# THE MECHANISM OF ANAPHYLACTIC SHOCK.

## STUDIES ON FERMENT ACTION. XXIII.

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Despite the intensive study which the phenomena of anaphylaxis have received, especially by immunologists, with considerable theorizing about the probability of protein splitting, and the source and the mechanism of production of the intoxicating agent, there have been published few experiments dealing with the actual ferment changes and the amounts of protein split products in the serum during shock.

Abderhalden and Pincussohn and their various coworkers (1) noted by means of the polariscope an increase in the power of the serum of sensitized animals to split the specific antigen. Pfeiffer and Mita (2) worked along similar lines. A summary of their work, together with detailed experiments carried out by means of the dialysis method, is found in a paper of Pfeiffer and Jarisch (3).

Auer and Van Slyke (4) have reported experiments in which they determined the amino-acid content of lungs of guinea pigs before and during shock. They found no noteworthy change.

In a recent paper Zunz and György (5) have confirmed the findings of Abderhalden and of Pfeiffer and Mita. Following the primary injection they found a gradual increase in a protease for beef protein, reaching a maximum in about 15 days; this ferment, according to their observations, disappears following shock, only to reappear on a further injection if made from 4 to 10 days after recovery from shock. They definitely determined an increase in amino-acids during acute shock in dogs, this being quite contrary to the supposition of de Waele (6).

In previous papers we have shown that the homologous serum may be toxic for animals under conditions which remove the antiferment and permit serum protease to act (7); that anaphylatoxin formation depends upon the adsorption of the serum antiferment (8); while an increase in antiferment tends to diminish the susceptibility to shock (9). During trypsin shock, which closely resembles anaphylactic shock, we have noted a marked increase in the serum protease and lipase (10).

The present experiments deal with the serum ferments and the protein split products during anaphylactic shock in dogs. The changes that occur are striking and have not, so far as we are aware, been recorded by other workers. We believe that the facts developed confirm the idea of a protein cleavage, in contradistinction to the supposition of a purely physical change, as advanced, for instance, by von Behring (11) and previous workers. But this cleavage concerns the proteins of the animal itself, rather than the antigen.

While these observations have been made on the serum, the deduction that the primary site of the reaction is to be found in the serum is not therefore warranted, for the serum changes may simply express the end results of cellular changes.

### *Technique.*

Dogs have been used exclusively in our experiments, not only because of the larger amounts of serum which are available and which are needed in work of this kind, but because the greater susceptibility of the bronchial musculature of the guinea pig is lacking to a large degree, so that death from asphyxia does not complicate the picture before serum changes become established. The dog, too, probably more closely parallels the human condition. The methods used to determine the ferments and the split products have been fully described in a previous paper (12). The proteoses present in the serum have been determined as follows: 5 cc. of serum are diluted with about 10 cc. of normal saline and 2 cc. of a mixture of 10 per cent acetic acid and 20 per cent salt solution are added. The serum is then boiled for 10 minutes, filtered through hard paper, and the filtrate saturated while boiling with sodium sulphate. The precipitate is collected and after washing with water saturated with sodium sulphate it is dissolved from the filter paper by means of a small amount of water made slightly alkaline with sodium carbonate. The dissolved proteoses are collected directly in the large Jena glass tubes used for oxidizing, and nitrogen determinations are made. The results charted therefore represent the amount of nitrogen present as proteoses in 5 cc. of serum. The

majority of the determinations made to determine the amount of non-coagulable nitrogen in the serum, together with the amount of protease activity, have been made in duplicate.

Purified horse serum albumin has been used as an antigen. This preparation, containing antiferment, shows considerable resistance to tryptic digestion. Injections have been intravenous; blood samples have been obtained from ear veins; during shock recourse to a suction pump has been found necessary to obtain blood in sufficient quantity.

### *The Effect of Primary Injection and Sensitization.*

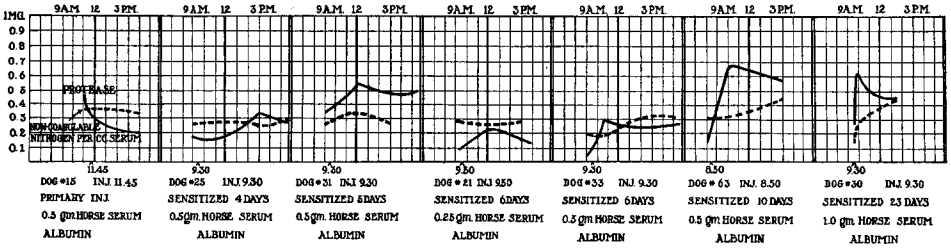
The first experiments illustrated the changes that occur in the serum following the first or sensitizing dose.

*Dog 25.*—Weight 8 kilos. Sensitized Feb. 27, 1915, and Mar. 2, 1915, with subcutaneous injections of horse serum albumin. On Mar. 3, 1915, at 9.30 a. m. 0.5 gm. of horse serum albumin was injected intravenously. There resulted an immediate rise in temperature to 106° F. at 11.10 a. m., together with an immediate leucopenia. The antiferment index was practically unaltered, as was also the amount of non-coagulable nitrogen in the serum. The serum protease showed no change until the afternoon, when a moderate increase was noted. The serum lipase increased during the same time. Blood drawn the following morning showed a slight increase in antiferment, a decrease in protease and lipase, and a slight increase in non-coagulable nitrogen.

*Dog 21.*—Weight 4 kilos. Sensitized in the same manner as Dog 25. 0.5 gm. horse serum albumin injected at 9.50 a. m., Mar. 5, 1915. The results are similar to those of the first animal, except that the rise in protease was noted a little sooner than in the previous dog. The non-coagulable nitrogen showed a considerable increase after 24 hours.

Inasmuch as the interest in anaphylaxis centers chiefly about the protease and the amount of split products, we have collected the results of a series of experiments dealing with the injections of the antigen at various intervals after sensitization in Text-fig. 1. From this it will be noted that there is a progressive decrease in the time interval of the mobilization of the protease in the serum. Thus in Dog 15, a single injection in a non-sensitized dog has resulted in a decrease in protease, and practically no change in the non-coagulable nitrogen. The dogs which received injections of the antigen from four to ten days after sensitization (Nos. 25, 31, 21, 33, and 63) showed an increase in protease at an interval after the in-

jection which becomes progressively less, while a rise in the non-coagulable nitrogen in the serum becomes evident in those animals which are sensitized longest. When the animal finally becomes fully sensitized the ferment mobilization is immediate, as is also the increase in non-coagulable nitrogen.




TEXT-FIG. 1. Mobilization of serum protease during the course of sensitization.

It should be emphasized that the ferment which we demonstrate is not specific, for when the serum is emulsified with chloroform and incubated, the ferment digests the serum proteins. The only evidence of specificity lies in the fact that the ferment is mobilized by the injection of the specific antigen.

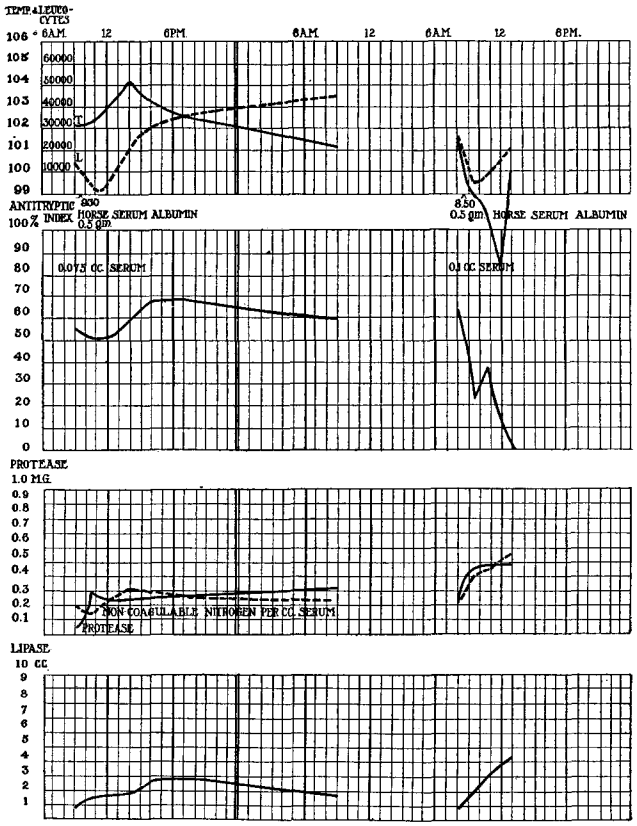
### *The Effect of Acute Anaphylactic Shock.*

The following experiments serve to illustrate the effect of the reinjection of the antigen after complete sensitization.

*Dog 15.*—Weight 4.5 kilos. Sensitized Feb. 9, 1915, with 1.0 gm. of horse serum albumin intravenously. The primary injection resulted in the usual rise in temperature and fall in leucocytes. The antiferment, which had a relatively high titer, fell slightly, then made a gradual recovery. The non-coagulable nitrogen showed practically no change, while there was a decided fall in the protease content. The lipase showed a gradual increase from 1.5 to 5 cc. N/100 sodium hydrate after 24 hours. When we now contrast this picture with that following the injection after sensitization, there has been noted a much greater fall in the antiferment titer, an immediate rise in non-coagulable nitrogen (from 0.27 to 0.45 mg.), together with a large increase in serum protease (from 0.47 to 0.87 mg.). The lipase increased from 0.8 to 6.8 cc. The animal died 4 hours after the injection.

*Dog 33.*—Weight 9 kilos. Sensitized, beginning Mar. 8, 1915, with three subcutaneous injections of horse serum albumin. The effect of the primary in-

travenous injection before complete sensitization is shown in Text-fig. 2. The injection (0.5 gm. of horse serum albumin) was made at 9 a. m., Mar. 15, 1915 (7 days after the first sensitizing dose). As a result there was noted a distinct rise in the protease, a slight increase in the lipase in the afternoon; a slight fall,



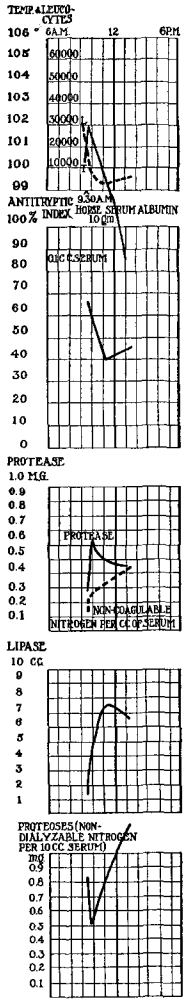

TEXT-FIG. 2. TEXT-FIG. 2 a.

TEXT-FIGS. 2 AND 2 a. Serum changes following antigen injection before and after complete sensitization.

followed by a rise in the antiferment titer; the non-coagulable nitrogen first showed a slight fall followed by a rise in the afternoon. The intoxicating injection was made Mar. 29, 1915, at 8.50 a. m. (0.5 gm.) (Text-Fig. 2 a). As a result there was a typical fall in the antiferment titer, and an immediate increase in the protease, lipase, and non-coagulable nitrogen. The animal died at 1.30 p. m. Of the total amount of the non-coagulable nitrogen in the blood serum at this time 33 per cent represented nitrogen present in the form of proteoses. It is possible that some of these proteoses may be toxic.

*Dog 30.*—Weight 7.7 kilos. Sensitized Mar. 7, 1915. Reinjected at 9.30 a. m.,

Mar. 30, 1915, with 1.0 gm. horse serum albumin. Died at 1.30 p. m. (Text-fig. 3). In this experiment a larger amount of antigen was injected for the intoxicating dose than in the two previous animals. The ferment picture is typical



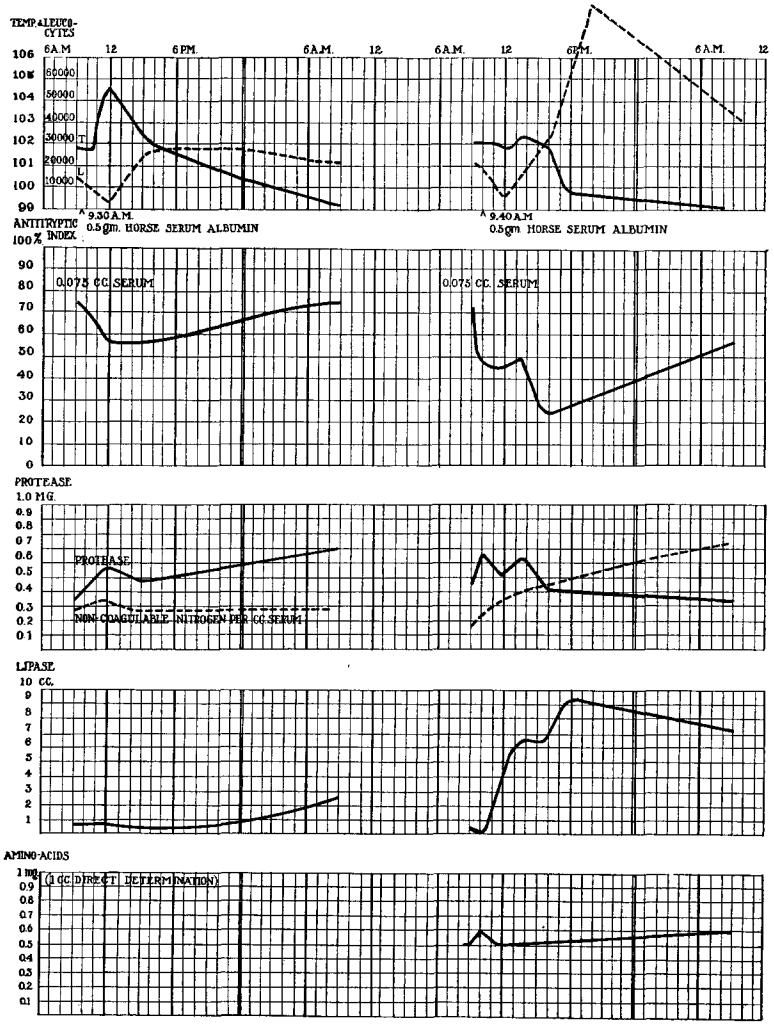

TEXT-FIG. 3. Serum changes during acute anaphylactic shock.

and similar to the other experiments. In this animal the amount of proteoses present in the serum was determined by dialyzing the filtrate from 10 cc. of serum after the coagulable nitrogen had been removed by acid and heat. Nitrogen determinations were then made on the undialyzable portion. This represents

the higher split products (proteoses). As will be observed from Text-fig. 3, there resulted an almost immediate drop in the amount present in the serum with an increase following later.

*Protracted Shock.*

The following experiments illustrate the conditions when the animal does not die within a few hours, but lives over a period of about twenty-four hours following the second injection.




TEXT-FIG. 4. TEXT-FIG. 4 a.

TEXT-FIGS. 4 AND 4 a. Serum changes during protracted anaphylactic shock.

*Dog 31.*—Weight 11 kilos. Sensitized Mar. 7, 1915. First intravenous injection at 9.30 a. m., Mar. 12, 1915 (Text-fig. 4). On Mar. 30, 1915, at 9.40 the second intravenous injection was made. The dog showed the usual malaise and prostration, but apparently recovered somewhat in the afternoon. The animal died at 8.30 the following morning. As will be observed from Text-fig. 4 a there was noted a marked leucocytosis (97,000) in the evening. The antiferment showed the usual fall with a recovery the next morning. The non-coagulable nitrogen increased from 0.17 mg. per cc. to 0.75 mg. the following morning. The protease showed an immediate increase with some fluctuations following, and a gradual decline. The lipase increased to a marked extent. The amino-acids (Van Slyke method) increased immediately after the injection, then fell to the original figure, and increased the following morning. Whatever may have been the factor which delayed exitus in this dog, it seems to have had some influence on the ferments and the antiferments, and was manifested possibly first in the fall in protease at noon. It will be observed that all the curves in the experiment are broken and in so far differ from the normal course of events during shock.

### *The Effect of Reinjection During the Refractory Stage.*

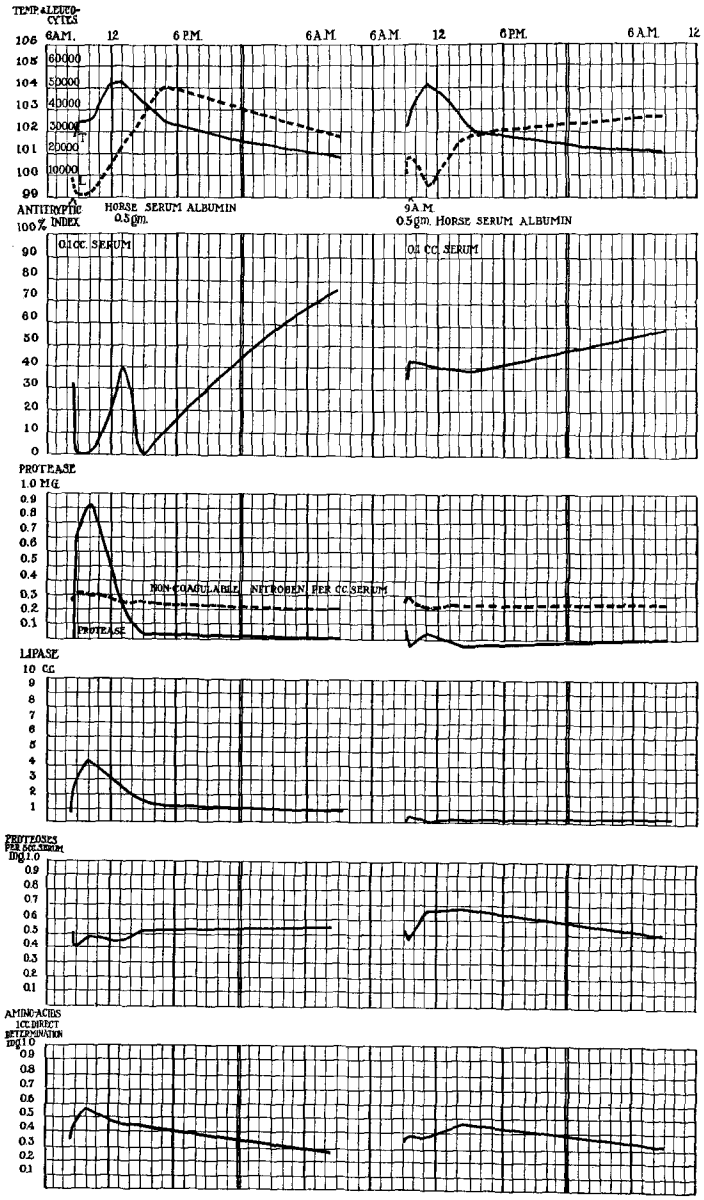
In the following experiment the effect of a single shock in the sensitized animal, followed by a second injection after two days, has been studied.

*Dog 65.*—Weight 6.2 kilos. Sensitized by an intravenous injection, Apr. 2, 1915. On Apr. 20, 1915, at 8.37 a. m., 0.5 gm. of horse serum albumin was injected, which dose was sufficient to cause a profound intoxication, but not death. The animal was bled at 8.50 and 10.00 a. m., 1, and 3 p. m., and the following morning. The resulting serum changes are quite typical (Text-fig. 5). The antiferment showed the usual immediate drop, with a following recovery and second drop. The non-coagulable nitrogen showed only a slight increase and remained low, thus differing from the other animals so far studied. The rise in protease was instantaneous and very marked, fell rather rapidly, and then remained low. The lipase showed the usual rise, but not to such a marked extent. The serum proteoses showed the immediate diminution which is characteristic, while the amino-acids increased quite markedly.

On reinjection two days later (9 a. m., Apr. 22, 1915) it will be noted (Text-fig. 5 a that the change in antiferment was absent. The protease showed some decrease and remained low, as did the lipase. The proteoses showed a slight decrease after 15 minutes with a distinct increase later. The amino-acid increased gradually and returned to normal the following morning.

### *Shock in the Pregnant Animal.*

During pregnancy animals are known to show a relative immunity to anaphylactic shock; the mechanism of the resistance is not understood. It is possible that the increase in antiferment which usually accompanies pregnancy might in a measure account for this




TEXT-FIG. 5. TEXT-FIG. 5 a.

TEXT-FIGS. 5 AND 5 a. Serum changes during acute anaphylactic shock and following antigen injection during the refractory period.

resistance, since a certain amount of resistance can be imparted to an animal by increasing its antiferment, as we have shown previously (9).

The following experiment was made on a pregnant animal.

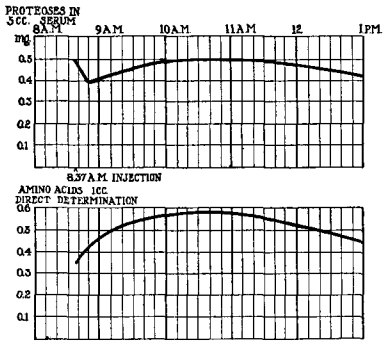
*Dog 56.*—Weight 6 kilos. Sensitized by an intravenous injection of horse serum albumin Apr. 2, 1915. A second injection, made three days later, resulted in no marked alterations with the exception of a rather sharp drop in the afternoon in the amount of proteoses contained in the serum. The intoxicating dose was made at 8.30 a. m., Apr. 22, blood being collected at 8.45 and 9.30 a. m., 12, and 3 p. m. The animal was somewhat nauseated and vomited several times, but remained in fair condition until the afternoon; it died suddenly at 4.30 p. m. In the first place it was observed that the antiferment instead of being high was quite low (0.15 cc. of serum inhibiting only about 10 per cent of the digestion). The protease, however, rose sharply, as in the other experiments, although there was no increase in non-coagulable nitrogen until towards noon. The lipase curve was very irregular, but continued to rise. The proteoses were practically unaltered for 15 minutes, later increased, decreased toward noon, and increased markedly toward the terminal stage. The amino-acids at first declined progressively, but increased during the afternoon.

*The Relation of Serum Proteoses and Amino-Acids to Shock.*

When the antigen is injected into the circulation of the sensitized animal proteolysis takes place. This fact has been definitely established through the work of Zunz and György in demonstrating an increase in amino-acids by the Van Slyke method during shock. The various experiments which we have detailed have also illustrated this phase, inasmuch as an increase in the non-coagulable nitrogen is an almost invariable accompaniment of the acute shock. The question arises whether Zunz and György and the other workers who have advanced the idea of specific protease action are justified in assuming that the increase in amino-acids proves that the specific antigen has been split.

Before the antigen reaches the amino-acid stage it must pass through the various cleavage phases,—proteoses, peptones, etc.,—and we should expect that there would be a preceding or concomitant increase in these split products in the serum. The contrary is true. This is illustrated in Text-fig. 6, in which the amino-acid and protease curves are illustrated immediately after the injection of the antigen at 8.37 a. m. (Dog 65). The immediate rise in

amino nitrogen is apparent, but is accompanied by an actual decrease of the amount of proteoses in the serum. We have repeatedly observed this relation and have never noted an increase in proteoses during the first fifteen minutes following the antigen injection,




TEXT-FIG. 6. Relation of serum proteoses and amino nitrogen during acute anaphylactic shock.

when the anaphylactic symptoms are, of course, already well advanced. The decrease of the proteoses at the instant of the shock, together with an increase in amino-acids, seems to warrant the assumption that the primary cleavage that occurs has its substrate in the proteoses already present in the serum.

### *Non-Specificity of Serum Protease.*

In the preceding paragraph attention has been called to the fact that when the increase in amino-acids occurs immediately after the injection of the antigen into the sensitized animal, there is a simultaneous decrease in the higher split products of the serum, a condition which is contrary to the view that it is the injected antigen that is being split. For if that were true we should expect an increase in the higher split products, produced during the process of cleavage of the antigen to the amino-acid stage. The idea that it is the antigen that is split has seemed in accord with the data at hand up to the present time. Thus Abderhalden and Pincussohn and Pfeiffer and Mita report positive results when the serum of sensitized animals is permitted to act upon the specific antigen *in vitro*.

And the experiments of Zunz and György confirm these findings by means of the increase in amino-acids demonstrable with the Van Slyke apparatus *in vivo* and when the specific serum is permitted to "digest" the antigen *in vitro*. So, too, they also report that immediately following shock this property disappears, affording an attractive explanation for the state of anti-anaphylaxis. This entire hypothesis concerning the mechanism of anaphylactic shock is based, therefore, upon the specificity of the serum proteases. In a previous paper we have shown that there is no element of specificity in so far as the proteases are concerned in the digestion occurring in the Abderhalden reaction (13). The digest is obtained from the serum proteins and is brought about by changes in the colloidal state of dispersion of the serum induced by the antigen introduced as a substrate, resulting in what may be termed local areas of antiferment deficiency, due either to an actual adsorption of antiferment by a formed substance, such as a precipitate, or to a change in the degree of dispersion of the unsaturated lipoids upon which the antiferment property depends, as we have previously demonstrated (14). If a protease is present in such sera a positive reaction can occur; if not, the digestion does not take place.

Since it is well known from various immunological experiments that minimal amounts of the antigen can call forth a specific reaction, as, for instance, in the precipitin test or in complement deviation, we can, by means of dilution of the specific substrate, show that the amount of digestion brought about by the addition of such a specific substrate may be greater by many times than the total amount of nitrogen introduced, thus positively proving that the digestive products are not derived from the specific substrate and can only be formed from the serum itself. Such an experiment is shown as follows: Fresh serum is used from a fully sensitized dog. A 1 per cent horse serum albumin solution, to which the dog has been sensitized, is used as a substrate and varying dilutions to 0.001 per cent are made. If we now add a constant amount of the ferment (the specific serum) to decreasing dilutions of the substrate, we should expect that with minimal dilutions there would not be enough substrate present for the ferment to act upon, and as a result

the amount of digestion products should become smaller. In the following experiment the serum was allowed to "digest" the horse serum albumin solution for twenty hours under toluol (Table I).

TABLE I.

| Tube No. | Serum 56/5. | Horse serum albumin solution. | | Nitrogen available as substrate. | Amino-acids determined in 1 cc. of mixture (direct). | Increase in amino-acids. |
|---|---|---|---|---|---|---|
| | *cc.* | *cc.* | *per cent* | *mg.* | *mg.* | *mg.* |
| 1 | 1 | 1 | 1 | 0.74 | 0.279 | 0.034 |
| 2 | 1 (inactivated) | 1 | 1 | 0.74 | 0.245 | |
| 3 | 1 " | 1 | 0.1 | 0.074 | 0.245 | 0.055 |
| 4 | 1 " | 1 | 0.1 | 0.074 | 0.190 | |
| 5 | 1 | 1 | 0.01 | 0.0074 | 0.256 | 0.078 |
| 6 | 1 " | 1 | 0.01 | 0.0074 | 0.178 | |
| 7 | 1 | 1 | 0.001 | 0.00074 | 0.268 | 0.078 |
| 8 | 1 " | 1 | 0.001 | 0.00074 | 0.190 | |

As will be observed, with an available total nitrogen from the specific substrate of about 0.00074 mg. (Tubes 7 and 8) we obtain a digest of 0.078 mg. of amino-acids per cc. of the mixture. This experiment, which we have repeatedly made, affords absolute proof that the theory of specific protease action is without warrant. The various methods of demonstrating specific protease action all have a common error in that the digestion of the serum proteins and of the higher split products present in the serum (proteoses) by the non-specific serum proteases is ignored. The serum protease should be regarded as a simple ferment, polyvalent in character, and in so far resembling the ordinary tryptic ferment. This is precisely the view that Fermi (15), working with a variety of methods, has reached. Serum ereptase should, however, be strictly distinguished from the protease.

### *The Serum Esterase.*

An invariable accompaniment of the shock has been a marked mobilization of the serum esterase. While the first injection of horse serum albumin may cause some increase in the ferment, it never causes so sudden nor so extensive a rise in the esterase curve. This increase is not specific for anaphylactic shock, but occurs following trypsin and peptone shock, as well as following intoxication by bacterial proteins of varied derivation. This increase prob-

ably represents a mobilization from tissue cells in general rather than the secretion of any one organ.

## DISCUSSION.

In a previous paper (8) we stated:

"The arguments made against the protein-intoxication conception, such as the time element and the minute amount of substance necessary, are not convincing, for we know that ferment action may be very rapid; and as far as the quantity of substrate is concerned the argument fails if we place the matrix of the poison in the serum proteins themselves. There is some reason to believe, however, that if ferment action is the basis of anaphylactic shock, these ferments may have a much wider range of action than merely on the introduced protein."

The facts brought out during the course of this work, have, we believe, established this view as essentially correct. In brief, these results can be stated as follows: The injection of the antigen in the non-sensitized animal is practically without influence on the serum ferments or the split products present in the serum.

During the period following the sensitizing injection and preceding the development of complete sensitization the organism responds to the injected antigen by a progressively increasing rapidity of mobilization of ferments, the amount of protease mobilized becoming greater as the maximum of sensitization is reached. This protease is not specific.[1]

The acute shock is accompanied by an immediate and marked increase in serum protease; by a fall in antiferment; by a rise in the amino-acid content and non-coagulable nitrogen of the serum, together with a primary decrease in the serum proteoses. The esterase rise is constant and progressive. When death occurs after a few hours the serum may contain a large amount of proteoses (33 per cent of the total non-coagulable nitrogen).

Before discussing further the mechanism of the various phenomena we must emphasize the fact that whatever change takes place during the course of the reaction is not due to specific pro-

[1] In a paper which has appeared after the completion of our work Pfeiffer (Pfeiffer, H., *Ztschr. f. Immunitätsforsch., Orig.,* 1915, xxiii, 515) has demonstrated an increase in the peptolytic power of the serum during anaphylactic shock. Because of the extent of the paper it cannot be adequately reviewed at this time.

teases (*Abwehrfermente*). The antigen which we used (purified horse serum albumin) is exceedingly resistant to the ordinary tryptic ferment, a ferment which is not only polyvalent but also very active; and it seems unreasonable that this same protein should be readily attacked by a specific protease when we know that the antiferment, to which the horse serum albumin owes its resistance, inhibits not only the tryptic ferment but also the so called specific ferment. The actual digestion is due to an entirely different mechanism,—to a non-specific ferment acting upon serum proteins. This digestion is brought about through colloidal changes which take place when the antigen is brought into contact with the serum. Either a precipitate is formed which may act as an adsorbing substance for the antiferment, or else the dispersion of the antiferment itself is also altered, so that local areas of antiferment deficiency are formed with a resulting immediate cleavage either from the serum proteins (especially the globulins) or from the proteoses already present in the serum. Bearing these conditions in mind, the mechanism of the production of the toxic substances which produce the acute shock becomes relatively simple, but involves not only the serum, but the cellular elements of the organism as well, and the latter probably primarily.

The mobilization of the protease must be a cellular phenomenon and during the course of sensitization this response is increased not only in intensity, but also in rapidity. This need not result in an intoxication, for the isolated cell, as the various investigators who have studied cellular phenomena in anaphylaxis have shown (Dale, Schultz, Weil, etc.), may be capable of repeated response to specific stimuli; so we must concede that the antigen is not in itself toxic for the cell either before or after sensitization. Associated, however, with the cellular response we find serum changes that do result in an intoxication. There is an immediate fall in the antiferment titer. This may in part be due to a saturation of the antiferment by the mobilized ferment. It is more probably due to a colloidal change with a resulting lessening of the dispersion of the unsaturated lipoids upon which the antiferment property depends. This lessening of the antiferment titer will, of course, facilitate

proteolysis. Furthermore, definite changes in the antibodies of the serum (especially the precipitins) occur at the time of shock. Whether or not an actual change to the final picture which is demonstrable *in vitro* with the precipitin reaction occurs is immaterial. The precipitin content which before the shock is present in the serum disappears instantly when shock is induced (Joachimoglu (16)). There is evident at least a colloidal rearrangement which must result in local areas of antiferment adsorption or diminution in which proteolysis can take place.

This proteolysis is made evident by the immediate increase in amino-acids. The only question relates to the substrate from which the amino-acids are derived. Contained in the serum of normal guinea pigs and dogs one finds a relatively large amount of higher split products (5 cc. of serum containing from 0.3 to 0.5 mg. of nitrogen in this form). Immediately after shock these split products are diminished in amount, but later increase progressively until death takes place. It is quite evident that if we were dealing with a shock primarily due to protein split products derived from the introduced antigen we should expect to find an immediate increase in the higher split products derived from that antigen.

Briefly, then, the mechanism of acute intoxication may be stated as follows: An immediate mobilization of a non-specific protease in large amounts; the formation of a state of antiferment deficiency through colloidal changes; a simultaneous cleavage of serum proteins (proteoses) through the peptone stage to amino-acids; an intoxication by these peptones with a resulting cellular injury, made evident by an increase in serum lipase, the fall in temperature, and other manifestations of acute shock. The elements of specificity lie in the mobilization of the non-specific cellular ferment and the colloidal changes in the serum, not in the production of specific ferments.

In a later paper we shall discuss more fully the mechanism of anti-anaphylaxis and other manifestations of protein intoxication.

## CONCLUSIONS.

1. The serum ferments are practically unaltered by a primary injection of foreign protein.

2. During the course of sensitization the injection of the antigen is followed by the mobilization of a non-specific protease which increases in rapidity and intensity as the maximum period of sensitization is reached.

3. Acute shock is accompanied by:

(a) The instantaneous mobilization of a large amount of non-specific protease; (b) a decrease in antiferment; (c) an increase in non-coagulable nitrogen of the serum; (d) an increase in amino-acids; (e) a primary decrease in serum proteoses.

4. Later there is a progressive increase in the non-coagulable nitrogen, in proteoses, and in serum lipase.

5. The acute intoxication is brought about by the cleavage of serum proteins (and proteoses) through the peptone stage by a non-specific protease.

6. The specific elements lie in the rapid mobilization of this ferment and the colloidal serum changes which bring about the change in antiferment titer.

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