SERUM FERMENTS AND ANTIFERMENT DURING TRYPSIN SHOCK.

STUDIES ON FERMENT ACTION. XXII.

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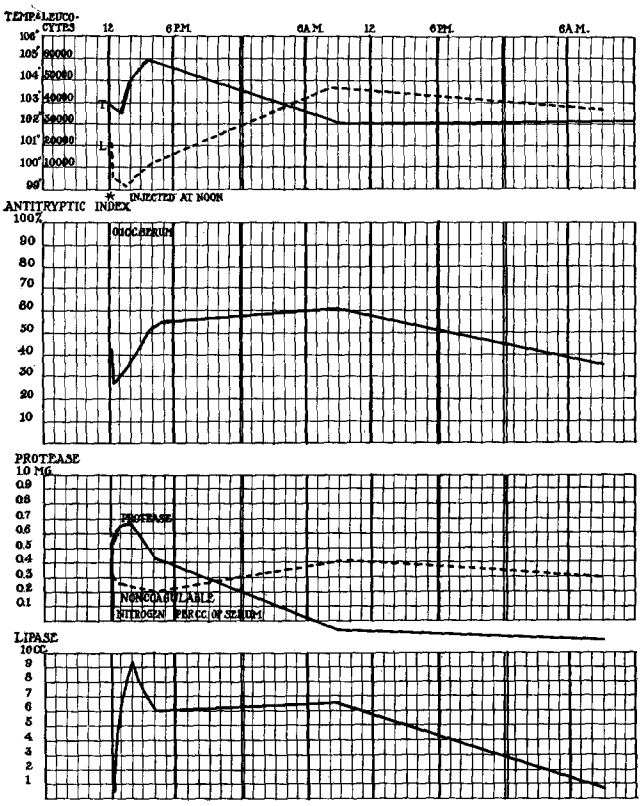
In an extended series of papers Kirchheim (1) has studied the question of the toxicity of trypsin in both its local and general effects. The resistance of living tissue to the local effect of trypsin has also been studied by Langenskiöld (2) and by Marie and Villandre (3). Kirchheim called attention to the similarity of trypsin intoxication to anaphylactic and peptone shock. He determined that the toxicity was destroyed when the ferment was inactivated by heat and that the fresh pancreatic secretion was not toxic unless activated by enterokinase. He concluded therefrom that the toxicity was not due to admixed protein split products. In order to determine whether the toxicity depended upon the effect of the ferment directly on the living cell, or whether split products were first formed from soluble proteins, in this way leading to an indirect intoxication, Kirchheim tried the effect of the ferment directly on spermatozoa. Since he found, however, that the spermatozoa were injured neither by the ferment nor by split products produced by the action of the ferment, Kirchheim drew no definite conclusion. In his work on the serum antiferment Kirchheim showed that both theories held (split products in the serum, and true antibody formation) were erroneous. Incidentally he noted that chloroform rendered the serum albumin more digestible by trypsin.

In view of the similarity of trypsin intoxication to anaphylactic and peptone shock we have undertaken a series of experiments to determine the effect of the ferment when injected into the blood stream of dogs. The trypsin used was either commercial pancreatin (for intestinal injection) or purified according to the method previously described (4). The latter ferment was very active and when dried retained its strength unimpaired. The serum ferments were titrated according to the method described fully in a previous paper

(5). Dogs of medium weight (five to nine kilos) were used throughout.

EXPERIMENTAL.

Dog 11.—(Text-fig. 1.) Weight 5.5 kilos. 0.27 gm. of purified trypsin was dissolved in 5 cc. of normal saline and injected intravenously at noon. The animal became ill immediately, cried with pain, and was nauseated. Bled after 10 minutes, at 2 and 4 p. m., and the following morning. As will be noted from Text-fig. 1 there was an immediate rise in serum protease, the blood taken 10 minutes after the injection digesting slightly more than 0.6 mg. of nitrogen



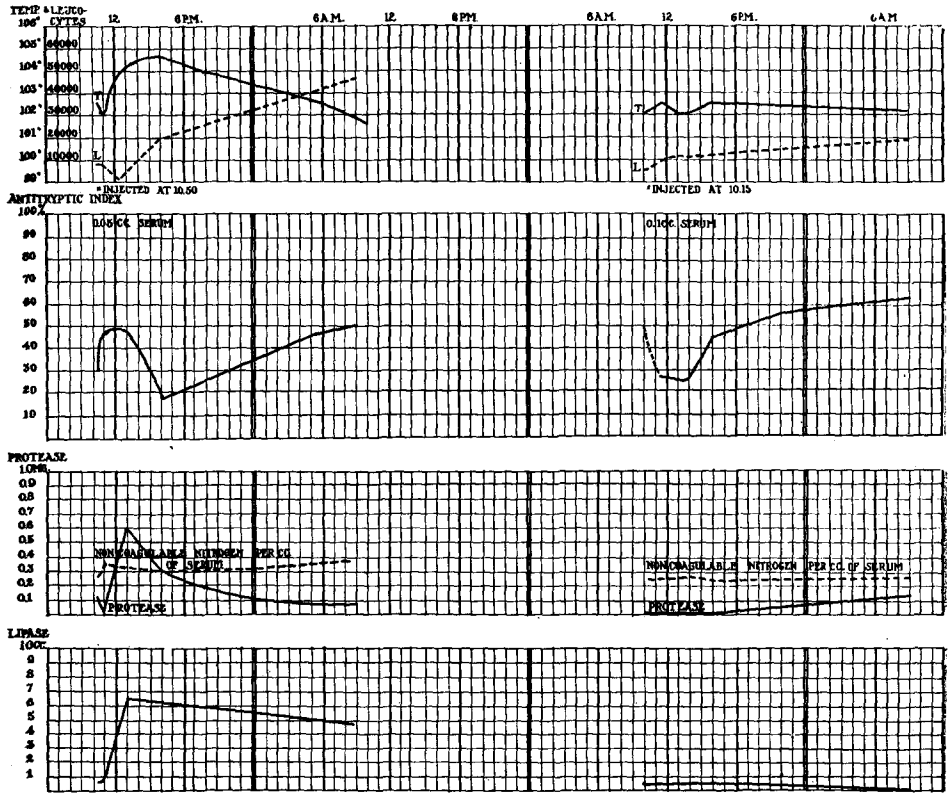
TEXT-FIG. 1. Effect of trypsin injection on the serum ferments and antiferment titer.

from serum proteins per cc. The maximum effect was noted after 2 hours, followed by a gradual decline. The non-coagulable nitrogen showed a gradual decrease, with an increase after 24 hours. The antiferment index fell immediately, recovered gradually, and increased after 24 hours, after which the original titer was reached.

The lipase curve shows a striking rise, this being progressive for 2 hours, after which the amount is lessened, but remains at a high level during the following day.

There was a marked leucopenia immediately following the injection, followed by a leucocytosis for the next 48 hours. The maximum rise in temperature was noted after 4 hours.

A similar experiment is shown in Text-fig. 2.



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

TEXT-FIG. 2. Effect of trypsin injection on the serum ferments and antiferment titer.

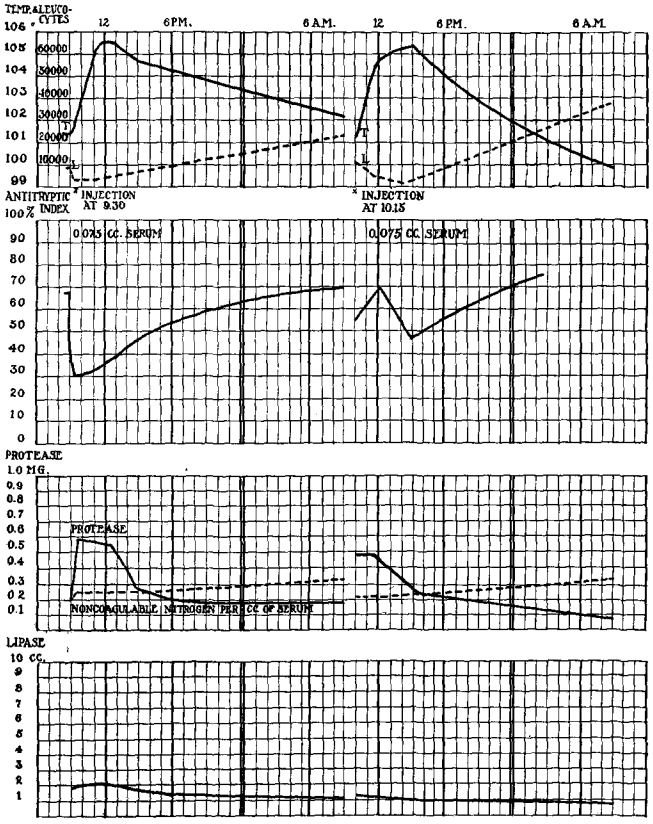
TEXT-FIG. 2 a. Effect of subcutaneous injection of trypsin on the serum ferments and antiferment titer.

Dog 14.—(Text-fig. 2.) Weight 9.4 kilos. Injected 0.1 gm. of very active trypsin at 10.55 a. m. Bled at 11 a. m., 1 and 4 p. m., and the following morning.

In this experiment the increase in protease was not noted immediately after

injection, but only at the time of the second bleeding, when the maximum was reached. The lipase, too, showed no immediate change, but remained constant, showing a marked increase at 1 p. m. The antiferment, instead of showing an immediate decline, rose at first, and the fall was noted only during the afternoon. The temperature and leucocyte count were similar to those in the previous animal.

Text-fig. 3 illustrates a similar experiment.



TEXT-FIG. 3. TEXT-FIG. 3 a.

TEXT-FIG. 3. Effect of trypsin injection on serum ferments and antiferment titer.

TEXT-FIG. 3 a. Effect of inactivated trypsin injection.

Dog 35.—(Text-fig. 3.) Weight 6 kilos. 0.2 gm. of an active trypsin preparation was given intravenously at 9.30 a. m. The animal became quite ill, the temperature reaching 105° F. The rise in the protease in the serum was imme-

diate. The antiferment dropped quite markedly. The animal showed no rise in lipase. The non-coagulable nitrogen rose almost immediately and remained high for 24 hours.

Inactive Trypsin.

According to Kirchheim, his inactive trypsin preparations were non-toxic in the animals which he used (guinea pigs and rabbits). In our experiments we have not been able to confirm this finding. Inasmuch as by this method the question as to whether the ferment is toxic because of its ferment property, or because of its inherent toxicity as a proteose, is to be decided, we have repeated our experiments under various conditions and with several different trypsin preparations. We have without exception found the inactivated ferment toxic for dogs, as indicated by the resulting malaise and the effect on temperature and leucocyte count. The effect on the ferments of the serum is shown in the accompanying charts.[1]

Dog 35.—(Text-fig. 3 a.) The same trypsin preparation was used as before, but inactivated by boiling for 10 minutes after alkalinizing with sodium carbonate. 0.2 gm. was given intravenously at 10.15 a. m. The animal became ill and showed a temperature and leucocyte count similar to that following the active trypsin injection. The antiferment showed a slight rise followed by a drop, with recovery the following day. The protease, which was high at the beginning of the experiment, showed a progressive decline. The non-coagulable nitrogen increased after 24 hours, as with the active preparation. The lipase showed practically no change.

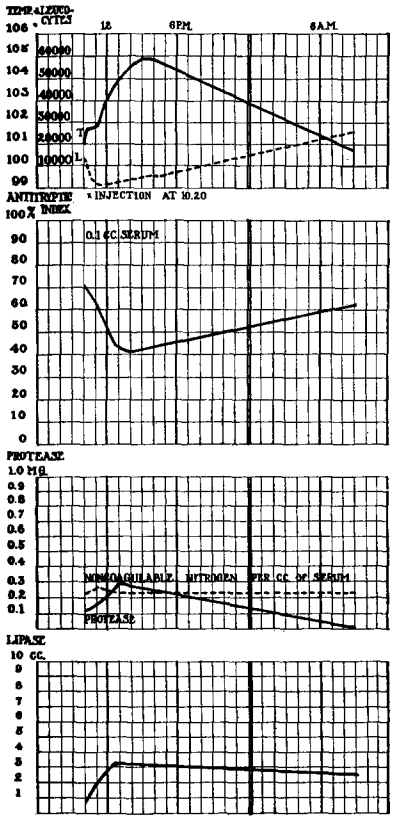
Dog 13.—(Text-fig. 4.) Weight 5.7 kilos. 0.25 gm. of inactivated trypsin was injected at 10.20 a. m. This animal responded with a picture of intoxication similar to that produced by the active preparation. The temperature and leucocyte curve are typical. There was a distinct fall in the antiferment, followed by a gradual recovery. There was a moderate rise in the serum lipase. The serum protease showed a slight increase, with a following decline to zero the next day. The non-coagulable nitrogen, with the exception of a slight initial rise, remained unchanged.

Subcutaneous Injections.

If the dose, instead of being brought directly into the blood stream, is injected subcutaneously, there are apparent no toxic effects as far as the general condition of the animal is concerned. Such an experiment is as follows:

[1] F. Ishiwara (abstracted in *Sei-I-Kwai Med. Jour.*, 1915, xxxiv, 19) has recently noted the toxicity of inactivated trypsin and considers it due to the presence of diamino-acids.

Dog 14.—(Text-fig. 2 a.) 0.1 gm. of active trypsin was injected subcutaneously at 10.15 a. m. (the intravenous dose is shown in Text-fig. 2). The temperature of the animal remained practically unaltered; the leucocyte count increased gradually. The antiferment curve showed rather a marked fall, with



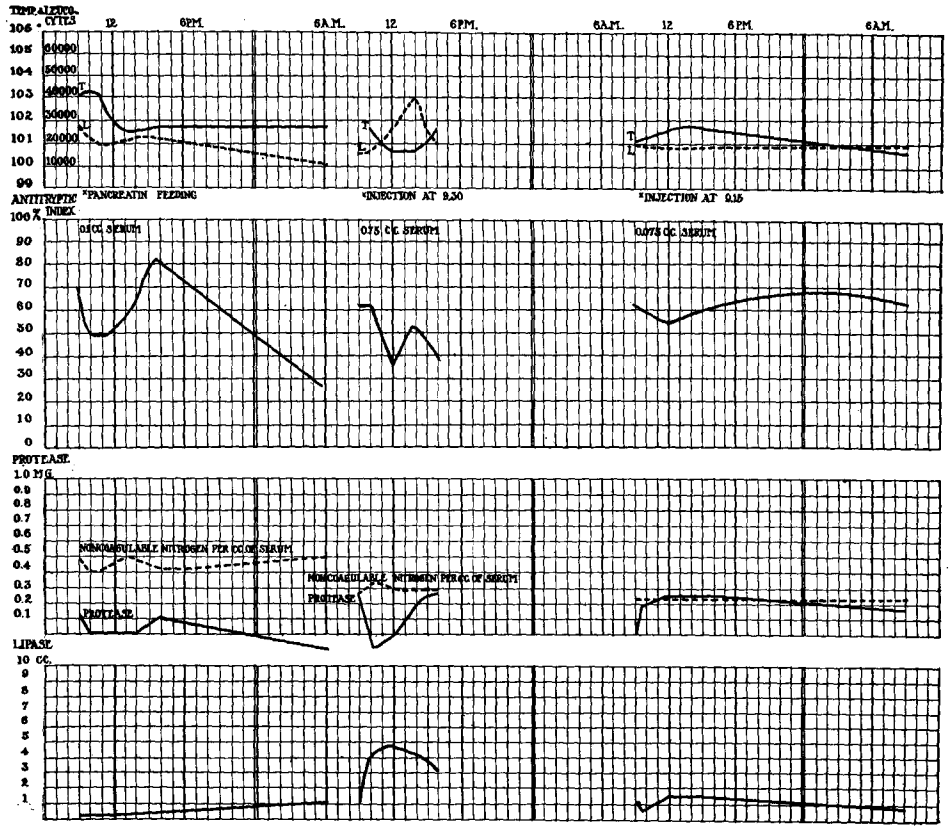
TEXT-FIG. 4. Effect of inactivated trypsin on serum ferments and antiferment titer.

an increase the following day. The protease was unaltered until the following morning when a slight increase was noted. The lipase showed a slight decrease.

Gastric Absorption.

When pancreatin is introduced into the stomach there is practically no increase in the ferments of the serum, the introduced ferment being probably destroyed before the duodenal tract is reached. The following experiment is illustrative.

Dog 14.—(Text-fig. 5.) 1 gm. of commercial pancreatin was dissolved in 25 cc. of water and injected into the stomach at 9.30 a. m. Bled at 10.00 and 11.30 a. m., 1.30 and 4.00 p. m., and the following morning. There resulted a gradual rise in temperature and leucocyte count, and a rather marked fall in antiferment, with rapid recovery, was noted. The lipase remained constant. The non-coagulable nitrogen showed first a slight decrease, followed by a rise in the afternoon. The protease, which fell to zero after injection, reached the original titer in the afternoon, but declined the following morning.



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

TEXT-FIG. 5. Effect of gastric absorption of pancreatin on serum ferments and antiferment titer.

TEXT-FIG. 5 a. Effect of intestinal absorption of trypsin on serum ferments and antiferment titer.

TEXT-FIG. 5 b. Effect of intestinal absorption of pancreatin on serum ferments and antiferment titer.

Intestinal Absorption.

In order to determine whether a toxic effect of the ferment could result from intestinal absorption the following experiment was undertaken in which the dissolved ferment was injected directly into a loop of the small intestines.

Dog 24.—(Text-fig. 5 a.) The animal was anesthetized and a loop of small bowel isolated through a small laparotomy wound under strict aseptic precautions; 0.5 gm. of a very active trypsin preparation was injected. First blood taken during anesthesia. Bled at 10.00 a. m., noon, 2 and 4 p. m., when the animal was killed. Text-fig. 5 a gives the ferment picture in detail. Contrary to the direct intravenous injection the dog showed a marked leucocytosis and only a slight fall in temperature. The antiferment showed the usual fall with partial recovery and secondary drop. The lipase curve showed the typical rise indicative of the intoxication. The serum protease, instead of a rise, showed a distinct fall, a recovery to the normal titer occurring in the afternoon.

In place of the very active and toxic preparation the following experiment was made with pancreatin (commercial).

Dog 40.—(Text-fig. 5 b.) Similar to the above; 0.5 gm. of commercial pancreatin was injected into a loop of the small bowel at 9.15 a. m. Bled at 9.30 a. m., noon, and 3.00 p. m. In this experiment there was no evidence of an intoxication in so far as the temperature and leucocyte count are concerned. There was practically no change in the antiferment and non-coagulable nitrogen of the serum; the protease showed an immediate increase, while the lipase showed at first a fall and then a slight rise in titer.

DISCUSSION.

The experiments have demonstrated that when active trypsin solutions are injected into the blood stream an intoxication results, manifested by marked gastro-intestinal irritation, a rise of temperature, with a primary leucopenia, followed by a leucocytosis. There is usually an immediate rise in serum protease and serum esterase, together with a lengthening of the coagulation time, which in some instances may lead to a complete inhibition of coagulation. In many ways the picture is not dissimilar to anaphylactic shock, which we shall discuss in a later paper. In the latter condition there is associated, however, a marked increase in the non-coagulable nitrogen of the serum, representing protein split products, and in so far differing from the effect with trypsin.

The antiferment change usually consists of an immediate drop,

with recovery following in a few hours. This lowering of the antitryptic titer is probably not due to a saturation of the antiferment by the injected trypsin, for the extent of the fall is not related to the amount of ferment injected nor to its activity. It is more probably the expression of a colloidal change in the serum whereby the lipoids are changed from a greater to a less disperse state. This would seem reasonable in view of the fact that a similar change occurs in anaphylactic and peptone shock and following the injection of various other substances,—bacteria, serum, etc. There is, of course, the possibility of a rapid oxidation occurring, with a resulting lowering of the antiferment strength because of an alteration of the unsaturated carbon bonds. A simple physical change seems, however, to account more readily for the rapidity of the various fluctuations that occur.

The question as to the toxicity of trypsin resolves itself into an endeavor to decide whether free protease activity in the blood stream is noxious; whether the trypsin molecule apart from its tryptic activity is toxic; whether due to admixed foreign toxic material (protein split products); or whether the ferment free in the serum is able to split non-toxic body proteins to toxic products and so induce an indirect toxic effect.

We have been able to demonstrate a considerable increase in serum protease immediately following the injection, an increase maintained for two or more hours and then rather rapidly disappearing. It would seem reasonable that this represents the injected ferment, were it not for the fact that the ferment activity so demonstrable is not active in an alkaline medium, but only in a neutral or acid reaction; that the increase may be progressive; that in one experiment the ferment was not demonstrable in blood drawn five minutes after the injection, but became apparent later. Furthermore, a similar, if not so marked an increase in serum protease may occur during peptone and anaphylactic shock. If we regard the ferment rise as a mobilization of ferments from the animal organism, the explanation that the lowering of the antiferment titer is due to a saturation with ferment finds a stronger basis, for the amount of ferment so liberated need have no relation to the amount and strength of the ferment injected. On the other hand, the inactivated preparations, while toxic, usually

do not produce the increase in serum protease, although the antiferment may show marked changes.

Our experiments differ from those of Kirchheim in that we have found the inactive trypsin solution toxic for dogs; Kirchheim found that his inactivated preparations were non-toxic for guinea pigs and rabbits. We are not able to account for this discrepancy. As evidences of the toxic effect we have observed the general conditions of the animals, together with the temperature and leucocyte count, and in no instance have we noted any great difference between the active and inactive preparations in these respects. From our results we are inclined to the view that the toxicity does not depend on the activity of the ferment, but rather on the ferment molecule itself. While it is true that the more active trypsin preparations are most toxic, the increase may in part be due to the elimination of protective colloids during the course of purification.

The rise in lipase noted after the injections is not peculiar to the trypsin intoxication, but occurs during similar intoxications from other causes (anaphylaxis, peptone, etc.).

As might be anticipated, the experiments with the feeding or subcutaneous injections of trypsin lead to no appreciable increase in the amount of serum protease demonstrable, nor were there any definite effects of intoxication from the amounts used.

On the other hand, when the ferment is brought directly into the small bowel, an influence on the serum protease is apparent. This may result in a simple increase when a large amount of a relatively weak trypsin preparation is used, without evidences of an intoxication. This would in a way simulate the normal condition during digestion, except that the ferment finds no substrate to which it can become attached. When, however, a very active preparation is injected, an intoxication may result. Instead of the blood showing an increase of protease in this case, an actual decrease may occasionally be observed, as shown in Text-fig. 5 a. When the liver of such an animal is examined histologically, profound fatty changes are observed. It is possible that the ferment, when absorbed, does not pass the liver into the general circulation. This would correspond to the condition which we have noted after feeding, in which the portal blood may contain considerable protease, while the peripheral blood may contain much less or none at all.

The possibility that intoxication may occur from the absorption of large amounts of free tryptic ferment from the bowel under pathological conditions must be considered. This would be more apt to occur if the intestinal tract were empty, so that the secreted ferment would remain unbound by proteins.

As will be noted from the text-figures, there is no fixed relation between the number of leucocytes and the ferments or antiferment, thus corroborating the recent finding of Rosenow and Färber (6), who noted that the leucocyte count and the destruction of leucocytes had no constant effect on the antiferment titer. Caro (7) noted that the number of lymphocytes (which are supposed to contain a lipolytic ferment) bore no relation to the lipolytic activity of the serum.

The questions which arise in view of the marked increase in both protease and lipase during these intoxications (inclusive of anaphylactic shock, peptone poisoning, etc.) concern their origin and effect on the organism. We are inclined to the view that they are mobilized from the tissue cells in general, and not from any specific organ. It seems certain at least that they do not come from the liver, for the hepatic blood usually contains the minimal concentration of the ferments under discussion.

In order to discuss their effect we must keep in mind certain fundamental factors concerned in protein intoxication. The true proteases act only on native proteins, which are split into certain definite components probably preexisting in the protein molecule (Levene). These may be toxic, certain of the proteoses being especially toxic for guinea pigs (8, 9), others, as far down as the peptones, being even more markedly toxic for dogs.

The proteases do not act in the presence of the antiferment. Inasmuch as the serum always contains an antiferment, protease action is normally held completely in abeyance. Therefore under normal conditions, even with proteases present in the serum, toxic split products can not be formed from the serum proteins. This protection is not necessarily intracellular.

Any condition which tends to remove the antiferment protection may result in splitting and a consequent intoxication. In this way the so called anaphylatoxins are formed when the antiferment is adsorbed by the various agents used,—bacteria, agar, kaolin, etc.

The released serum protease then can simply split the serum protein (10). In a similar manner when the antiferment is removed by lipoidal solvents the serum becomes toxic (11); and the splitting which takes place in the Abderhalden test has its basis along similar lines (12).

It is possible that a lowering of the antiferment titer may have some part in producing an intoxication. Such a lowering takes place not only following trypsin injections, but following many other conditions, some of which have been noted by Pfeiffer and Jarisch (13). The toxicity of kaolin (14) and certain colloids can be explained in this way. Conversely the raising of the antiferment titer may prevent an intoxication if that intoxication depends upon the formation of toxic split products and not upon the introduction of preformed toxic substances. We have previously called attention to such conditions (15).

We may sharply differentiate the true serum protease from the ereptase action of the serum, which is solely concerned with the split products of the protein molecule (casein being an exception to the general rule that the native proteins are not split by this ferment). The ereptase is not influenced by the antiferment, so that under normal serum conditions it is only the ereptase which can be active. It is this ferment which is chiefly responsible for the changes occurring in the Abderhalden reaction. The ereptase may be responsible as an intoxicating, as well as a detoxicating agent. Thus a serum may contain non-toxic proteoses which may be rapidly split to toxic peptones by the ferment. Later, however, the serum ferment may split these same toxic substances to amino-acids and thus cause a complete detoxication.

The ereptase must not be supposed to be only lytic in action. There is considerable evidence, which we shall discuss in a later paper, that the ferment is actually synthetic under certain conditions. From these conditions it would seem probable that the increase in protease and ereptase which occurs during the various so called protein intoxications may be of considerable aid in the process of detoxication.

CONCLUSIONS.

1. The intravenous injection of trypsin in dogs results in a shock similar in many respects to anaphylactic and peptone shock.

2. The injection is followed by a marked rise of serum protease and lipase.

3. The antiferment usually shows a distinct drop in titer, with a recovery following in from four to twenty-four hours.

4. The non-coagulable nitrogen shows no constant alteration, but is never greatly changed in amount.

5. Inactivated preparations were in some respects followed by symptoms similar to those following the injection of the active preparation.

6. Subcutaneous and gastric absorption was practically without effect.

7. Intestinal absorption was followed by an increase in serum protease without evidence of intoxication, or by typical symptoms of acute poisoning.

8. The leucocyte curve bears no constant relation to the serum protease or lipase.

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