STUDIES IN FERMENT ACTION.

VII. Toxic Split Products of Bacillus Typhosus.*

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The study of the various toxic substances liberated by or derivable from bacteria has stimulated a large amount of work since the discovery of the specific relations between particular microorganisms and definite pathological conditions, for it was early believed that the mere mechanical presence of bacterial bodies in the tissues of the infected individual could not be held responsible for the damage wrought. But notwithstanding the early recognition of the significance of these substances, and their continued study by many workers, there is much left undetermined in regard to the nature of bacterial toxins, and the field of specific serum therapy is yet largely before us.

Bacterial toxic substances are easily divided into different classes. In one class we can put the true soluble toxins of Bacillus diphtheriae, Bacillus tetani, etc. These toxins are considered specific secretory products of certain bacterial cells, just as pepsin is a specific secretory product of certain animal cells. Animal organisms react to these toxins by producing specific counteracting bodies that neutralize the toxic bodies in definite multiple proportions, however high the multiple runs. In this type of infection a wide distribution of the causative organisms is not necessary for the production of general disturbances, since the toxic products are so easily separated from the bacterial bodies and so readily soluble.

The toxins or toxic substances of a second class of bacteria bear a very different relation to the bacterial bodies by which they are produced and the reaction products of inoculated animals are different from those produced by toxins of the first class. Bacillus typhosus, Bacillus coli, etc., produce toxins of this class. It is hardly

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conceivable that typhoid bacteria do not produce specific poisons that
give rise to the symptom-complex which characterizes the disease as
a definite clinical entity. Yet it has not been definitely determined
that these bacteria manufacture and liberate a secretory toxin. Toxic
substances have been obtained from typhoid bacteria, it is true, but
a mechanical or chemical destruction of the bacterial bodies was
necessary for their liberation. None of the various methods used
to isolate toxins from *Bacillus typhosus* has given a product of any
degree of purity or one which produced an antitoxin of great value
as a protective or therapeutic agent. Therefore, any additional in-
formation concerning the nature and source of the toxic substances
derivable from this class of organisms is of first importance, since
it is only through a biological and chemical knowledge of these
substances that we may hope to develop methods by means of which
an effective curative agent can be produced.

**HISTORICAL PART.**

A complete review of the extensive literature concerning the
toxins obtainable from typhoid bacilli will not be undertaken here.
Only the publications bearing more or less directly upon the subject
at hand will be referred to.

Pfeiffer (1) first advanced the opinion that typhoid toxins are liberated by
a destruction of the bacterial bodies through the action of lytic substances in
the body of the infected animal. He found no toxin in the filtrate of young cul-
tures. His endotoxin produced bactericidal substances in treated animals. It
was not destroyed by chloroform vapors, and it was thermolabile. Pfeiffer also
believed that the endotoxin acted as an antigen in natural cases of the disease,
giving rise to the antibodies in the blood of convalescents. Buchner (2) pre-
pared poisonous bacterial proteins by evaporating emulsions of different bacteria
to dryness, rubbing them up thoroughly in hot water, boiling for an hour in a
reflux condenser, filtering through *Kieselguhr*, and condensing. The product
thus obtained produced pus when placed in tubes under the skin of animals,
fever and apathy in dogs, and very severe local and general reactions in tuber-
culous animals. Antigenic substances were prepared by Brieger (3) from three
to four day cultures of typhoid bacilli by saturation with ammonium sulphate,
allowing the mixture to stand in a dark place for from one to four days, collect-
ing the precipitate and shaking it in water for several hours, and then passing
the water through a Berkefeld filter. The final filtrate gave Millon's reaction,
and produced agglutinins and precipitins in treated animals. Krehl and Matthes
(4) in their work on the production of fever in animals by different protein
fractions found that the deutoalbumose fraction from various bacterial pro-
teins was the only portion which had any influence upon the temperature. These authors found that the deuteralbumoses from fibrin, casein, muscle, egg albumen, etc., had the same properties as those from bacteria. Vaughan and his co-workers (5) washed bacterial bodies with alcohol, extracted them with ether, and pulverized them by thorough rubbing up in agate mortars. Bacteria thus prepared were boiled in a reflux condenser for an hour or more with several volumes of 2 per cent. sodium hydroxide in absolute alcohol. This procedure gave them a toxic and non-toxic portion; the former soluble and the latter insoluble in absolute alcohol. The toxic portion was collected by evaporating the alcohol at a low temperature. It was largely precipitated by saturation with ammonium sulphate, and gave all the ordinary protein reactions, except that of Molisch. It was termed an alcohol soluble albumose. The lethal dose for guinea pigs varied from 8 to 60 mg. when given intraperitoneally. The animals suffered a slight initial rise and a subsequent greater fall of temperature, and became comatose some time before death. By this procedure toxic substances were obtained from pathogenic and non-pathogenic bacteria and proteins from other sources. The toxicity of a product was largely independent of the source of the protein from which it was derived, all producing practically the same symptoms and giving the same chemical reactions. Many other investigators, among whom are Macfayden (6), Besredka (7), Chantemesse (8), Sirotinin (9), and others have done more or less extensive work on the toxic substances of typhoid bacteria.

In regard to the symptoms and pathological changes noted in the experiments to be described later, it is necessary to state that these same conditions have been observed by Pearce and Eisenbrey (10) in a study of anaphylaxis in dogs. These authors used foreign serum to produce the anaphylactic state, and the symptoms and pathology as described by them are in every detail similar to the conditions observed in the present experiments. Schittenhelm and Weichardt (11) produced the same symptoms and pathological conditions in dogs by first injecting peptone or bacterial emulsions and by injecting egg albumen into dogs previously sensitized with the same substance. Rosenow and Arkin (12) obtained similar results by giving dogs intravenous injections of pneumococcic extracts. These authors observed that the lungs of the injected animals were especially affected, being hyperemic and hemorrhagic.

In considering the entire literature on this subject, one finds, with few exceptions, that the selection of the various methods used to obtain toxic substances from typhoid bacteria has been governed by the idea that a toxic body is stored in the innermost parts of the bacterial bodies, and sufficient mechanical division or pressure should liberate such a body. Even where digestion experiments were employed, the idea prevailed that such processes would break down the containing structures and thus liberate the stored up toxic body. Some workers, however, have believed that at least part of the toxic symptoms of natural typhoidal infections are due to split products of the native bacterial proteins, the hydrolysis of the proteins being caused by the action of ferments of the infected animal. Vaughan and his students have strongly advocated this idea.
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In the present work the relation of the toxic substances obtained from *Bacillus typhosus* to the native bacterial proteins has been especially considered. Do these substances exist preformed in the bacteria, or are they formed by a cleavage of the protein constituents of the bacterial bodies? Can typhoid toxic products be isolated in any degree of chemical purity? And if processes more closely simulating those which operate under natural conditions are used to liberate these toxins, can antigenic agents be obtained that will produce clinically successful curative sera?

**EXPERIMENTAL PART.**

It has been observed by Schittenhelm and Weichardt that if certain quantities of typhoid bacteria are injected intravenously into dogs a definite set of symptoms develop a short time after the injection. The autopsy findings are also characteristic. The symptoms consist principally of an initial rise and subsequent fall of body temperature, vomiting, bloody diarrhea, coma, and finally death of the animal. At autopsy the abdominal organs are found to be greatly congested, the intestines being chiefly involved.

In order to observe the results of the intravenous injection of large quantities of typhoid cultures into dogs, to determine the effects of boiling the bacterial emulsion previous to injection, and to see if a solution of the bacteria by the addition of an alkali would in any way modify the results, the following three experiments were carried out.

*Preparation of Bacterial Emulsions.*—The bacteria used in the experiments were obtained by growing a typical strain of *Bacillus typhosus* on plain agar for twenty-four hours, washing the bacteria with physiological salt solution, and centrifuging and washing them a second time. From the washed bacteria emulsions of approximately two and one half billions per c.c. were made in physiological salt solution. Such an emulsion will be referred to as a standard bacterial emulsion.

*Experiment 1.*—2 c.c. of a standard emulsion of typhoid bacteria (five billion bacteria) were injected intravenously into a dog weighing ten pounds. Twenty minutes after the injection the animal became very sick and manifested, successively, vomiting, severe diarrhea which soon became exceedingly bloody.

*Experiment 2.*—We wish to acknowledge our obligations to Dr. A. Goldfine for preparing a large proportion of the bacterial emulsions used in the experiments.

All injections were given intravenously, and dogs were used as the experimental animals. The individual experiments were repeated many times with slight variations.
signs of exhaustion, labored breathing, stupor, and death. The animals died from two to six hours after the injection.

Autopsy.—A small amount of bloody fluid is found in the peritoneal cavity, the mesenteric vessels are hyperemic, the visceral peritoneum is rough and dull, subserosal hemorrhages are scattered over the small intestine, the kidneys are dark and congested, and the lumen of the small intestine contains much blood.

Experiment 2.—A standard bacterial emulsion was boiled for ten minutes over a free flame previous to injection. Otherwise the technique was the same as that of experiment 1. The symptoms and autopsy findings were not distinguishable from those obtained in the first experiment.

Experiment 3.—The bacteria of a standard emulsion were put into solution by the addition of 10 per cent. sodium hydroxide, neutralized with hydrochloric acid, and injected. The results were the same as those in experiments 1 and 2.

The results of the experiments show that a definite amount of typhoid bacterial bodies produces characteristic results when injected intravenously into a dog, that the effect of the injection is not appreciably modified by boiling the bacterial emulsion before the injection, and that a complete solution of the bacterial bodies with an alkali does not destroy the toxic properties. Therefore, it is evident that the toxic substance with which we are here dealing is bound up in the bacteria, not being removed by repeated washings, and, further, that we are apparently not dealing with a secretory toxin of any kind since such toxins have been found to be more or less highly thermolabile.

This evidence that the toxic substance that causes the symptoms and pathological conditions described in the above experiments is not a soluble, secretory toxin of the nature of diphtheria or tetanus toxin, and that its association with the bacterial bodies is an intimate one, led us to investigate the exact nature of the relation between the bacterial bodies and the toxic substance in question. As already stated, the idea prevails that a toxic body is stored in the structure of the bacterial bodies. To determine the relation of this toxic body to the coagulable proteins of freshly washed whole bacteria and bacteria put into solution by sodium hydroxide, experiments 4 and 5 were performed.

Experiment 4.—One or two drops of 1 per cent. alcoholic solution of phenolphthalein were added to about 100 c.c. of a standard bacterial emulsion and the mixture made definitely alkaline with 10 per cent. sodium hydroxide. After bringing this to the boiling point over a free flame, 5 per cent. acetic acid in 10 per cent. sodium chloride was added until no more precipitate formed.
The boiling was continued for a few minutes and the mixture was filtered through kaolin while hot and then passed through a Berkefeld filter. The filtrate was neutralized with sodium hydroxide and allowed to stand for several hours to permit the precipitation of any acid albuminates which might have been formed by the presence of the acetic acid used in the precipitation. The neutral solution was again passed through a Berkefeld filter. Intravenous injections of from 10 c.c. to 15 c.c. of this filtrate were without toxic effects upon the injected animals.

Experiment 5.—The bacteria of a standard emulsion were put into solution by adding sodium hydroxide and then precipitated and filtered as in the preceding experiment. The results of injection of this filtrate into dogs were the same as those of experiment 4.

The experiments show that a removal of the coagulable proteins from a young, freshly washed bacterial emulsion gives a comparatively non-toxic filtrate. The same results obtain even after a complete solution of the bacterial bodies before the removal of the coagulable constituents. Hence the toxic substance is either derived from the precipitable proteins or its association with them is such that it is mechanically carried down with the precipitate.

The mechanical and chemical methods used by many investigators to obtain toxic substances from typhoid bacteria are certainly different from any processes that could possibly occur in natural cases of the disease. This may explain, in part, the negative results of the active and passive immunization experiments with derivatives of these bacteria. In order to obtain a toxic product by processes which are more closely related to those that occur in nature, the following experiments were carried out.

Experiment 6. Leucocytic Ferment.—Inflammatory pus containing a high percentage of polymorphonuclear leucocytes was received from the operating rooms of the hospital immediately after removal from patients. After three washings with physiological salt solution, the cells were washed with alcohol and dried with ether. Thus prepared, the ferment can be kept in stock for several months without material deterioration. The ferment was prepared for use by extracting definite amounts of the dried cells with a weak sodium carbonate solution and centrifuging off the undissolved portion. The clear, supernatant fluid was used as the digesting agent, and a solution 0.3 c.c. of which would digest 2 c.c. of a 0.1 per cent. casein solution beyond the coagulable stage was termed a standard ferment solution. Jobling and Strouse (13) found that a leucocytic ferment prepared in this way contained no eretic element and, therefore, left the zymolyte largely in the proteose stage.

To 25 c.c. of a standard bacterial emulsion were added 5 c.c. of a standard ferment solution. About 2 c.c. of toluol or chloroform were added as a pre-
servative, and the mixture was kept at 37° C. for five days. The digested material was now freed of coagulable proteins and acid albuminates, as described in previous experiments. The filtrate of this digested mixture was fatal to dogs in amounts varying from 1 to 2 c.c. The animals died from two to six hours after injection. The symptoms and pathological conditions were identical with those obtained by using the whole bacteria.

Experiment 7.—A bacterial emulsion was dissolved with 10 per cent. sodium hydroxide, neutralized with hydrochloric acid, and then digested, like the emulsion in the preceding experiment. After the coagulable proteins and acid albuminates were removed, the filtrate was as toxic as that obtained in experiment 6.

Experiments 6 and 7 show that the toxic substance of the bacterial bodies is not removed by an acid and heat precipitation after the emulsion has been exposed to the action of leucoprotease. Previous experiments showed that precipitation of the coagulable proteins removes the toxic substance from a fresh emulsion or from an emulsion put into solution with an alkali. Two possibilities confront us here: (1) a toxic body was liberated by the destruction of a containing structure, or (2) a toxic substance was formed from the coagulable proteins by a hydrolysis effected by the leucoprotease. The results of experiments yet to be described make the latter possibility the more plausible one.

To get a more accurate knowledge of the rapidity of the formation of the non-coagulable toxic substance, to eliminate the possibility of the mere presence of the ferment being responsible for the toxicity of the filtrate of the digested mixtures, and to determine whether continued digestion would destroy the toxic properties of a mixture, the following experiment was carried out.

Experiment 8.—A large quantity of a digestive mixture of bacterial emulsion and ferment was prepared. The emulsion and ferment solution were in the same proportion as those of the previous digestion experiments. A test specimen was removed immediately after the mixture was prepared, the coagulable proteins and acid albuminates were removed in the usual way, and the filtrate was tested for toxicity. Only slight symptoms were manifested in animals receiving from 10 c.c. to 15 c.c. of the filtrate. The mixture was kept at 37° C., and a specimen removed and tested at forty-eight hour intervals. The specimen removed after forty-eight hours was much more toxic than the first one; 10 to 12 c.c. being the lethal dose. Each succeeding preparation was more toxic until the fifth. From 1 to 3 c.c. of the four day specimen killed the animals injected. The toxicity gradually diminished, the twenty day test specimen being only slightly toxic.

These results show that the mere presence of the ferment solution is not responsible for the toxicity of the filtrate, that interaction of
the ferment and bacterial substance is necessary to change the toxic substance from a coagulable to a non-coagulable condition, and that continued digestion destroys the toxic substance.

To sum up: Freshly washed, unheated typhoid bacilli intravenously injected into dogs cause the development of definite symptoms as early as twenty minutes after the injection. Boiling for ten minutes does not destroy the toxic effects of a freshly washed bacterial emulsion. Complete solution of the bacteria of a fresh emulsion does not prevent the removal of the toxic substance with the coagulable proteins. The action of leucoprotease splits the toxic substance to a non-coagulable state, the digested mixtures being toxic after removing the coagulable portion. The mere presence of the leucocytic ferment is not responsible for the toxicity of the filtrate from the digested mixture, and continued digestion destroys the toxicity of a previous toxic mixture.

From these observations it is concluded that the toxic properties of freshly washed typhoid bacteria are not entirely due to pre-formed secretory toxic bodies that are stored in the bacterial bodies, but that these properties are due largely to products formed by hydration of the bacterial proteins through the agency of ferments present in the circulation of the animal previous to the injection, or which become mobile subsequent to the entrance of the foreign bodies into the blood stream. Since leucocytic ferments can attack the bacterial proteins in vitro, it is possible that the leucocytes are a source of the ferments which are active in experimental and natural cases of intoxication with the whole bacteria.

Having determined that the toxic substances of the digested bacterial emulsions are no longer precipitated with heat and acid, but remain in the coagulable protein-free filtrate, we decided to determine more definitely the chemical nature of the toxic substances. To this end the following procedures were adopted.

Large quantities of standard bacterial emulsions were digested with leucoprotease, as in the previous digestion experiments. These digested mixtures were freed of coagulable proteins and acid albuminates in the usual way. The lethal dose of the digested bacterial filtrates for dogs was then determined by intravenous injection. The remaining portion of the toxic filtrate was saturated with neu-
tral, recrystallized ammonium sulphate and allowed to stand until the precipitate thus obtained had collected into coagula. The precipitate was then collected by centrifuging at high speed, dissolved in water and precipitated, and collected a second time. This sediment was termed the whole proteose fraction. After removing as much of the adhering sulphate solution as possible, it was dissolved in water and tested for toxicity by the usual method. The whole proteose fraction was found to be very toxic. It was not possible to compare in a strict quantitative way the toxicity of the proteose fraction with that of the filtrate from which it was derived, since an unavoidable loss always occurred in the precipitation and collection. However, in several instances a lethal dose of whole proteose was obtained from less than two lethal doses of bacterial filtrate.

In precipitating and collecting the proteoses all the ammonium sulphate was not removed by the centrifuging previous to its solution in water and a small amount was, therefore, injected into the circulation of the animals. To eliminate the possibility of the toxicity of the proteoses being increased by the presence of the sulphate, ten times the largest possible amount present was given to dogs intravenously. No noticeable symptoms followed the injections. Thus dialysis or other procedures to remove all traces of the sulphate before injecting the proteose solution were rendered unnecessary.

The supernatant fluid from the first saturation was also tested for toxicity after the sulphate was removed. It was first passed through a Berkefeld filter to remove all traces of precipitate. The sulphate was then precipitated with barium hydroxide, and any excess of the barium was removed with carbon dioxide. The filtrate was kept at incubator temperature for several hours to evaporate the ammonia that had been liberated by the action of the barium hydroxide upon the sulphate. Large quantities of this salt-free solution were injected into dogs without toxic effects.

Further fractionation of the toxic product was attempted. Solutions of the whole proteose fraction prepared as already described were treated with an equal volume of a saturated ammonium sulphate solution. The precipitate was removed as usual, dissolved in water, and precipitated a second time. The supernatant fluid from the first precipitation was saturated with ammonium sulphate crystals
and the precipitate removed. Both sediments were now dissolved in water and tested for toxicity. It was found that the primary proteoses (half saturation precipitate) possessed a toxicity almost equal to that of the whole proteose solution from which it was derived, the secondary proteoses (saturation precipitate) being relatively non-toxic. More exact chemical methods would probably give a complete separation of the proteoses into a toxic and non-toxic fraction.

GENERAL DISCUSSION.

The severity and regularity of the intestinal lesions produced by intravenous injections of typhoid bacteria, or toxic products derived from them, incline one to believe that a special affinity exists between these substances and the intestinal tissues, especially the lymphoid tissue. In fact many investigators of this subject have considered these lesions to be absolutely specific. In a recent publication Arima (14) describes in some detail the symptoms and pathological lesions produced by injecting the toxic products of typhoid bacteria into rabbits, and he concluded that these results are due to a specific chemical affinity between the toxin and the tissues affected. The intestinal symptoms and pathology produced by Arima’s typhoid toxins are similar to Schittenhelm and Weichardt’s enteritis anaphylactica and can be produced by injecting sensitized rabbits or dogs with a toxic dose of the sensitizing protein or by injecting them with a first dose of typhoid, colon, or other bacteria, and are, therefore, not peculiar to typhoid bacteria or toxins.

At first we were inclined to believe that the swollen, hyperemic, hemorrhagic condition of the Peyer’s patches and mesenteric lymph glands was due to a specific action of the toxic filtrate from the digested typhoid bacteria. Further experiments showed that the same condition could be produced by injecting a similar filtrate from Bacillus coli, meningococci, or Staphylococcus aureus. In these experiments the acute action of non-specific toxic proteins overshadows the action of any specific toxin that may be present, and the pathological picture is a non-specific one. Hence it is evident that lesions peculiar to the toxic product used in these experiments and resembling those of natural typhoidal infections cannot be pro-
duced by an acute intoxication of the experimental animals. It is possible, however, that a chronic treatment would give different results.

As shown by Pearce and Eisenbrey (10) and Schittenhelm and Weichardt (11), anaphylaxis in dogs is characterized by a fall of the blood pressure, vomiting, and bloody diarrhea. It is noteworthy that these symptoms are also produced by a single intravenous injection of freshly washed emulsions of typhoid, colon, and other bacilli. If the coagulable nitrogen is removed from these fresh emulsions, the filtrate is without effect. On the other hand, the filtrate from a digested emulsion produces the same results as the whole bacteria. Hence the coagulable proteins, or some derivative of them, are necessary to produce the anaphylactic results. Remembering, also, that a single injection of egg albumen, casein, etc. does not produce anaphylactic symptoms, and that Abderhalden (15) has shown that the sera of animals sensitized with native proteins possess proteoclastic ferments not found in the sera of normal animals, some interesting points are suggested. If the sensitizing dose of egg albumen, etc. acts by calling forth a ferment capable of attacking the native protein, the protein constituents of bacterial bodies are attacked by ferments incapable of acting upon the proteins ordinarily used in animal experimentation, since a sensitizing dose is not required. This biological difference between bacterial and other proteins may be due to the action of antiferments or to a denaturation of the proteins by the methods used in their isolation and purification. It is conceivable that the stability of the molecules of living, growing protoplasm is quite different from that of protein substances which have gone through the process of precipitation, coagulation, or dehydration. The influence of such physical or chemical states upon the parenteral introduction of proteins into animals and upon the toxic symptoms of general infections deserves investigation.

The classification of the toxic substances derived from typhoid bacilli and the particular part that each plays in natural typhoidal infections remain unsettled questions. The preponderance of opinion seems to be in favor of a true extracellular toxin to which, under proper conditions, an antitoxin is produced, and a so called endo-
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The part played by these toxins individually in the production of the general symptoms and the local and general pathological conditions has never been positively determined. The thermostability of the toxic substance obtained by us does not permit its being classed with the extracellular secretory toxins. Pfeiffer's endotoxin was highly thermolabile, and all bacterial endotoxins are considered to be more or less thermolabile, being destroyed at 70°C. and below. As is shown by the experimental procedures we have described, the toxic substance obtained by digesting typhoid bacilli with leucoprotease is not destroyed by a heat and acid precipitation of the coagulable proteins, and withstands boiling for ten minutes and longer. Hence its relation to the toxic substances described as extra- and intracellular toxins cannot be decided without further experimentation.

In a consideration of the part this toxic split product plays in natural cases of the disease, a field for speculation arises.³ The toxic substances of a fresh emulsion of the bacteria being removed by precipitating the coagulable proteins and not being thus removed from a digested emulsion, they are apparently formed by a hydrolysis of the native proteins. Moreover, this split product produces the same symptoms and pathology as the whole bacteria, and a further digestion destroys its toxicity. Moreover, the primary proteoses possess a large part of the toxicity of a digested mixture, an extensive cleavage not being necessary to reach the toxic stage. This is in harmony with the rapidity of the development of toxic substances following an intravenous injection of the bacteria and proves the presence of ferments capable of causing the first cleavage. The severe toxic effects that follow are evidence of an accumulation of these toxic compounds and prove the absence of agents capable of causing a rapid cleavage to non-toxic combinations. If these inferences are correct, it may be possible to produce this lacking ferment by active and passive immunizations. Experiments to test this and other points concerning the immunization with bacterial split products are now under way.

³ The fact that typhoid fever is a septicemia justifies still more our drawing inferences from the intravenous injection of the bacteria.
SUMMARY.

1. A single intravenous injection into dogs of a sufficient number of freshly washed typhoid bacteria produces the symptoms and pathology that characterize anaphylaxis in these animals.

2. These effects are not produced by the coagulable protein-free filtrate from a fresh emulsion, while a similar filtrate from an emulsion digested with leucoprotease is very potent, the toxic portion of the bacterial bodies being changed from a coagulable to a non-coagulable state.

3. The symptoms and pathology described are not specific, since they can be produced by substances other than typhoid toxins.

4. Digestion with leucoprotease furnishes a method of liberating toxic substances from typhoid bacteria resembling the processes of nature more closely than the methods heretofore used.

5. The toxic substances thus liberated are not destroyed by a heat and acid precipitation of the coagulable proteins, and are of the nature of primary proteoses.

BIBLIOGRAPHY.