STUDIES ON FERMENT ACTION.

VIII. The Toxicity of Some Proteoses.*

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In a previous article (1) we showed that the products of digestion of pneumococci by the ferment of leucocytes were capable of producing immune bodies when injected into the rabbit. By analogy with the digestion of other proteins these products should have consisted mainly of proteoses; for we have shown by our studies on the proteolytic action of leucoprotease (2) that leucocytic ferments as used in our experiments split mainly to the proteose stage. We then attempted to carry the experiments further in order to separate, if possible, the individual cleavage products and to test their biological action in the same way as was done by Jobling and Bull (3) with *Bacillus typhosus*. Jobling and Bull showed that the toxic element of *Bacillus typhosus* resided in the primary proteose fraction resulting from digestion with leucoprotease. It was important to attempt to fractionate the products of digestion of pneumococci by leucoprotease in order to determine whether the same fraction is toxic as with the typhoid bacillus. Perhaps the variations in the clinical course of certain diseases can be brought into relation with variations in the response of the bacteria to body ferments; or, in other words, the primary proteose derived from *Bacillus typhosus* may be toxic, and the same chemical compound from pneumococci may be non-toxic.

Attempts to pursue this line of investigation were begun, but the technical difficulties of securing pneumococcus protein free from mixtures of culture medium in sufficient quantity to carry on exhaustive experiments made it advisable to use other protein for preliminary experiments.

Witte's peptone, as is well known, contains a variable proportion of proteoses as well as of peptone and amino acids, and it was

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decided to separate the various components and to make biological tests of the toxicity of the proteose fractions. It has been shown that the injection of Witte's peptone produces symptoms similar to those of anaphylactic shock, and much work has been done to elucidate the problems of anaphylaxis by means of the study of the chemistry of the reaction with Witte's peptone. As anaphylaxis will be discussed in this paper only as the phenomenon occurs in the course of our experiments, the literature on the subject will not be reviewed. The exhaustive study of Zunz (4) contains a complete bibliography. However, the conception that anaphylaxis and immunity are merely different stages in the same process makes it necessary to attach importance to studies attempting to define in terms of chemical formulæ the nature of any biological phenomenon. Most studies on the relation of protein digestion to immunological problems will be neglected, but we shall briefly review some of the more important contributions bearing directly on the question of the toxicity of proteoses.

De Waele (5) gave peritoneal injections of serum into sensitized guinea pigs, and at the height of anaphylactic symptoms, he withdrew the peritoneal exudate. This exudate on injection into normal guinea pigs produced acute symptoms resembling those of anaphylaxis. The exudate after removal of protein gave Millon's and the biuret reaction which were not present in the serum injected. Consequently, he argued, proteolysis had occurred in the sensitized guinea pig and the symptoms of anaphylaxis were due to protein digestion products.

Biedl and Kraus (6) and others have shown that analogies exist between the condition of anaphylaxis and intoxication by Witte's peptone. Vaughan and Wheeler's (7) method of treating protein resulted in hydrolysis. The separation into a non-toxic portion insoluble in alcohol and a toxic alcohol-soluble portion perhaps may be compared to the separation of proto- and heteroalbumose by the method of Pick (8). The work of Wells (9), of Pick and his co-workers (8) shows that the effect of digestion of albumen on its sensitizing and toxic properties varies with the nature of the ferment and the time of digestion; in other words, it depends on what substances result from digestion. Hartoch and Ssirenskij (10) treated serum protein with trypsin and produced substances toxic for rabbits at the first injection. The symptoms produced were similar to those of anaphylactic shock. Abderhalden and Kämpf (11) found that the portion of peptone precipitated by ammonium sulphate was toxic for guinea pigs. Friedberger (12) claimed that the toxicity of Witte's peptone was due to "primary proteose." During the course of our work Zunz (4) published his comprehensive report on the relation of anaphylaxis to proteoses, but in his studies no especial importance was attached to primary intravenous injection of the various proteoses. His protocols, however, show that control injections of all the substances were made, and that toxic symptoms arose only from the thioalbumose fraction.

Probably the most successful method of separating the various proteoses from a digestion mixture is that of Pick, but even with this procedure, according to Zunz (13), it is not a question of "chemically defined substances," but only of "groups, complexes, or combinations of proteoses." In the first experiments a 10 per cent. solution of Witte's peptone was used, its toxicity tested by injection into the jugular vein of guinea pigs, and then it was submitted to the following chemical procedures. Any free protein was removed by acidifying with acetic acid and boiling. The filtrate was neutralized, filtered after standing, and then the filtrate was treated with an equal amount of saturated ammonium sulphate. The precipitate (primary proteoses) was used in the experiments or further divided as follows: It was dissolved in a small amount of water, to which two parts of 95 per cent. alcohol were added. The heteroalbumoses are precipitated by this method, and the protoalbumoses remain in the alcohol. The protoalbumoses were removed from the alcohol by distillation at low temperature under diminished pressure. The filtrate remaining after half saturation of the original peptone solution was saturated with ammonium sulphate crystals and the precipitate (secondary proteoses) obtained. In some instances the thioalbumoses were first precipitated by two thirds saturation with ammonium sulphate. Throughout the work each step was repeated in order to purify the products. For most of the experiments the material was not thoroughly dried, nor was it free from traces of ammonium sulphate. The exact dosage cannot be given with precision, especially since a certain amount of material is always lost by filtering. In sufficiently large doses ammonium sulphate is not without toxic effects on guinea pigs, but this was controlled by a series of experiments to be described subsequently.

Experiment I.—1 c.c. of 10 per cent. Witte peptone solution was injected into the jugular vein of a small guinea pig. The animal developed symptoms of anaphylactic shock—dyspnea, convulsions, etc.—and died within a few minutes. Autopsy showed dilated lungs.

0.1 c.c. produced symptoms (dyspnea, restlessness).

The primary proteoses from this solution were dissolved in water so that 1 c.c. was equivalent to 2 c.c. of the original peptone solution. Of this 1 c.c. injected into the jugular vein of a guinea pig caused immediate death. Autopsy showed lungs and stomach distended.

0.4 c.c. produced symptoms, but the animal survived.

Of the secondary proteoses an equivalent of 4 c.c. of the original peptone solution produced immediate toxic symptoms, with recovery. The symptoms with this injection were more convulsive than those obtained with the primary proteoses. A larger dose caused immediate death, but the autopsy showed no dilated viscera. The lungs were the seat of many small hemorrhages.

Heteroalbumoses.—Doses equivalent to 8 c.c. and to 12 c.c. of original peptone solution were without effect on intravenous injection into guinea pigs.

Protoalbumoses.—The equivalent of 3 c.c. of original peptone solution produced immediate anaphylactic symptoms with death in five minutes. Autopsy showed typical distention of lungs.

From this experiment the suggestion is derived that both proteose fractions of Witte's peptone are inherently toxic. Of the two components of the primary proteoses, that precipitable by alcohol does not seem toxic, while that soluble in alcohol is decidedly toxic. The symptoms and autopsy findings resulting from the injection of secondary were not exactly identical with those produced by the primary proteoses.

Experiment 2.—This differed from the preceding experiment only in that each of the various fractions was prepared and laid aside until all were ready, and the injection of the peptone solution and of the proteose fractions took place at the same time. The same results were obtained. The protoalbumose produced immediate marked symptoms, the heteroalbumose no symptoms, and the secondary albumose convulsions, prostration, death. At autopsy the lungs were retracted, the stomach and colon distended, and the peritoneal surface of the stomach and intestines injected.

These experiments were repeated with constant results so that they can be accepted. In order to rule out the possible harmful effect of the ammonium sulphate, an experiment was performed with fractions, all of which had been dialyzed for forty-eight hours. The toxic effects of the primary proteose fraction and of the protoalbumose fraction were verified. The secondary proteose fraction produced immediate severe symptoms, with recovery. In the undialyzed material the method of procedure would result in this fraction containing more sulphate than the other fractions, and it is important to find that the purified substance still causes toxic symptoms. In this experiment the heteroalbumose fraction produced death, this being the only time in our series that symptoms were produced by this portion. Perhaps, however, the intensity of the toxic symptoms of secondary proteose were increased by the ammonium sulphate present in the undialyzed material, and the small

pulmonary hemorrhages may have been due to the salt. The injection of the dialyzed material resulted in typical anaphylactic death, with typical autopsy, whereas an injection of a small amount of ammonium sulphate likewise produced convulsions and death. The autopsy on the last guinea pig showed numerous small hemorrhages in the lung, so that undoubtedly some of the symptoms following injection of the undialyzed secondary proteoses were due to ammonium sulphate.

In order to see whether proteoses from other sources than Witte's peptone had the same effects an experiment was performed with casein. A 1 per cent. solution of casein (technical, Merck) in 0.1 per cent. sodium carbonate was digested with leucoprotease for eight days at 37° C. The digestion mixture was then treated exactly as above outlined to separate the primary and secondary proteoses. The amount of primary proteose was comparatively large, while the amount of secondary proteose was small. Intravenous injections of these substances purified, in both cases produced marked symptoms and death. In the case of the primary proteoses the symptoms and autopsy were typically anaphylactic. With the pig dying from the injection of secondary proteoses there were marked convulsions and death in four minutes. The lungs were distended and somewhat hemorrhagic.

Experiments were also performed with the digestion products from egg albumen treated for one week with leucoprotease, and the toxicity of primary and secondary proteoses was studied. With the egg albumen definite toxic symptoms were produced by the primary proteose, but no marked symptoms with the secondary proteose, although the latter animal died in eighteen hours. In another experiment, however, definite symptoms and death were produced by the secondary proteoses. Further splitting of the proteoses derived from casein and from egg albumen was not attempted because of the small amounts of material.

TABLE I.

Injections.	Death.	No symptoms.	Symptoms. Recovery.
Witte's peptone	4	0	3
Primary proteose	4	1	2
Secondary proteose	6	1	2
Protoalbumose	3	0	1
Heteroalbumose	1?	3	1

Table I summarizes the results of injection of the various proteoses from all sources.

DISCUSSION.

In any discussion of the toxicity of proteoses it must be emphasized, as was stated in the text, that we are not dealing with chemical entities. In all probability the "groups" which are precipitated by various strengths of ammonium sulphate are not constant, but contain chemical substances grading one into the other. In order to fix biological properties on any one of these fractions the experiments would have to be absolutely conclusive and constant. In our work we obtained results pointing to toxic properties in both primary and secondary proteoses. Of the primary proteoses that group precipitated by alcohol seems to possess the least toxic effect.

BIBLIOGRAPHY.

1. Jobling, J. W., and Strouse, S., *Jour. Exper. Med.*, 1912, xvi, 860.
2. Jobling, J. W., and Strouse, S., *ibid.*, 269.
3. Jobling, J. W., and Bull, C. G., *Jour. Exper. Med.*, 1913, xvii, 453.
4. Zunz, E., *Ztschr. f. Immunitätsforsch., Orig.*, 1912–13, xvi, 580.
5. De Waele, H., *Bull. de l'Acad. roy. de méd. de Belgique*, 1907, xxi, series 4, 715.
6. Biedl, A., and Kraus, R., *Wien. klin. Wchnschr.*, 1909, xxii, 363.
7. Vaughan, V. C., and Wheeler, S. M., *Jour. Infect. Dis.*, 1907, iv, 476.
8. Pick, E. P., and Yamanouchi, T., *Ztschr. f. Immunitätsforsch., Orig.*, 1909, i, 676.
9. Wells, H. G., *Jour. Infect. Dis.*, 1909, vi, 506.
10. Hartoch, O., and Ssirenskij, N., *Ztschr. f. Immunitätsforsch., Orig.*, 1910, vii, 253; 1911, viii, 420.
11. Abderhalden, E., and Kämpf, E., *Ztschr. f. physiol. Chem.*, 1911, lxxi, 421.
12. Friedberger, E., *Deutsch. med. Wchnschr.*, 1911, xxxvii, 481.
13. Zunz, E., in Abderhalden, E., Handbuch der biochemischen Arbeitsmethoden, Berlin and Vienna, 1910, iii, 239.