THE EFFECT OF KILLED BACTERIA ON THE SERUM FERMENTS AND ANTIFERMENT.

STUDIES ON FERMENT ACTION. XXVII.

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In a study of the serum reactions brought about by the injection of foreign substances, interest naturally centers on changes produced by the injection of various bacteria. That, apart from the well known immunity reactions, certain changes occur in the ferments of the serum when bacteria cause a reaction on the part of the host has become evident during the past few years, largely through a study of anaphylaxis and its correlation with immunological phenomena. The work of Vaughan, of Kraus, Pfeiffer, Friedberger, de Waele, Zunz, and many others, has emphasized the importance of protein intoxication, and, as a corollary, that of the ferments which bring about a lysis of the bacterial protein. In this way the study of the phenomena of infection and immunity has gradually centered about the idea that the hydrolyzed protein of the bacterium was responsible for the intoxication, while the unaltered protein was the cause of the immunity reaction.

More recently the subject has been somewhat confused rather than aided by the endeavor to demonstrate specific protease action and in this way possibly to connect the simple antibody reactions with the hydrolysis which results in an intoxication. In a recent paper (1) we have sought to demonstrate that the methods used and the results obtained in the course of such studies were seriously to be questioned; that the serum protease was not specific, but polyvalent; that in many instances the antigen was not the substrate

which yielded the split products, but that they were derived from the serum supposed to contain the specific ferment.

The older idea of Pfeiffer that the bacterium contained a preformed toxic substance which became evident on lysis has been gradually superseded, and the emphasis placed on the theory that the non-toxic proteins of the bacteria cell are hydrolyzed to toxic substances. There are, however, certain facts which have not been taken into consideration in the development of this hypothesis. They are as follows: (1) bacteriolysis has been confused with proteolysis, despite the fact that intact organisms are quite resistant to proteolytic ferments; (2) bacteria adsorb antiferment from the serum and in this way become even more resistant to proteolytic influence; (3) the serum contains an antiferment which prevents the splitting of native proteins, but does not prevent the splitting of the higher split products to amino-acids; (4) simple lysis of cells, as, for instance, by grinding and freezing, as Cole (2) has recently shown for pneumococci, may free substances which are toxic in a degree and manner similar to the protein split products derived in other ways; (5) the amount of preformed split products in the cell, which are set free by simple lysis, has been ignored.

In the following experiments we have used dried, killed organisms so that we might work with constant amounts and the factor of intravital multiplication be obviated.

The technique used in the determinations of the serum ferments, antiferment, and split products has been previously described (3).

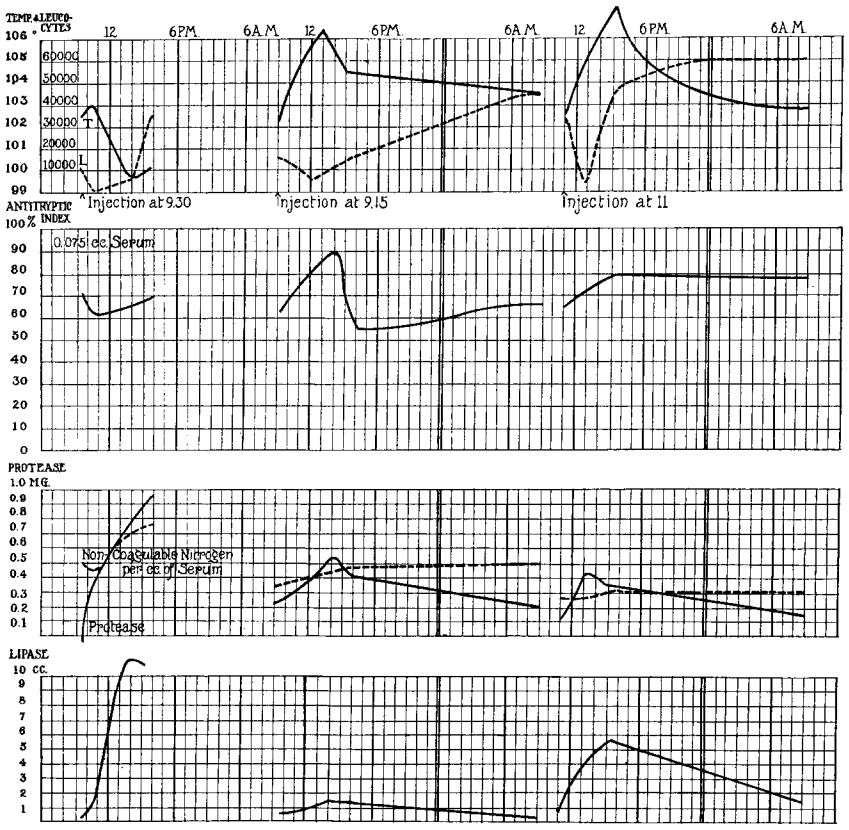
EXPERIMENTAL.

Typhoid Bacteria.

Dog 20.—Weight 7 kilos. 20 mg. of dried typhoid organisms were suspended in saline solution and injected intravenously at 9.30 a. m. (Text-fig. 1). The animal became ill shortly after the injection, was prostrated gradually, and died during the course of the night.

As will be observed from Text-fig. 1, the temperature, except for a slight initial rise, decreased during the afternoon, while the leucocyte curve at first declined and later increased. The antiferment showed little alteration. The most striking changes are noted

in the serum protease and lipase which increased to more than ten times their original titer. The non-coagulable nitrogen also increased after a slight initial decrease.



TEXT-FIG. 1. TEXT-FIG. 1 a. TEXT-FIG. 1 b.

TEXT-FIGS. 1, 1 a, AND 1 b. Serum changes following intravenous injection of typhoid bacilli; (Text-fig. 1), 20 mg., (Text-fig. 1 a), 10 mg., (Text-fig. 1 b), 10 mg., second injection.

The next dog was given a similar dose but in two injections.

Dog 38.—Weight 4 kilos. 10 mg. of dried typhoid bacteria were injected at 9 a. m.; a second injection of 10 mg. was made at 11 a. m. Died at 5 p. m.

This experiment showed a similar increase in serum lipase and protease, although following the second injection there was no

further increase in protease, and a drop occurred. The antiferment showed no marked changes.

In the following dog a small dose was given after an interval of several days.

Dog 22.—Weight 6.5 kilos. 10 mg. of dried typhoid bacteria were injected at 9.15 a. m., Feb. 23, 1915 (Text-fig. 1 a). A second injection was made after 8 days, on Mar. 3, at 11 a. m. (Text-fig. 1 b).

The animal in both instances showed the usual evidences of illness. From Text-figs. 1 a and 1 b it will be observed, however, that, following the second injection, the mobilization of the protease was more rapid than after the first injection, and the rise in lipase was more marked. This is the condition usually occurring during sensitization (4). The antiferment showed less change the second time than the first, while the temperature and the leucocyte count showed evidence of even a more marked intoxication.

Various Organisms.

Dog 35.—Weight 4 kilos. 10 mg. of dried subtilis bacilli were injected at 9 a. m.

In this instance, while there was some change in the leucocyte count and a slight rise in temperature the only marked change occurred in the antiferment, which fell following the injection but increased later. The lipase increased slightly.

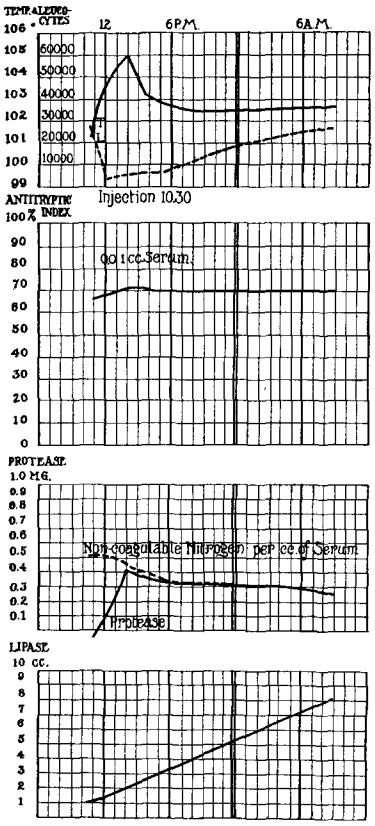
Dog 21.—Weight 4 kilos. 50 mg. of dried diphtheria bacilli were injected at 10.30 a. m. (Text-fig. 2).

The animal responded by a distinct rise in temperature and leucopenia. There was an immediate rise in protease, while the lipase increased progressively until the following morning. The antiferment was unaltered.

These two organisms differ widely in their resistance to tryptic digestion. *Bacillus subtilis* contains only a small amount of lipoids, autolyzes readily, and contains preformed a considerable amount of non-coagulable nitrogen. Diphtheria bacilli, on the other hand, contain considerable antiferment, are very resistant to tryptic digestion, and contain only a very small amount of non-coagulable nitrogen.

Typhoid organisms occupy rather an intermediate position (5). It is interesting that the rise in lipase following the diphtheria injection occurs at a much later period than after typhoid injections.

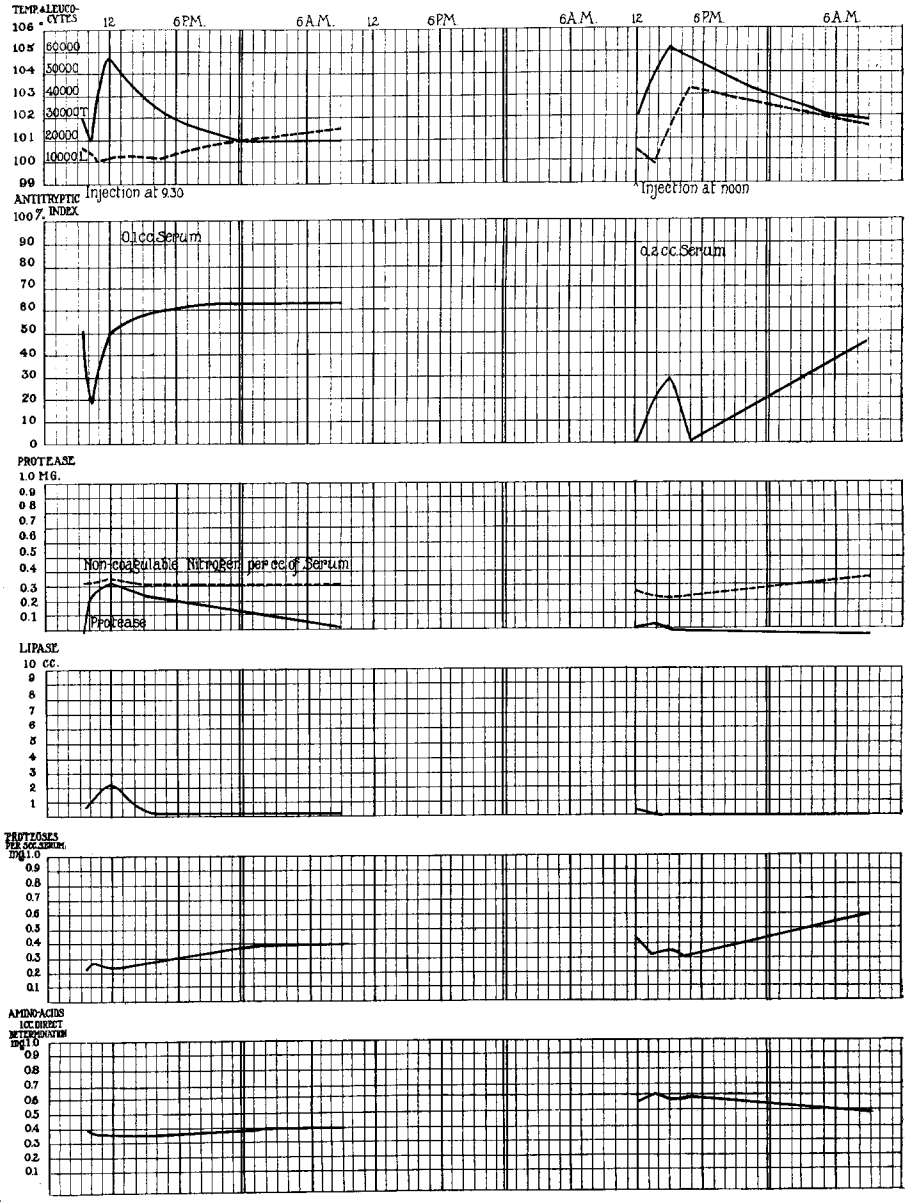
Dog 52.—Weight 4.5 kilos. 20 mg. of dried pneumococci (Neufeld strain) were injected at 11 a. m.



TEXT-FIG. 2. Serum changes following intravenous injection of diphtheria bacilli.

There resulted a rise in temperature to 105° F., and the leucocytes showed a primary rise. The serum, however, showed no marked changes; it is quite evident from this experiment that the temperature curve bears no direct relation to the ferment changes.

In the following experiments the serum proteoses and amino nitrogen have been determined in addition to the ferment changes.



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

TEXT-FIGS. 3 AND 3 a. Serum changes following intravenous injection of (Text-fig. 3) staphylococci and (Text-fig. 3 a) tubercle bacilli.

Dog 76.—Weight 5.4 kilos. 10 mg. of typhoid bacteria were injected at 9 a. m. Death at 4 p. m.

The ferment changes were typical, the serum protease increasing from 0.05 to 0.7, the lipase from 1.2 cc. to 7 cc. at the time of death. There was a slight increase in serum proteoses while the amino nitrogen showed a primary decrease.

Dog 78.—Weight 6 kilos. 50 mg. of dried *Staphylococcus aureus* were injected at 9.30 a. m. The animal remained well and showed no evidence of the intestinal irritation always observed after injections of typhoid bacteria.

There will be observed, however, a sharp rise in temperature (Text-fig. 3), together with a marked fall in the antiferment titer. Protease and lipase increased, but returned to normal the following day. Serum proteoses increased, while the amino nitrogen showed a slight decrease.

Dog 79.—Weight 5.5 kilos. 100 mg. of dried tubercle bacilli (human) were injected at noon (Text-fig. 3 a). While the temperature and leucocyte curve rose considerably, the animal remained quite well and was free from nausea or gastro-intestinal symptoms.

The serum ferment remained unaltered, while the antiferment titer showed some changes. The proteoses at first decreased, but returned to slightly above normal the following day. The amino nitrogen showed a slight decrease after twenty-four hours.

DISCUSSION.

Incidental to the study of anaphylaxis (4), we have shown that the primary injection of protein, while it may be associated with malaise, a rise in temperature, and a leucopenia, is not associated with a change in protease, whereas the split products of proteins, as illustrated in the preceding paper,[1] may give rise to such a mobilization. It becomes quite evident, therefore, when we examine the effects produced, for instance, by typhoid bacteria, that the toxicity cannot be due to the effect of the native protein unless that protein is immediately hydrolyzed to its toxic components. We must assume either that such splitting takes place immediately on injection, or that

[1] Jobling, J. W., Petersen, W., and Eggstein, A. A., *Jour. Exper. Med.*, 1915, xxii, 597.

a simple lysis due to other causes sets free preformed toxic substances. Inasmuch as specific ferments are quite out of question at the time of the primary injection, we must acknowledge that whatever splitting may occur is due to a non-specific protease. Pfeiffer (6) has recently made observations following intoxication by burns and photodynamic agents, in which he determined the proteolytic strength of the serum with glycyltryptophane. He noted an increase following the intoxication, and discusses the possible relation of such ferments to the so called "*Abwehrfermente.*"

It is quite evident from the text-figures that if the development of toxicity depends on soluble substances derived from the killed organisms, these substances are at least in part preformed.

The relative toxicity bears some relation to the degree of resistance of the organism to the proteolytic ferment. This is noted with the tubercle bacillus (Text-fig. 3 a), which in large doses has caused a rise in temperature but no change in the ferments. The diphtheria organism (Text-fig. 2), which is also quite resistant, shows a maximum rise in lipase only after twenty-four hours, while the staphylococcus (Text-fig. 3), less resistant, shows a prompter effect. When we compare the toxicity of subtilis and typhoid bacilli, however, resistance to digestion cannot be the most important factor, for the subtilis bacillus is rather easily digested, at least as easily as the typhoid bacillus, whereas the toxic manifestations are quite out of proportion in the case of typhoid injection.

Under these circumstances we are rather of the opinion that proteolysis, in so far as it relates to the production of toxic substances from bacteria *in vivo,* is not to be emphasized as the sole agent in the mechanism, but rather that certain organisms contain preformed toxic split products which may be liberated during bacteriolysis, a reaction in which proteolysis has no part as yet demonstrable. On the contrary, it would seem warrantable to suppose that in the serum the protease might act rather as a detoxicating agent, in that it is able to split the toxic fragments to the non-toxic amino-acids. In this reaction the antiferment plays no part, for it seems to act only in inhibiting the splitting of the native proteins to toxic fragments. The mobilization of the protease, as of the lipase, occurs as a result of cellular injury, but is not necessarily associated with the febrile reaction.

CONCLUSIONS.

1. The intravenous injection of killed organisms is followed by the mobilization of a non-specific protease and lipase; the rapidity and extent of this reaction depend upon the toxicity of the organism and on the resistance of the organism to proteolysis.

2. The temperature and leucocytic curve bear no relation to the ferment changes.

3. The serum antiferment is usually increased after the injection.

4. Of the organisms studied, the typhoid bacilli produced the most marked ferment changes, and the tubercle bacilli the least.

5. The toxicity of the dried organisms cannot depend wholly upon proteolysis *in vivo,* but must depend in part on the preformed toxic substances liberated on lysis.

6. Serum protease should not be considered as the sole exciter of intoxication through the production of protein split products; it seems possible that its function may in part be one of detoxication.

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5. Jobling and Petersen, *Jour. Exper. Med.,* 1914, xx, 452.
6. Pfeiffer, H., *Ztschr. f. Immunitätsforsch., Orig.,* 1915, xxiii, 473.