 1912, 8, p. 1355.
[15] Biochem. Ztschr., 1914, 65, p. 381.
[16] Ztschr. f. Immunitätsforsch., 1910, 7, p. 223.
[17] Biochem. Ztschr., 1910, 30, p. 227.
[18] Compt. rend. de la Soc. de Biol., 1908, 66, p. 23.
[19] Ztschr. f. Immunitätsforsch., 1909, 4, p. 410.
[20] Cent. f. Bakteriol., I, O., 1911, 61, p. 238.

Dogs which received intravenous injections of Witte peptone also showed a marked fall in their opsonic index which varied with the severity of the symptoms.

Friedberger and Mita[21] injected 0.1-0.5 c c of sheep serum intraperitoneally or into the dorsal sac of frogs. One to four weeks later the animals received an intravenous injection of 0.1 c c of the homologus serum. Following the second injection there were characteristic changes in the heart action. Very soon after injection there was a definite slowing of the rate, marked irregularity, and finally the heart stopped in diastole. These changes are attributed to anaphylactic shock, and the authors point out that definite circulatory changes have been observed in anaphylactic shock in warm blooded animals. The effect of various commercial peptones and a sample of pure silk peptone obtained from Abderhalden was then studied. The peptone mixtures had a toxic effect on the isolated frog heart the intensity of which was much less than that of true anaphylactic shock.

Hirschfelder[22] reports that by the intravenous injection of from 5-8 c c of 10% Witte peptone into guinea-pigs he was able to produce the changes in the lungs described by Auer and Lewis as characteristic of anaphylactic shock. This occurred only when the injection was made rapidly. As much as 55 c c intraperitoneally produced no effect.

Manwaring[23] sensitized three dogs against horse serum and after a second injection the animals recovered. They were in no way refractory to Witte peptone but reacted in the usual way. If the mechanism for both reactions were the same we should expect that when animals fail to react against the one they would also fail to react against the other. Manwaring is unwilling to admit that the two phenomena are different, but concludes that the toxic substance formed or set free may be identical in both cases. Loewit[24] repeated the experiments of Manwaring, using rabbits and guinea-pigs. He also found that animals which had been made anti-anaphylactic by recovery from a second injection of horse serum reacted to a single injection of Witte peptone in a typical way. So that in the guinea-pig and rabbit as well as in the dog the exhaustion of one mechanism leaves the other still intact.

Nolf[25] was able to confirm the findings of Biedl and Kraus in dogs, and also believes that anaphylaxis is identical with "propeptone" intoxication.

Calvary[26] found that during anaphylactic shock in the dog there was a lessened flow of lymph which failed to clot. A single injection of protein had no such effect, neither did a mere fall in blood pressure. Witte peptone, on the other hand, had a lymphagogue action. He concludes that if both reactions were the same, their effect on the lymph ought to be the same.

Graetz[27] made an extensive study of the anatomic changes in guinea-pigs dying of anaphylactic shock. He found that the circulatory changes and the changes in the lungs were the same as those following a single injection of Witte peptone.

In a study of the blood changes following intravenous injection of egg-white into dogs, Schittenhelm, Weichardt and Grisshammer[28] found that the

[21] Ztschr. f. Immunitätsforsch., 1911, 10, p. 362.
[22] Jour. Exper. Med., 1910, 12, p. 586.
[23] Ztschr. f. Immunitätsforsch., 1911, 8, p. 589.
[24] Arch. f. Exper. Path. u. Pharm., 1911, 65, p. 337.
[25] Arch. Internat. de Physiol., 1910, 10, p. 37.
[26] München. med. Wchnschr., 1911, 58, p. 670.
[27] Ztschr. f. Immunitätsforsch., 1911, 8, p. 740.
[28] Ztschr. f. Exper. Path. u. Ther., 1912, 10, p. 412.

first injection produced no marked changes. In sensitized animals, following a second injection, there was a marked leukopenia depending on the severity of the anaphylactic reaction. They report similar blood changes following the first injection of large doses of Witte peptone. Silk peptone had no appreciable effect. In their experiments they used as a sensitizing dose 20 c c of egg-white, and repeated this dose at varying intervals. They do not describe the symptoms accepted as anaphylactic, but apparently any animal becoming acutely ill was considered anaphylactic. They also obtained a leukopenia after a third injection of egg white without anaphylaxis resulting, so that leukopenia is not specific for the anaphylactic state.

Salus[29] sensitized guinea-pigs with 0.01 c c of horse serum and found that a nontoxic dose of Witte peptone (1-1.5 c c of a 10% solution) produced no effect. The same animals, however, died of acute anaphylactic shock after receiving an injection of 0.25 c c of horse serum. The horse serum did not sensitize to peptone, nor did an injection of peptone render the animal refractory to a second injection of horse serum. Similar results are reported by Besredka, Ströbel and Jupille.[30] Peptone shock, using Witte peptone, did not in the least protect guinea-pigs sensitized with horse serum against a second injection of horse serum. They also are of the opinion that the mechanism by which the symptoms are produced in "peptone intoxication" is entirely different from that concerned in anaphylactic shock.

Ritz[31] observed that the injection of hypertonic (10%) salt solution into guinea-pigs sensitized with horse serum resulted in milder symptoms of anaphylaxis after the second injection of horse serum. Guinea-pigs received from 1.5-2.2 c c of a 10% Witte peptone solution which was followed by 0.9-1 c c of 30% sodium chlorid solution. The protective effect was not so marked nor as constant as in the case of true anaphylaxis. This author believes that his results furnish additional evidence of a close relationship between anaphylaxis and "peptone intoxication."

Kumagai and Odaira[32] were not able to produce a specific anti-anaphylaxis by the use of Witte peptone. Guinea-pigs were sensitized with sheep serum, and after an incubation period the intoxicating dose was determined. By injecting sub-lethal doses of Witte peptone into sensitized animals they were able to inject twice the usual intoxicating dose of serum without killing the animal. Three times this dose was fatal. Animals which had recovered both from the injection of peptone and from a second injection of sheep serum were given a toxic dose of peptone. The animals showed only a slight protection against "peptone intoxication." The injection of peptone leads only to a slight nonspecific resistance to anaphylaxis, and from the results of their experiments the authors believe that peptone intoxication and anaphylaxis must be considered as separate and distinct phenomena.

In the laboratory of Schittenhelm and Weichardt,[33] an assistant developed severe respiratory symptoms from Witte peptone, and gave a local reaction when it was spread on the skin of his hand. Silk peptone had no such effect. This reaction might easily be accounted for by the presence of a minute amount of histamine.

[29] Biochem. Ztschr., 1914, 65, p. 381.
[30] Ann. de l'Inst. Pasteur, 1913, 27, p. 185.
[31] Ztschr. f. Immunitätsforsch., 1912, 12, p. 644.
[32] Ibid., 1912, 14, p. 391.
[33] Deutsch. med. Wchnschr., 1911, 37, p. 876.

(*b*) *Anaphylaxis with Products of Protein Digestion.* — Rosenau and Anderson[34] found that guinea-pigs sensitized with a mixture of toxin and antitoxic horse serum died when injected 27 days later with antitoxic horse serum to which various ferments had been added and allowed to stand over night at 15 C. The ferments used were takadiastase, pancreatin, rennin, mycrosin, invertin, emulsin, pepsin in acid solution, pepsin in alkaline solution, ingluvin, malt and papain. No attempt was made to determine to what extent, if at all, the proteins had been affected by enzyme action.

Realizing that the discrepancy in results obtained by investigations with Witte peptone might be due to the fact that it is such a heterogeneous mixture of substances, other workers have attempted to study the effects of proteolysis on the anaphylactogenic properties of proteins by using products prepared in the laboratory and purified as nearly as was possible by the use of existing chemical methods.

In his studies of the chemistry of anaphylaxis, Wells[35] found that tryptic digestion of bovine serum until but 8% of its nitrogen remained in coagulable form greatly reduced its sensitizing power. Such a serum sensitized guinea-pigs to normal bovine serum in doses of 0.004 c c but not in doses of 0.0004 c c. Normal bovine serum sensitized to itself in doses of 0.00001 c c. Sensitized guinea-pigs received 5 c c of the digestion mixture intraperitoneally without developing symptoms, showing that it had little or no intoxicating properties. Its effect on animals sensitized to bovine serum was the same. Digestion of serum to this point did not destroy its specificity, in that guinea-pigs sensitized to the digestion mixture did not react to horse serum or milk and were rendered refractory to these substances. After digesting for over 16 months[36] the mixture still contained traces of coagulable material (admixed with the trypsin which had been added?), but gave no biuret reaction. Guinea-pigs receiving doses of 1-5 c c were sensitized so that they reacted slightly but typically, to bovine serum injected 3 weeks later, the most marked reaction occurring in the pigs that had received the 5 c c doses; in no case was the reaction at all severe.

Similar experiments showed that peptic digestion of egg-white destroys its power to intoxicate sensitized guinea-pigs only when practically all coagulable protein has been destroyed. Egg albumin which had been acted on for 26 days by pepsin-HCl until no more coagulable protein was recognizable on heating still was able to sensitize guinea-pigs so that a subsequent injection of egg albumin produced moderate symptoms of anaphylaxis.

Albumoses, peptones, crystallizable amino-acids, etc., obtained by digesting egg white with pepsin and trypsin possessed no power to sensitize or intoxicate guinea-pigs. Some of the products of hydrolysis of coagulated egg albumin possessed a slight power of sensitizing to egg albumin. These experiments indicate that proteins cannot be decomposed much, if any, beyond the coagulable form without losing their anaphylactogenic properties.

Pick and Yamanuchi[37] found that beef serum digested with pepsin-HCl for 15 minutes was still able to sensitize to itself and to undigested beef serum. Rabbits sensitized with the digested serum showed symptoms of anaphylaxis when injected 6 days later with the same mixture and 9 days later when injected with normal serum. The sensitizing and intoxicating

[34] Hyg. Lab., Bull. 36, U. S. Pub. Health Serv., 1907.
[35] Jour. Infect. Dis., 1908, 5, p. 449.
[36] Ibid., 1909, 6, p. 506.
[37] Ztschr. f. Immunitätsforsch., 1909, 1, p. 676.

doses in young rabbits for native serum were not given so that a comparison between the anaphylactogenic properties of the original serum and the digested serum cannot be made. The dosage employed, however, is large so that apparently both the sensitizing and intoxicating properties of beef serum digested with pepsin-HCl for 15 minutes are low. A trypsin-digested mixture, free from coagulable protein and proteoses, yielded results which were not constant. The mixture was not able to sensitize to itself, but in one of a series of animals did sensitize to beef serum. They conclude from their experiments that pure native proteins yield most constant results in anaphylaxis. As the protein content diminishes, fewer positive results are obtained, and the sensitizing and intoxicating doses greatly increased, while the results become less constant.

In their studies of the antigenic properties of the split-products of casein, Gay and Robertson[38] found that a guinea-pig sensitized with 1 c c of 3% casein showed marked symptoms of anaphylactic shock when injected after 23 days with 5 c c of paranuclein. Paranuclein is a product of the partial digestion of casein by pepsin. In a similar manner paranuclein was found to sensitize animals to milk, and to a second injection of paranuclein itself.

A mixture of casein which had been digested with pepsin for 10 days at 36 C., did not sensitize either to a subsequent injection of the same mixture, or to a second injection of paranuclein.

Jobling and Strouse[39] obtained primary and secondary proteoses from Witte peptone by removing all coagulable material and then precipitating with one-half and full saturation of ammonium sulphate. Both fractions were toxic for guinea-pigs and produced death with necropsy findings similar to those in anaphylaxis. Egg white and casein were digested with leukoprotease and the proteoses obtained in the same way, yielded similar results.

Working with proteoses obtained by peptic digestion of beef fibrin prepared by the method of Adler (heteroalbumose and protoalbumose), of Haslam (heteroalbumose, protoalbumose alpha and beta, deuteroalbumose alpha and beta), of Pick (heteroalbumose, protoalbumose, synalbumose and thioalbumose), pepsinfibrinpeptone-beta of Siegfried, and a mixture of abiuret products obtained by the digestion of fibrin with pepsin-trypsin-erepsin, Zunz[40] found that the heteroalbumoses and protoalbumoses were able to sensitize and intoxicate guinea-pigs and rabbits, while synalbumose sensitized only. In animals treated with heteroalbumose, protoalbumose or synalbumose the symptoms of anaphylactic intoxication are not generally so marked as in serum sensitized animals. They usually appear after a definite latent period and are not always marked. In these cases it was necessary to use the lowering of rectal temperature as a criterion of anaphylaxis in guinea-pigs.

If the serum of an animal injected with heteroalbumose or protoalbumose be withdrawn after a suitable interval and incubated with the proteose used for sensitization, a solution is obtained which produces the symptoms of anaphylactic intoxication when injected into a normal animal.

The results of Zunz' experiments clearly show that proteoses are not as effective as serum in the production of anaphylaxis, much larger doses being required to produce less marked symptoms. Specificity is not marked, since an animal sensitized with one proteose will react with any of the other anaphylactogenic proteoses and with beef serum. Species specificity, how-

[38] Jour. Exper. Med., 1912, 16, p. 470.
[39] Ibid., 1913, 18, p. 591.
[40] Ztschr. f. Immunitätsforsch., 1913, 16, p. 580.

ever, is shown, inasmuch as an animal sensitized with proteoses obtained from beef fibrin does not react with horse serum.

Friedberger and Joachimoglu[41] sought to repeat Zunz' work with heteroalbumoses and protoalbumoses. They used preparations furnished them by Zunz. On examining the protocols in the latter's report, they were surprised at the large dose of beef serum used, a dose which in their experience proved toxic to normal animals; 0.15-0.20 c c per 100 gm. body weight was lethal for young guinea-pigs. The lethal dose for animals injected 11 days previously with heteroalbumose or protoalbumose was the same. Such animals showed no symptoms when injected with large doses of horse serum. They therefore attribute the greater susceptibility of guinea-pigs to beef serum as compared with horse serum to the difference in toxicity of the two serums, and not to true sensitization. As compared with normal controls, pigs previously treated with albumose do not show an increased susceptibility to beef serum.

Zunz and György[42] then repeated some of the experiments with proteoses, and again reported that guinea-pigs sensitized with either hetero- or protoalbumose gave anaphylaxis reactions when injected with either or with ox serum, but not with horse serum. The question of the primary toxicity of beef serum as a possible explanation of these results was not discussed.

The sensitizing power of heteroalbumoses seemed to be more marked than their ability to produce anaphylaxis in animals treated with these proteoses. Thus, in such animals the intravenous injection of hetero- or protoalbumose resulted only in mild symptoms of intoxication or even no symptoms whatever. They suggest that perhaps there are varying grades in the sensitizing ability of two heteroalbumose or protoalbumose preparations obtained in exactly the same manner.

A large series of experiments with peptic, tryptic and acid hydrolytic cleavage products of beef and hog muscle is reported by Hailer.[43] In addition, a number of commercial peptone and meat extract preparations were used. The products were tested for coagulable protein, biuret reaction, nitrogen content and precipitability by a homologous precipitin serum. From the results obtained Hailer concludes that it is undoubtedly possible to sensitize guinea-pigs with completely digested protein mixtures (free from coagulable protein, and broken down to the final building stones—the amino-acids—by boiling with sulphuric acid); but that this sensitization is by no means specific since anaphylactic symptoms develop after reinjection of totally unrelated proteins. Despite intensive treatment with a particular kind of protein specific sensitization did not occur when the solution contained large quantities of cleavage products. Similar results were obtained with the commercial products except that reactions were more marked when native protein was present in addition to the cleavage products.

These results are not in agreement with those obtained by Wells[86] who found that tryptic digested serum containing but 8% coagulable nitrogen sensitized to beef serum (40 times the dose of native beef serum required) but did not sensitize to horse serum or milk and were not rendered refractory to these substances. Hailer's conclusions appear to be based on insufficient experimental evidence. Thus, he says that relatively toxic doses were selected because it seemed necessary to flood the animal with large quantities of

[41] Ibid., 1914, 22, p. 522.
[42] Ibid., 1914, 23, 296.
[43] Arb. a. d. k. Gsndhtsamte, 1914, p. 527.

native protein when using substances of relatively weak sensitizing power. As much as 0.6 c c of beef serum was injected intracardially. Friedberger and Joachimoglu[41] found that the lethal dose of beef serum for normal guinea-pigs was 0.15-0.20 gm. per 100 gm. body weight, and while Hailer inactivated his serum, definite symptoms might have resulted from the injection of such doses of beef serum. Since none of his animals died, this remains as a possibility. Suitable controls were not used, as the author depended on the toxic doses of human, horse and swine serums as determined by Uhlenhuth and Händel. No attempt was made to test the intoxicating properties of the digestion mixtures or commercial preparations. The statement that the presence of native protein interferes with the specificity of anaphylaxis by digestion mixtures is contrary to the experience of Wells, whose findings in this respect have already been quoted.

In a series of experiments reported by Schmidt,[44] deuteroalbumose obtained from Witte peptone according to the method of Kutcher was unable to sensitize or intoxicate a sensitized animal.

(*c*) *Vaughan's Protein Poison.*—The work of Vaughan and his students also represents an attempt to prove that anaphylaxis is the result of parenteral digestion of proteins. His theory of anaphylaxis as recently stated in a publication from his laboratory, is that the parenteral introduction of a protein into an animal, the guinea-pig for example, leads to the production of a specific proteolytic ferment by the cells of that animal. Following a suitable incubation period, the injection of a second dose of this same protein, parenterally, results in the liberation of the specific enzyme which then digests the protein molecule with the immediate production of large quantities of poison, sufficient under proper conditions, to kill the animal (Pryer[45]). The experiments of Vaughan on the "protein poison" have served as the basis of this theory.

By extracting egg-white with boiling alcohol (78 C.) containing 2% sodium hydroxid, Vaughan and Wheeler[46] were able to split the protein into a poisonous and nonpoisonous fraction. Earlier experiments (1903) had shown that colon bacillus protein could be thus broken up, and subsequent work in Vaughan's laboratory[47] has shown that all proteins, animal, bacterial and vegetable may be broken up in this manner. When injected into animals, the toxic fraction produces symptoms identical in every particular with those following a second injection of egg white into an animal sensitized against egg white. The minimum lethal dose of the purest preparation of protein poison thus far isolated is 0.0005 gm. (Pryer). Wells[48] found the minimal lethal dose of egg white injected into the circulation of guinea-pigs to be $\frac{1}{10}$ to $\frac{1}{20}$ mg.

Chemical examination of the poisonous product indicated only that the carbohydrate group was absent. For a time Vaughan considered it best to look on the poisonous fraction merely as a cleavage product of whole protein, but still a protein. Recently, however, he has expressed the opinion that "the so-called peptone poison, proteoses and the protein poison are closely related bodies" (Vaughan[49]).

[44] Univ. of Cal. Pub. in Pathology, 1916, 2, p. 157.
[45] Jour. Lab. and Clin. Med., 1916, 1, p. 490.
[46] Jour. Infect. Dis., 1907, 4, p. 476.
[47] Jour. Lab. and Clin. Med., 1916, 1, p. 400.
[48] Jour. Infect. Dis., 1908, 5, p. 449.
[49] Jour. Am. Med. Assn., 1916, 67, p. 1559.

Armit[50] was unable to confirm Vaughan's original experiments. He found that when pure crystallized egg albumin is employed the portion corresponding to the haptophore fraction is unable to sensitize normal guinea-pigs to itself, to the other fraction, or to whole egg albumin. On the other hand, the toxophore fraction is capable of sensitizing to a slight extent. Under certain circumstances it is able to intoxicate when used for a third injection. The hypersusceptibility to pure albumin was not interfered with by the injection of either fraction. On subsequent testing with egg white the guinea-pigs were found to be hypersensitive.

Based on a wide experience in the study of proteoses and "peptone intoxication," Underhill and Hendrix[51] report that Vaughan's crude soluble poison from casein has a more marked physiologic action than any proteose with which they have worked. Its action on blood pressure and blood clotting closely resembles that of the proteoses. Vaughan's preparation was found to be toxic for rabbits in relatively small doses, differing in this respect from the proteoses. By boiling with dilute HCl the toxicity of the protein poison was destroyed, indicating that it is a toxic product of protein hydrolysis.

(*d*) *Precipitins with Derived Proteins.*—In his immunological studies of eel serum, Tchistovitch[52] attempted to produce precipitins against peptone (presumably Witte's). After 5 or 6 injections of 5 c c each of 10% peptone solution into a rabbit, no evidence of a precipitin against peptone could be obtained. The first positive results with the use of Witte peptone as antigen were those of Myers.[53] The commercial product was dissolved in salt solution and the coagulable protein removed by boiling. The cooled filtered solution (concentration not mentioned) was injected intraperitoneally into rabbits. The details of the experiments are not given. Immunization led to the appearance of substances in the serum which produced a precipitum in Witte peptone at 37 C. Control experiments gave absolutely negative results. Heating at 56 C. for half an hour weakened the precipitating power of the serum, but the addition of normal serum restored the original strength. Peptone solution to which normal rabbit serum was added remained perfectly clear. Since the details of these experiments have not been published, a critical discussion is not possible. Later work has not confirmed the results with Witte peptone. Heat inactivation and serum reactivation is not a phenomenon generally observed with precipitins, and this seems to be the only recorded instance in which it occurred.

The statement of Myers that the precipitate formed by the reaction of Witte peptone with a serum immunized against it did not give a biuret reaction, was investigated by Bashford[54] who was able to immunize two goats against Witte peptone, and obtained a large quantity of precipitate by treating the serum with Witte peptone. Comparison of analyses of the precipitated product and the mother substance did not show any striking difference in composition. Bashford points out that proteoses, in the presence of other protein substances in neutral solutions, may yield precipitates which are not the product of a specific immune reaction.

Obermayer and Pick[55] worked with tryptic digested mixtures of pure proteins that did not show the presence of unaltered protein. The mother sub-

[50] Ztschr. f. Immunitätsforsch, 1910, 6, p. 703.
[51] Jour. Biol. Chem., 1915, 22, p. 465.
[52] Ann. de l'Inst. Pasteur, 1899, 13, 406.
[53] Lancet, 1900, 2, p. 98; Cent. f. Bakteriol., 1900, 28, p. 237.
[54] Quoted by Nuttall, Blood Immunity and Relationship, Appendix, Note 2.
[55] Wien, klin. Rund., 1902, 16, p. 277.

stances were egg white, a globulin, conalbumin—the noncrystallizable portion of egg white, and ovomucoid. The injection of trypsin digestion products of these substances into animals led to the rapid appearance of immune products. They believe, therefore, that precipitinogen is not complete protein. The action of pepsin-HCl on proteins which readily produce immune bodies is to destroy their antigenic properties, even while there are considerable quantities of albumoses and peptones present in solution. Witte peptone in their experience had no antigenic properties. It is impossible to give a critical review of this paper by Obermayer and Pick because they give only the results of their experiments and omit a detailed description of the digestion mixtures, the methods used in immunization and the number of experiments on which their results were based. A second paper by the same authors[56] consists of a discussion of the biochemistry of the precipitin reaction with experiments designed to determine on what chemical group in the protein molecule species specificity depends. A biuret-free preparation obtained by long continued autolysis of beef pancreas appeared totally inactive in one experiment. On the other hand, if coagulated beef serum or egg white be subjected to the action of trypsin an immune serum can be obtained with the products of digestion even after the biuret reaction has disappeared. Such an immune serum has a very narrow range of reaction in that it precipitates only the digestion mixture. Its species specificity remains intact, since it does not react with the products of tryptic digestion of horse serum. Immunization of rabbits with products of oxidation of proteins by potassium permanganate in alkaline solution led to the formation of precipitins which were strongly species specific.

After many attempts Michaelis and Oppenheimer[57] were unable to obtain a precipitin by the use of peptic digestion products of proteins which would precipitate the mother substance. They used Riedel peptone, which is obtained through peptic digestion of beef fibrin, Merck egg peptone (peptic digested egg white precipitated with alcohol and dried in vacuo) and pure deuteroalbumose obtained from beef by acid hydrolysis. Both commercial preparations probably consisted of mixtures of albumoses. Fifteen animals were used in their experiments. In no instance did the serum of these animals show a specific precipitin. The ability to form a precipitate with a specific immune serum was lost even when considerable heat coagulable material was present in the digestion mixture.

In the case of tryptic digestion, as long as coagulable protein was demonstrable in the solution, it was precipitable by precipitin. After digestion for several weeks with large quantities of trypsin, until protein had entirely disappeared, the precipitability of the mixture by precipitin was completely lost, and it was no longer possible to obtain a precipitin for the mother protein by injecting this substance.

Rostoski and Sacchonagi[58] worked with pepsin and trypsin digestion products of horse serum albumin. They were not able to get rid of the last traces of coagulable protein in the tryptic digestion. Albumoses were obtained by half and full saturation with ammonium sulphate. The filtrates contained peptones. The serum of injected animals yielded a definite precipitate when added to the solutions used for injection. Very marked results were obtained with the ring test of Ascoli, in which serum and solution were placed in narrow

[56] Wien. klin. Wchnschr., 1906, 19, p. 327.
[57] Arch. f. Physiol., 1902, Supplement, p. 343.
[58] Ztschr. f. klin. Med., 1903-04, 51, p. 187.

test tubes. After several minutes a heavy ring-shaped precipitate formed, almost diffuse, finally falling to the bottom. Numerous control experiments using normal serum against the various substances were always negative. The precipitins in this case were not specific, in that the serum of an animal immunized against one of the solutions reacted with all of them. Rostoski and Sacchonagi conclude from their experiments that precipitins can be produced against the products of gastric and pancreatic digestion, even the peptones. They ascribe the failure of previous workers to the use of related proteins.

Michaelis[59] injected a preparation of beef serum partially digested with pepsin-HCl, intraperitoneally into a rabbit at 4-day intervals. Six days after the third injection the serum of the animal was tested. It showed an active precipitin for the digested beef serum producing a marked opalescence almost as soon as the mixture was added. This rapidly turned to a precipitate and settled out. The reaction did not occur in as high dilution as native serum precipitin. With small amounts of normal horse serum a precipitate was formed which dissolved on the addition of an excess of serum. When tested with pseudoglobulin there was no reaction; it reacted well, however, with euglobulin and albumin. If enough serum was added to redissolve the precipitate first formed, the addition of a drop of digestion mixture produced an opalescence. The precipitin against partially digested serum did not react with a digestion mixture from which all coagulable material had been removed either by prolonged digestion or by heating.

Pozerski and Pozerska[60] were unable to demonstrate the presence of specific precipitins in the serum of a dog immunized against Witte "peptone,"

Levene[61] worked with proto- and deuteroalbumoses obtained from Witte "peptone" by precipitation with one-half and full saturation with ammonium sulphate. The serum of rabbits injected with either proteose formed a precipitate with both antigens. Relatively large quantities of serum (as much as 0.4 c c) and antigen (2% solution) were used in the tests. Schmidt[62] used deuteroalbumose from Witte "peptone" and did not obtain a precipitin reaction with the antiserum.

Recently Lampé[63] has reported positive results with various peptones. These were obtained from the crystalline lens, brain, placenta, thyroid, thymus, lung, silk and gliadin. The smallest quantities used activated hemolysis. Normal serum was found to contain no antibodies to these peptones, but antibodies to some may occur in human serum in disease or in rabbits after immunization. Specificity was not absolute in that following protein injections antibodies to the corresponding peptones appeared. Unfortunately I have been unable to gain access to the original article and am thus unable to determine just what tests for antibody production were employed, but the ability of a substance to activate hemolysis is not a sufficient test of its antigenic properties.

(*e*) *Complement Fixation with Protein Derived Products.*—Friedberger and Gay and Robertson using the substances mentioned under the discussion of the anaphylaxis reaction were unable to demonstrate the presence of an antibody to any of the substances except whole protein. Schmidt also reports negative results with his deuteroalbumose.

[59] Deutsch. med. Wchnschr., 1902, 28, p. 733.
[60] Compt. Rend. Soc. Biol., 1911, 70, p. 592.
[61] Jour. Med. Research, 1904, 12, p. 195.
[62] Univ. of Cal. Pub. in Pathology, 1916, 2, p. 157.
[63] Deutsch. Arch. f. klin. Med., 1916, 119, p. 113. Physiological Abstracts, 1916, 1, p. 224.

By digesting proteins (horse serum) with an alcoholic solution of sulphuric acid (48 hours at 62-63 C.), Landsteiner and Prasek[64] were able to produce an immune serum in rabbits which reacted with similarly modified serum proteins of various animals (beef, chicken, rabbit) and even with edestin but not with unaltered horse serum.

Landsteiner and Jablons[65] report that rabbit serum treated with alcoholic sulphuric acid produces complement binding antibodies when injected into rabbits.

SUMMARY OF LITERATURE

The bulk of evidence indicates that "peptone shock" is not an anaphylactic phenomenon. Other substances totally unrelated to anaphylactogens, such as saponins and hirudin may give similar symptoms. The work of Underhill and others has shown quite conclusively that the proteoses contained in the various "peptone" preparations are responsible for their physiologic action. "Peptone shock" differs fundamentally from anaphylaxis in the absence of the phenomenon of sensitization following a suitable incubation period, and in the relatively large quantity of "peptone" required to produce a reaction. Experiments with products of protein digestion have shown that proteins cannot be disintegrated much if any beyond the coagulable form without losing their sensitizing and intoxicating properties. Positive experiments reported with proteoses as anaphylactogens have not been fully confirmed.

The single recorded instance of precipitin formation against Witte peptone has never been confirmed, all subsequent work showing that it has no antigenic properties.

Most of the precipitin experiments with protein digestion products were undertaken to determine on what group in the protein molecule specificity depends, rather than to test the effect of disintegration on their antigenic properties. The entire literature on precipitins is in a chaotic state, the great bulk of it consisting of theoretical discussions. Instead of testing against the digestion mixtures, the serums obtained by their use have often been tested against the mother substance alone. Separating the wheat from the chaff, what little remains seems to parallel the results with the anaphylaxis reaction. Precipitinogen seems to be more resistant to tryptic digestion than to peptic digestion, corresponding to proteins in general in this respect.

Experiments with the complement fixation reaction have been negative.

[64] 'Ztschr. f. Immunitätsforsch., 1913, 20, p. 211.
[65] Ibid., 1914, 20, p. 618.

We might mention at this point that the evidence for the proteose nature of toxins is doubtful, and that Whipple[65a] considers intestinal intoxication due to proteoses.

THE CHEMISTRY OF PROTEOSES

The proteoses are not a chemically definable group of substances, inasmuch as their exact chemical composition is practically unknown. Our knowledge of proteoses has resulted largely from attempts to determine the structure and composition of the protein molecule through methods of analysis. By hydrolytic cleavage, proteins are decomposed, yielding products of lower molecular weight. Among the first of these are the proteoses regardless of the agent employed for hydrolysis, whether by the action of enzymes, dilute acids or superheated steam. So that proteoses, or albumoses, as they are often called have been defined as a group of derived proteins. Analysis has shown that they differ but little in their fundamental composition from the mother proteins.

Albumoses are identified almost wholly by their physical properties and physiologic action. That they consist of smaller molecules than the proteins from which they are derived is evidenced by the fact that they are somewhat more diffusible. Their separation and classification have been largely worked out by Kühne and his pupils, and is based on the fact that they are precipitated by solutions of neutral salts of different strengths.

Kühne and Chittenden[66] divide the primary albumoses into proto- and hetero-albumose. During peptic digestion these give rise to the secondary albumoses or deutero-albumoses. Differences in solubility and precipitability by sodium chlorid led to the separation of four different albumoses. Kühne's[67] method of separating albumoses from peptones consists in saturating with ammonium sulphate while hot, a solution containing a mixture of the two of neutral, alkaline and acid reactions. Although apparently simple, their method does not always succeed, and Kühne and Chittenden have found that the filtrates which are supposed to contain only peptones sometimes contain definite quantities of proteoses. The difficulty seems to be in the adjustment of the reaction at the different stages of precipitation.

Proteoses thus obtained were found to be water-soluble; with sodium chlorid and nitric acid they formed precipitates in the cold which dissolved on heating, and they all gave the biuret reaction.

The work of Kühne and Chittenden was carried a step farther by Neumeister[68] who perfected a method of separating deuteroalbumoses from a

[65a] Jour. Am. Med. Assn., 1916, 67, p. 15.
[66] Ztschr. f. Biol., 1884, 20, p. 11.
[67] Ibid., 1892, 29, p. 1.
[68] Ibid., 1887, 23, p. 381.

mixture of albumoses. Neumeister's method is based on the conception that, in the process of protein cleavage, albumoses are formed in two stages. The first stage results in the formation of proto- and hetero-albumoses, which he has termed primary albumoses; in the second stage each of the primary albumoses yields a deuteroalbumose, or secondary albumose.

E. P. Pick[69] working with Witte peptone was able to isolate four fractions by precipitate with ammonium sulphate of varying degrees of saturation.

The method of fractional precipitation of proteoses has been severely criticized by Haslam[70] who called attention to the fact that there are no chemical tests by which we are able to prove that the various fractions are not mixtures, and that reprecipitation does not serve to remove all traces of impurities, especially other albumoses. In order to avoid this difficulty, Haslam suggests that where possible, the filtrate should be tested for the substance which it is desired to remove by precipitation. Where no such test exists, the amount of organic nitrogen should be determined by the Kjeldahl method in the original filtrate after resolution and reprecipitation. The substance should not be considered pure until the organic nitrogen in the filtrate becomes constant. Washing the precipitate was found to be of no value because the impurities are intimately admixed with the albumoses and washing affects only the surface particles.

PRELIMINARY EXPERIMENTS WITH WITTE "PEPTONE" PRODUCTS

It seemed desirable to test the antigenic properties of Witte "peptone," in the first place to get an idea of the activity of proteoses in this respect, and in the second place to determine if possible why the results of other workers are so conflicting. Three preparations were obtained as follows:

1. Hot alcohol soluble proteose prepared according to the method of Gibson[71] who proved by its physiologic activity on dogs that it was a true proteose. Witte "peptone," 150 gm., were boiled several hours with 1 liter of 80% alcohol under a reflux condenser. The alcoholic solution was filtered through a hot water funnel and the proteose precipitated in a semi-crystalline form in a freezing mixture. The extraction was repeated several times and the fractions combined. The final product was pulverized in a mortar, yielding a brownish-white powder readily soluble in water. From a watery solution it could be precipitated by ⅔ saturation with ammonium sulphate and the filtrate gave only an opalescence with an excess of ammonium sulphate. The filtrate from ¾ saturation gave no further precipitate on the addition of saturated ammonium sulphate. This preparation was used as antigen 1.

2. Cold alcohol soluble material prepared by evaporating to dryness the filtrate from the above and pulverizing in a mortar. This was a light yellow powder, readily soluble in water, and was used as antigen 2.

3. The residue from the alcohol extraction was dissolved as much as possible in hot water, evaporated to a syrupy consistence, and precipitated with 3 times its volume of 95% alcohol, yielding a sticky mass. This was washed with absolute alcohol, followed by ether and dried in a desiccator. The dried material was powdered as above and used as antigen 3.

4. Beef serum was used as control antigen 4.

[69] Ztschr. f. Physiol. Chem., 1898, 24, p. 246.
[70] Jour. Physiol., 1905, 32, p. 267; 1907, 36, p. 164.
[71] Philippine Jour. Science, 1914, 9B, p. 499.

ANIMAL EXPERIMENTS

The first series of animals were immunized by the intensive method. Intraperitoneal injections were given on three successive days of 0.5, 1.0 and 1.5 gm., respectively, and the animals bled on the 10th day after the first injection. The serums were then tested for precipitins and complement binding substances. For the precipitin reaction a 1% antigen solution was used in dilutions varying from 0.5-0.0009 c c with the usual controls. In testing out each serum all the antigens were used to determine nonspecific reactions. For the complement fixation reactions a 1% solution of the antigens were used in dilutions ranging from $\frac{1}{20}$ to $\frac{1}{10,240}$ c c with suitable controls. In this case also all the antigens were tested against each serum. The serums of animals injected with antigens 1, 2 and 3 failed to show the presence of antibodies for any of the antigens used. Animals treated in the same way, using 5, 10 and 15 c c of beef serum showed in the case of precipitins a positive reaction down to the 5th tube, and complete inhibition in the first 4 tubes with partial inhibition in the rest. With the other preparations as antigens, using the same system, there were no reactions.

In order to be certain that the negative results were not due to insufficient treatment of animals, another series of rabbits were injected with increasing doses of antigen at 3-day intervals, beginning with 0.5 gm., and increasing by 0.1 gm. at each subsequent injection. These animals were injected over a period of one month. The results were the same, and no evidence of antibody formation could be demonstrated. Therefore, the three fractions prepared from Witte's "peptone" possessed no power to stimulate the production of precipitins or complement binding antibodies, either for themselves or for beef serum — the mother substance. Neither did they react with the serum of rabbits immunized against beef serum.

Anaphylaxis experiments showed that the Witte "peptone" antigens possessed only very slight anaphylactogenic properties. Guinea-pigs received intraperitoneal injections of 25 mg. of the substance to be tested and 3 weeks later a second injection of 50 mg. In only one or two cases did the animals become sick. In no case did such severe reactions result as follow on the re-injection of beef serum into an animal sensitized to beef serum. For the most part the reactions consisted of vigorous scratching 15 minutes after reinjection. Guinea-pigs sensitized with Witte "peptone" products did not react to beef

serum, nor was the reverse combination any more effective. Preliminary experiments with normal guinea-pigs showed that the preparations in the dosage employed were nontoxic. The protocols are contained in table 1.

TABLE 1

ANAPHYLAXIS EXPERIMENTS WITH PRODUCTS FROM WITTE'S "PEPTONE"

Sensitizing Dose (Intraperitoneally)	Days Interval	Second Injection	Results
1. 0.025 gm. Antigen 1	19	0.050 gm. Antigen 1	Slight roughing, scratching
2. 0.025 gm. Antigen 1	19	0.050 gm. Antigen 1	Scratching
3. 0.025 gm. Antigen 1	19	0.050 gm. Antigen 1	Scratching
4. 0.025 gm. Antigen 1	19	0.050 gm. Antigen 1	No symptoms
5. 0.025 gm. Antigen 2	19	0.050 gm. Antigen 2	No symptoms
6. 0.025 gm. Antigen 2	19	0.050 gm. Antigen 2	No symptoms
7. 0.025 gm. Antigen 2	19	0.050 gm. Antigen 2	Scratching
8. 0.025 gm. Antigen 2	19	0.050 gm. Antigen 2	No symptoms
9. 0.025 gm. Antigen 3	19	0.050 gm. Antigen 3	Roughened hair, scratching
10. 0.025 gm. Antigen 3	19	0.050 gm. Antigen 3	Scratching
11. 0.025 gm. Antigen 3	19	0.050 gm. Antigen 3	Slightly upset, rapid resp.
12. 0.025 gm. Antigen 3	19	0.050 gm. Antigen 3	Scratching
13. 0.025 gm. Antigen 1	19	0.1 c c heated beef serum	No reaction
14. 0.025 gm. Antigen 2	19	0.1 c c heated beef serum	Scratching
15. 0.025 gm. Antigen 3	19	0.1 c c heated beef serum	No reaction
16. 0.1 c c heated beef serum	21	0.1 c c heated beef serum	Typical shock, recovery
17. 0.1 c c heated beef serum	21	0.050 gm. Antigen 1	No reaction
18. 0.1 c c heated beef serum	21	0.050 gm. Antigen 2	No reaction
19. 0.1 c c heated beef serum	21	0.050 gm. Antigen 3	Scratching, late paralysis, not specific
20. 0.1 c c heated beef serum	21	0.050 gm. Antigen 3	No reaction
21. 0.1 c c heated beef serum	21	0.050 gm. Antigen 3	No reaction

Beef serum does not sensitize to products of Witte "peptone," nor does it produce "shock" in animals sensitized to such products.

Witte "peptone" preparations, therefore, possess a slight power of sensitizing to themselves. They are not able to sensitize to beef serum, nor do they produce symptoms of anaphylactic intoxication in guinea-pigs sensitized to beef serum.

EXPERIMENTS WITH PROTEOSES FROM EGG WHITE

(*a*) *Method of Preparation.*—In order to avoid the possibility of introducing protein substances which might be difficult to get rid of in a digestion mixture, enzymes were not used. The method employed was essentially that of Chittenden, Mendel and Henderson.[72] The whites of 6 dozen eggs were coagulated by pouring slowly into a large volume of boiling water to which enough acetic acid had been added to make it distinctly acid to litmus paper. The coagulum was collected on a clean towel, the water squeezed out as much as possible and ground through a fine-meshed copper sieve, in order to obtain the maximum surface for hydrolytic action. The coagulated egg white in a finely divided state, was suspended in an equal volume of water and autoclaved for 10 hours with steam under 10 lbs. pressure. The mixture was then filtered and the filtrate slightly acidified with acetic acid to precipitate any coagulable protein present, again filtered, and the filtrate rendered slightly alkaline with ammonium hydroxid. The neutralization precipitate was filtered

[72] Am. Jour. Physiol., 1898-99, 12, p. 142.

off, and the solution boiled on the water-bath to drive off any excess ammonia, and finally concentrated to a small volume. This final product was filtered until clear. To the filtrate was then added enough cold saturated $(NH_4)_2SO_4$ solution to make it 1/4 saturated, that is, to every 300 c c of filtrate 100 c c of saturated sulphate solution was added. The brownish sticky precipitate was filtered off and allowed to dry on the filter paper. In the same way products were obtained by adding to the filtrate enough saturated $(NH_4)_2SO_4$ solution to yield 1/3, 1/2, 2/3, 3/4 and full saturated fractions, that is, to the filtrate of the 1/4 saturated fraction was added the calculated volume necessary to bring the concentration to 1/3 saturation, etc. The insoluble residue from the original filtrate was again suspended in water and again autoclaved. When the yield in proteoses became small a final hydrolysis with 0.8% HCl was attempted and the residue brought into solution. In order to obtain enough material for immunizing animals it was necessary to repeat the process many times. The yield of proteoses was inconstant at different times, in some instances being abundant, while in others quite disappointing. This has been the experience of most workers with proteoses, and makes the task of obtaining proteoses by hydrolysis tedious and time-consuming. Obviously we are dealing here with a chemical reaction which cannot be controlled. At one time the process may be interrupted at a point when the maximum yield of proteoses results, at another time when the disintegration of the molecule has gone beyond this stage. In the case of a compound of the complex structure of the protein molecule there is no way of determining the stage of optimum production of proteoses. Where the conditions of chemical equilibrium are changing as constantly as in the hydrolysis of proteins, the time when the process should be interrupted is largely a matter of trial and error.

The preparations were purified by dissolving the dried material on the filter papers, discarding the insoluble portions, and determining the amount of ammonium sulphate present by the Folin aeration method.[73] For this purpose 10 c c of the solution to be tested was placed in the receiving cylinder of the Folin apparatus. This was then covered with 5 c c paraffin oil, and 5 gm. dry Na_2CO_3 dusted over the layer of oil. The cylinder was stoppered quickly, and by means of a current of air the liberated ammonia was passed into another cylinder containing 20 c c of N/20 H_2SO_4. Vigorous aeration was continued for 1½ hours, after which the acid solution was titrated with N/10 NaOH using congo red as indicator. From the amount of ammonia thus determined, the concentration of $(NH_4)_2SO_4$ in the total volume of solution was calculated and sufficient saturated $(NH_4)_2SO_4$ solution added to bring it to the desired concentration (1/4 saturation, 1/3 saturation, etc., in the corresponding fractions). The filtrate was added to the succeeding fraction. The precipitates were washed with the corresponding saturation of $(NH_4)_2SO_4$ solution and the whole process repeated. The final products were obtained by precipitation with 3 volumes of alcohol, washing with a small amount of ether, and drying in a desiccator. The result in all cases was a white amorphous powder readily soluble in water. The yield was as follows: 1/4 saturated fraction, 0.7 gm.; 1/3 saturated fraction, 4.2 gm.; 1/2 saturated fraction, 11 gm.; 2/3 saturated fraction, 25 gm.; 3/4 saturated fraction, 12 gm.; 4/4 saturated fraction, 49 gm.

[73] Mathews, Physiological Chemistry, 1916, p. 961.

Sufficient material for testing the physiologic action of the preparations was obtained only with the ½, ⅔, ¾ and ¼ saturated products. These were injected intravenously into dogs in concentrations of 0.06 gm. per kg. of body weight and showed in all cases the typical drop in blood pressure characteristic of all proteoses.

The protocols of our immunization experiments show that the small amounts of ammonium sulphate contained in the preparations did not interfere with the biologic reactions.

(*b*) *Animal Experiments.*—Rabbits were used to test for the production of precipitins and complement binding antibodies. In the first series of experiments, rabbits were injected in pairs with each fraction as follows: 0.1 gm. dissolved in 5 c c of water was injected intravenously on each of 3 successive days, the animals allowed to rest 3 days, after which 3 injections of 0.1 gm. each were again given on successive days. Each animal thus received 0.6 gm. of the preparation to be tested. A second series of rabbits was injected intravenously with proteose preparations, a pair of animals for each fraction, starting with 0.1 gm. and increasing by 0.1 gm. at each succeeding injection, space at 3-day intervals over a period of 1 month. The rabbits were bled and their serums tested for antibodies during the course of treatment, and the final injection consisted of an intraperitoneal injection of 0.5 gm., the deciding test being made with serum withdrawn on the 10th day after the last injection. As controls, rabbits were treated with filtered fresh egg white solution, in exactly the same way using 0.1 c c egg white for each 0.1 gm. proteose. Owing to the small yield of proteoses of 1/4 saturation with ammonium sulphate, only a limited number of experiments were possible with this fraction.

The technic in testing for precipitins and complement binding antibodies was the same as described in connection with the experiments on Witte "peptone" products. The serums of aniamls injected with 1/4, 1/3, 1/2 and 2/3 saturation products showed no evidence of production of either precipitins or complement binding antibodies.

The results of the experiments show that the ¾ and ¼ saturated products were not as efficient antibody producers as egg white, but that they possess definite antigenic power for both precipitin and complement binding antibodies. In the complement fixation experiments the possible anticomplementary effect of ammonium sulphate contained in the preparations was carefully controlled. In treating rabbits for antigen production we found that two of our animals died suddenly following the third intravenous injection, with typical symptoms of anaphylactic shock. Such reactions never occurred with any of the other fractions, and furnish additional evidence that the ¾ and ¼ saturated fractions possess antigenic properties not possessed by the others.

The following are typical protocols of experiments with 3/4 and 4/4 saturation products and egg white as control.

TABLE 2

COMPLEMENT FIXATION TESTS.* EGG-WHITE IMMUNE RABBIT SERUM

Antigen† Dilution 1% Solution	1/4 Saturated	1/3 Saturated	1/2 Saturated	2-3 Saturated	3/4 Saturated	4/4 Saturated	Egg-white
1. 1:20	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete	++	++	++++
2. 1:40	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete	+	Slight fixation	++++
3. 1:80	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete	+	Slight fixation	++++
4. 1:160	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete	+	Slight fixation	++++
5. 1:320	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete	+	Slight fixation	++++
6. 1:640	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete	+	Slight fixation	++
7. 1:1,280	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete	Slight fixation	Slight fixation	+
8. 1:2,560	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete	Slight fixation	Slight fixation	+
9. 1:5,120	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete	Slight fixation	Slight fixation	+
10. 1:10,240	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete
11. Normal salt	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete
12. 1:20 + normal rabbit serum (56 C.)	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete
13. 1:20 + normal salt	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete	Hemolysis complete

* The hemolytic system employed was: Amboceptor—rabbit serum immune to sheep's corpuscles, 2 units. Complement—fresh guinea-pig serum, titrated before use, 1.2 units. Sheep's corpuscles, thoroughly washed fresh, 2.5% suspension. Anticomplementary dose of antigen determined and 1/4 anticomplementary dose used.

† 0.1 c c antiserum (56 C. ½ hr.) in each of first 10 tubes, normal salt sol. q. s. 4 c c in all tubes.

TABLE 3

PRECIPITIN TEST. EGG-WHITE-IMMUNE RABBIT SERUM. 0.1 C C ANTISERUM IN EACH OF FIRST 10 TUBES, NORMAL SALT SOLUTION Q. S. 2 C C IN ALL TUBES

Dilution of Antigen 1% Solution C C	Antigens						
	1/4 Saturated	1/3 Saturated	1/2 Saturated	2/3 Saturated	3/4 Saturated	4/4 Saturated	Egg-white Saturated
1. 0.5	—	—	—	—	slight	slight	+++
2. 0.25	—	—	—	—	slight	slight	+++
3. 0.125	—	—	—	—	slight	slight	+++
4. 0.0625	—	—	—	—	slight	slight	+++
5. 0.03125	—	—	—	—	—	—	+
6. 0.0156	—	—	—	—	—	—	+
7. 0.0078	—	—	—	—	—	—	+
8. 0.0039	—	—	—	—	—	—	+
9. 0.0019	—	—	—	—	—	—	+
10. 0.0009	—	—	—	—	—	—	+
11. no antigen	—	—	—	—	—	—	—
12. 0.5 normal rabbit serum	—	—	—	—	—	—	—
13. 0.5 normal salt	—	—	—	—	—	—	—

TABLE 4

PRECIPITIN TEST: ¾ SAT. RABBIT IMMUNE SERUM. 0.1 C C ANTISERUM IN EACH OF FIRST 10 MIXTURES, NORMAL SALT SOLUTION Q. S. 2 C C IN ALL TUBES

	Dilution of Antigen 1% Solution, C C	3/4 Saturation as Antigen	Egg white as Antigen
1.	0.5	+	+
2.	0.25	+	+
3.	0.125	+	+
4.	0.0625	+	+
5.	0.03125	+	+
6.	0.0156	+	+
7.	0.0078	Faint trace	+
8.	0.0039	Faint trace	+
9.	0.0019	Faint trace	No precipitation
10.	0.0009	Faint trace	No precipitation
11.	no antigen	No precipitation	No precipitation
12.	0.5 + normal rabbit serum	No precipitation	No precipitation
13.	0.5 + normal salt	No precipitation	No precipitation

TABLE 5

PRECIPITIN TEST: 4/4 SAT.-RABBIT-IMMUNE SERUM. 0.1 C C ANTISERUM IN EACH OF FIRST 10 MIXTURES, NORMAL SALT SOLUTION Q. S. 2 C C IN ALL TUBES

	Dilution of Antigen 1% Solution, C C	4/4 Saturation as Antigen	Egg white as Antigen
1.	0.5	+++	++
2.	0.25	++	—
3.	0.125	+	—
4.	0.0625	—	—
5.	0.03125	—	—
6.	0.0156	—	—
7.	0.0078	—	—
8.	0.0039	—	—
9.	0.0019	—	—
10.	0.0009	—	—
11.	no antigen	—	—
12.	0.5 + normal rabbit serum	—	—
13.	0.5 + normal salt	—	—

TABLE 6

COMPLEMENT FIXATION TESTS: ¾ SAT.-RABBIT-IMMUNE SERUM, 0.1 C C ANTISERUM (56 C., ½ HOUR) IN EACH OF FIRST 10 MIXTURES, NORMAL SALT SOLUTION Q. S. 4 C C IN ALL TUBES

	Antigen Dilution 1% Solution	3/4 Saturation as Antigen	Egg white as Antigen
1.	1:20	+++	+++
2.	1:40	+++	+++
3.	1:80	+++	+++
4.	1:160	+++	+++
5.	1:320	+++	+++
6.	1:640	+++	+++
7.	1:1,280	+++	+++
8.	1:2,560	+++	+++
9.	1:5.120	+++	+++
10.	1:10,240	Hemolysis complete	Hemolysis complete
11.	Normal NaCl	Hemolysis complete	Hemolysis complete
12.	1:20 + 0.1 normal rabbit serum	Hemolysis complete	Hemolysis complete
13.	1:20 + normal NaCl	Hemolysis complete	Hemolysis complete

TABLE 7

COMPLEMENT FIXATION TESTS: 4/4 SAT.-RABBIT-IMMUNE-SERUM, 0.1 c c ANTISERUM (56 C., ½ HOUR) IN EACH OF FIRST 10 MIXTURES, NORMAL SALT SOLUTION Q. S. 4 c c IN ALL TUBES

	Antigen Dilution 1% Solution	4/4 Saturation as Antigen	Egg white as Antigen
1.	1:20	++++	++
2.	1:40	++	Hemolysis complete
3.	1:80	+	Hemolysis complete
4.	1:160	Slight inhibition	Hemolysis complete
5.	1:320	Slight inhibition	Hemolysis complete
6.	1:640	Slight inhibition	Hemolysis complete
7.	1:1,280	Slight inhibition	Hemolysis complete
8.	1:2,560	Slight inhibition	Hemolysis complete
9.	1:5,120	Hemolysis complete	Hemolysis complete
10.	1:10,240	Hemolysis complete	Hemolysis complete
11.	Normal NaCl	Hemolysis complete	Hemolysis complete
12.	1:20 + 0.1 normal rabbit serum	Hemolysis complete	Hemolysis complete
13.	1:20 + normal NaCl	Hemolysis complete	Hemolysis complete

TABLE 8

ANAPHYLAXIS EXPERIMENTS WITH PROTEOSES FROM EGG WHITE*

	Sensitizing Dose	Days Interval	Second Injection	Results	Subsequent Injections
1.	0.010 gm. 1/4 sat.	21	0.025 gm. 1/4 sat.	No reaction	4 days later, 1 c c 50% egg white, no reaction
2.	0.010 gm. 1/4 sat.	21	0.025 gm. 1/4 sat.	No reaction	4 days later, 1 c c 50% egg white, no reaction
3.	0.010 gm. 1/4 sat.	21	0.025 gm. 1/4 sat.	No reaction	4 days later, 1 c c 50% egg white, no reaction
4.	0.010 gm. 1/3 sat.	21	0.025 gm. 1/3 sat.	No reaction	4 days later, 1 c c 50% egg white, sick in 15 min., recovered in 30 minutes
5.	0.010 gm. 1/3 sat.	21	0.025 gm. 1/3 sat.	No reaction	
6.	0.010 gm. 1/3 sat.	21	0.025 gm. 1/3 sat.	No reaction	
7.	0.010 gm. 1/2 sat.	21	0.025 gm. 1/2 sat.	No reaction	1 c c 50% egg white, no reaction
8.	0.010 gm. 1/2 sat.	21	0.025 gm. 1/2 sat.	No reaction	1 c c 50% egg white, no reaction
9.	0.010 gm. 1/2 sat.	21	0.025 gm. 1/2 sat.	No reaction	1 c c 50% egg white, no reaction
10.	0.010 gm. 1/2 sat.	21	0.025 gm. 1/2 sat.	No reaction	1 c c 50% egg white, no reaction
11.	0.010 gm. 2/3 sat.	21	0.025 gm. 2/3 sat.	No reaction	1 c c 50% egg white, no reaction
12.	0.010 gm. 2/3 sat.	21	0.025 gm. 2/3 sat.	No reaction	1 c c 50% egg white, no reaction
13.	0.010 gm. 2/3 sat.	21	0.025 gm. 2/3 sat.	No reaction	1 c c 50% egg white, no reaction
14.	0.010 gm. 2/3 sat.	21	0.025 gm. 2/3 sat.	No reaction	1 c c 50% egg white, no reaction
15.	0.010 gm. 3/4 sat.	23	0.025 gm. 3/4 sat.	Vigorous scratching	1 c c 50% egg white, vigorous scratching
16.	0.010 gm. 3/4 sat.	23	0.025 gm. 3/4 sat.	Vigorous scratching, roughing of fur	1 c c 50% egg white, vigorous scratching
17.	0.010 gm. 3/4 sat.	23	2 c c 10% egg white	Vigorous scratching	1 c c 50% egg white, heaving respiration
18.	0.010 gm. 3/4 sat.	23	2 c c 10% egg white	Scratching	

TABLE 8—*Continued*

ANAPHYLAXIS EXPERIMENTS WITH PROTEOSES FROM EGG WHITE*

	Sesitizing Dose	Days Interval	Second Injection	Results	Subsequent Injections
19.	0.010 gm. 4/4 sat.	23	0.025 gm. 4/4 sat.	Immediate convulsive breathing, recovery, 5 minutes	
20.	0.010 gm. 4/4 sat.	23	0.025 gm. 4/4 sat.	Roughing of fur, slight difficulty in breathing	
21.	0.010 gm. 4/4 sat.	23	2 c c 10% egg white	Scratching	1 c c 50% egg white, vigorous scratching
22.	0.010 gm. 4/4 sat.	23	2 c c 10% egg white	Scratching	1 c c 50% egg white, vigorous scratching
23.	2 c c 5% egg white	21	0.050 gm. 3/4 sat.	No reaction	1 c c 50% egg white, heaving resp.
24.	2 c c 5% egg white	21	0.050 gm. 3/4 sat.	No reaction	1 c c 50% egg white, vigorous scratching
25.	2 c c 5% egg white	21	0.050 gm. 4/4 sat.	No reaction	1 c c 50% egg white, vigorous scratching
26.	2 c c 5% egg white	21	0.050 gm. 4/4 sat.	No reaction	1 c c 50% egg white, no reaction
27.	2 c c 5% egg white	21	0.050 gm. 3/4 sat.	Vigorous scratching	1 c c 50% egg white, no reaction
28.	2 c c 5% egg white	21	0.050 gm. 3/4 sat.	Vigorous scratching	4 days later, 1 c c egg white, no reaction
29.	2 c c 5% egg white	21	2 c c 25% egg white	Exitus, typical shock	
30.	2 c c 5% egg white	25	2 c c 25% egg white	Exitus, typical shock	

* The sensitizing injections were given intraperitoneally, the intoxicating doses, intracardially. The various substances tested proved non-toxic for normal animals in the doses employed.

Anaphylaxis experiments with guinea-pigs served to confirm the lack of any antigenic properties possessed by the ¼, ⅓, ½ and ⅔ ammonium sulphate saturated fractions, and showed that the ¾ and 4/4 saturated products possessed slight sensitizing and intoxicating properties, the latter being apparently the more active.

In all forms of antibody production the antigenic proteoses were not specific, in that egg white could be used as antigen in place of either fraction when carrying out the tests. The reverse was not so marked.

(*c*) *Toxin-Antitoxin Reaction.*—According to Mitchell and Reichert[74] (1883) cobra venom contains 2% globulin which is the hemolysin and 98% of substances resembling "peptone," by which they probably meant substances now classed as proteoses. Antitoxin against cobra venom is quite efficient, and the possibility remains that we are dealing here with an anti-albumose. A further possibility is suggested, namely, are whole proteins necessary for anaphylaxis, precipitins and complement binding antibodies, while antitoxins are the result

[74] Cited by Wells, Chemical Pathology, 1918, p. 148.

of antigens consisting of derived proteins-albumoses? Weichardt[75] in connection with his studies of fatigue toxins (kenotoxins) obtained considerable evidence that the substances against which he was able to produce antitoxins represented the first stages in the disintegration of the protein molecule, and therefore closely related to proteoses.

One form of the epiphenin reaction reported by Weichardt[76] seemed to furnish a method of demonstrating the toxin-antitoxin reaction in vitro. He found that catalyzers (hemoglobin, platinum black) are markedly affected by bacterial toxins. If a water soluble toxin such as tetanus toxin be added to a much diluted blood, allowed to stand at 37 C. for 30 minutes, and then tincture of guaiac and hydrogen perox'd be added, the well known guaiac reaction does not occur. If the toxin is previously treated with its specific antitoxin there is no interference with the guaiac reaction and the solution turns blue. Nonspecific reactions may be avoided by quantitative means (dilution of antigen and serum). In addition to toxins, protein derived products including kenotoxin also inhibited the guaiac reaction.

I have test Weichardt's reaction, using the detailed protocol contained in his article. Diphtheria toxin and antitoxin, tetanus toxin and antitoxin, and all our proteose fractions and their corresponding serums were used. In the case of the bacterial toxins relatively large amounts of toxin were required for inhibition (500 units of diphtheria toxin and 1,000 of tetanus toxin). Much larger quantities of antitoxin were required to neutralize their action. The proteose preparations even as much as 0.1 gm., did not give the characteristic reaction. In my experience, the reaction was not of sufficient delicacy to be used in any sense as a quantitative test for the determination of toxin-antitoxin reactions.

SUMMARY

The results of these experiments indicate that Gibson's alcohol-soluble proteose obtained from Witte "peptone" is unable to stimulate the production of precipitins or complement binding antibodies when injected into rabbits. Two other fractions obtained from Witte "peptone" yielded similar results.

Anaphylaxis experiments with guinea-pigs showed that the Witte "peptone" preparations possessed only very slight power of sensitizing to themselves. They are not able to sensitize to beef serum, nor do they produce symptoms of anaphylactic intoxication in animals sensitized to beef serum. Beef serum did not sensitize to products of Witte "peptone," nor did it produce "shock" in animals sensitized to such products.

[75] Ueber Ermüdungsstoffe, 1910.

[76] Münch. med. Wchnschr., 1911, 58, p. 1662.

Proteose preparations were obtained by hydrolysis of coagulated egg white and fractional precipitation with ammonium sulphate in the manner described. There seems to be ground for disagreement with Haslam when he claims that constant nitrogen values as shown by Kjeldahl determinations are an index of purity of proteose preparations. A mixture of proteoses which is constant in its proportions will show constant quantities of nitrogen provided the proportions in the mixture remain the same. Kjeldahl nitrogen determinations of my preparations showed a maximum difference of less than 2% between all of them. When injected into dogs they showed the characteristic physiologic action of proteoses.

Experiments with rabbits indicated that the ¼, ⅓, ½ and ⅔ saturation products possessed no power of stimulating the production of precipitins or complement binding antibodies. They also were unable to sensitize or intoxicate guinea-pigs either to themselves or to egg white, the mother protein.

The ¾ and ¼ saturation products were not as efficient antibody producers as egg white, but showed definite antigenic power for both precipitins and complement binding substances. In guinea-pigs the ¾ and ¼ saturated products possessed slight sensitizing and intoxicating properties, the latter being apparently the more active.

In all forms of antibody reaction the antigenic proteoses were not specific, in that egg white could be used as antigen in place of either fraction. The converse was not so marked.

Experiments with Weichardt's epiphanin reaction (interference with the guaiac blue reaction of blood) indicated that it is not sufficiently delicate to be used as a quantitative determination of the toxin-antitoxin reaction in vitro.

If the immune reactions are reliable indicators of chemical relationships, these results would seem to indicate that the ¾ and ¼ saturated fractions are chemically but little different from the mother substance, or at least contain similar antigenic groups.