THE RELATION OF BACTERIOLYSIS TO PROTEOLYSIS.

STUDIES ON FERMENT ACTION. XVI.*

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The relation of the proteolytic ferments of serum to immunity re-actions, particularly to bacteriolysis, has interested several inves-tigators, and in view of the recent work of Abderhalden and his collaborators it merits attention quite apart from a theoretical con-sideration. The study of possible proteolytic cleavage during bac-teriolysis, has, because of the manifest technical difficulties entailed by the accurate determination of nitrogen in the small amounts available, been limited to rather general statements, based, in most instances, on experiments carried out with the Sörensen method. This method, being an index of the total cleavage of the protein molecule, gives no information of the change from coagulable to non-coagulable forms, and is therefore of use in estimating peptoly-tic or ereptic enzyme activity rather than proteolytic changes. For a like reason it represents the splitting of the protein bodies to non-toxic fragments in contradistinction to the splitting of the whole non-toxic protein molecule to the higher non-coagulable but toxic forms (proteoses). Since the microchemical methods devised by Bang (1) and Folin (2) were described there are available no ex-periments dealing with this subject, and we have therefore under-taken the following experiments using the Folin method, which lends itself admirably to work of this kind.

The bactericidal and bacteriolytic property of fresh serum due to the action of complement and amboceptor has been assumed, more particularly by French and German writers, to be enzymotic in character and in the nature of a protease. There is, however, no experimental basis for this idea; indeed the fact that complement

action is not identical with a protease action can be demonstrated in a very simple experiment. When chloroform is added to fresh serum and thoroughly mixed, the complement is very rapidly destroyed; the serum protease, however, first becomes active under these conditions (Delezenne and Pozerski (3)). The activation under the influence of chloroform or other lipoid solvents is due to the fact that the antiferment is removed in this way. There should therefore be no confusion as to the non-identity of these serum constituents. If complement activity is enzymotic in character, we should rather seek to identify it with the lipases or allied enzymes. Thus the objection made that complement acts in direct proportion to the concentration, and is therefore unlike other ferments, fails in view of the fact that serum esterase acts in a like manner (Loevenhart (4)). And the ease with which lipoid solvents inactivate the complement finds an analogy in plant lipases which are either destroyed by lipoidal solvents or are in such intimate relation to the lipoids that they are taken up with them into the solvent. The substrate offered for complement action—red blood cells, animal cells, bacteria—is lipoidal in nature, the potentially lipoidal character of the limiting membranes of the corpuscles and animal cells being well established and rendered more than probable for bacterial cells. The fact that powerful proteolytic enzymes are without effect on such substrata is almost conclusive evidence that the surfaces of these substrata are not protein in nature. An observation of Metchnikoff (5) is significant in this connection. Metchnikoff, noting that the intestinal tract of certain insects (moths) was free from bacteria, an exception to the condition found in other insects, concluded that these insects must secrete a powerful fat-splitting enzyme to enable them to utilize waxes and fats readily, and surmised some connection between the character of this intestinal secretion and the freedom from bacteria.

EXPERIMENTAL.

The experiments, designed to determine the amount of proteolysis occurring during bacteriolysis, were carried out in the following manner. The organisms used, *Bacillus coli, Bacillus typhosus,* and staphylococci, were grown on agar in large bottles. They were

washed repeatedly with salt solution and used as fresh emulsions, or dried at low temperature and made up in a 1 per cent. emulsion as used. In either case the total nitrogen and the total non-coagulable nitrogen per cubic centimeter of the emulsion were determined for several samples. Immune sera were obtained by bleeding rabbits when the blood contained the greatest concentration of immune bodies. The immune sera so obtained were kept without preservatives on ice and were inactive. Fresh guinea pig serum was used as complement.

One cubic centimeter of the bacterial emulsion was measured into small centrifuge tubes, the various sera were then added, and digestion was permitted at 37° C. under toluol for varying periods of time. The tubes were then thoroughly centrifuged until the supernatant fluid was clear. The fluid was carefully pipetted from the bacteria, these were washed once with salt solution, and the fluid was added to that already withdrawn. The total nitrogen of the bacterial rest was then determined and also the total non-coagulable nitrogen of the supernatant fluid. The difference between this latter figure and the amount of total non-coagulable nitrogen in the serum controls gives the amount of nitrogen which has autolyzed, *i. e.,* the bacterial nitrogen digestion. The difference between the total nitrogen of the bacteria originally introduced and the amount recovered indicates the amount of bacteriolysis.

In using the relatively large amounts of organisms necessitated by the character of the work, the amount of serum is by comparison small, but the bacteriolytic effect is in most cases quite evident by the greater loss of bacterial nitrogen in the tubes containing the immune serum and complement.

The work has been carried out in duplicate and in some instances four or more determinations were made on corresponding mixtures. For the sake of brevity the protocols have been condensed and show only single determinations. The variations were seldom greater than a few hundredths of a milligram. The figures indicated in the protocols express nitrogen in milligrams.

In numerous experiments the bacterial rests remaining after serum treatment were digested by trypsin after determinations on similar mixtures showed the amount of substrate remaining. The

PROTOCOL I.

	Action of immune serum and complement on bacteria and relation of resulting lysis to proteolysis.									Action of trypsin on bacteria following immune serum and complement action.					
Tube No.	Typhoid bacilli.	Colon bacilli.	Colon immune serum.	Complement.	Bacterial nitrogen recovered.	Loss in bacterial nitrogen.	Total non-coagulable nitrogen in supernatant fluid.	Gain in non-coagulable nitrogen in supernatant fluid.		Typhoid bacilli.	Colon bacilli.	Trypsin.	Bacterial nitrogen (substrate).	Nitrogen digestion.	Digestion.
1	1.0 c.c.				Total nitrogen = 1.65 mg. per c.c.			Total non-coagulable nitrogen = 0.25 mg. per c.c.							
2		1.0 c.c.			Total nitrogen = 2.80 mg. per c.c.			Total non-coagulable nitrogen = 0.38 mg. per c.c.							
3	1.0 c.c.				1.4 mg.	0.25 mg.	0.2 mg.		} Autolysis in salt solution						
4		1.0 c.c.			2.46 mg.	0.34 mg.	0.3 mg.								
5	1.0 c.c.				1.51 mg.	0.14 mg.	0.2 mg. } Total nitrogen in			1.0 c.c.		0.1 c.c.	1.45 mg.	0.78 mg.	49%
6		1.0 c.c.			2.34 mg.	0.46 mg.	0.53 mg. } supernatant fluid				1.0 c.c.	0.1 c.c.	2.4 mg.	0.75 mg.	31%
7			1.0 c.c.	2.0 c.c.	1.25 mg.	0.40 mg.	0.87(−0.625) mg.	0.24 mg.	} Typhoid bacilli, colon immune serum complement	1.0 c.c.		0.1 c.c.	1.25 mg.	0.48 mg.	38%
8			0.1 c.c.	2.0 c.c.	1.25 mg.	0.40 mg.	0.66(−0.437) mg.	0.22 mg.		1.0 c.c.		0.1 c.c.	1.25 mg.	0.50 mg.	40%
9		1.0 c.c.	1.0 c.c.	2.0 c.c.	2.10 mg.	0.7 mg.	1.0 (−0.625) mg.	0.375 mg.	} Colon bacilli, colon immune serum complement		1.0 c.c.	0.1 c.c.	2.10 mg.	0.77 mg.	34%
10		1.0 c.c.	0.1 c.c.	2.0 c.c.	2.10 mg.	0.7 mg.	0.8 (−0.437) mg.	0.363 mg.			1.0 c.c.	0.1 c.c.	2.10 mg.	0.508 mg.	24%
11	1.0 c.c.			2.0 c.c.	1.1 mg.	0.55 mg.	0.61(−0.417) mg.	0.2 mg.	} Complement	1.0 c.c.		0.1 c.c.	1.1 mg.	0.45 mg.	40%
12		1.0 c.c.		2.0 c.c.	2.34 mg.	0.46 mg.	0.8 (−0.417) mg.	0.383 mg.			1.0 c.c.	0.1 c.c.	2.34 mg.	0.57 mg.	24%
13	1.0 c.c.		1.0 c.c.		1.2 mg.	0.45 mg.	0.5 (−0.207) mg.	0.293 mg.	} Immune serum	1.0 c.c.		0.1 c.c.	1.2 mg.	0.55 mg.	45%
14		1.0 c.c.	1.0 c.c.		2.4 mg.	0.4 mg.	0.56(−0.207) mg.	0.353 mg.			1.0 c.c.	0.1 c.c.	2.4 mg.	1.0 mg.	40%
15			1.0 c.c.				0.207 mg.		} Non-coagulable nitrogen in serum controls						
16				2.0 c.c.			0.417 mg.								

per cent. of digestion was determined by carefully acidifying and boiling and filtering through kaolinized hard paper filters to remove the coagulated protein. The solution of trypsin used was prepared each time from a dried preparation and used in such dilution that 0.1 of a cubic centimeter of the solution would digest two cubic centimeters of a 1 per cent. casein solution in two hours. Protocol I will serve as an example of such an experiment.

PROTOCOL I—A.

Autolysis in Physiological Salt Solution. Relation of Solution of Bacteria to Autolysis.

Tube No.	Typhoid bacilli.	Colon bacilli.	Total nitrogen in supernatant fluid after		Total non-coagulable nitrogen in supernatant fluid after		
			24 hrs.	48 hrs.	24 hrs.	48 hrs.	
1	1.0 c.c.	....	0.37 mg.	....	....	....	} Solution.
2	1.0 c.c.	....	....	0.6 mg.	....	....	
3	1.0 c.c.	....	....	....	0.2 mg.	....	} Autolysis.
4	1.0 c.c.	....	....	....	....	0.2 mg.	
5	....	1.0 c.c.	0.57 mg.	....	....	....	} Solution.
6	....	1.0 c.c.	....	0.8 mg.	....	....	
7	....	1.0 c.c.	....	....	0.35 mg.	....	} Autolysis.
8	....	1.0 c.c.	....	....	....	0.5 mg.	

Fresh typhoid and colon bacilli were used. The typhoid emulsion contained 1.65 milligrams of total nitrogen and 0.25 of a milligram of total non-coagulable nitrogen per cubic centimeter. The colon emulsion contained 2.80 milligrams of total nitrogen and 0.38 of a milligram of total non-coagulable nitrogen per cubic centimeter. Bacteria were permitted to autolyze in salt solution over night (tubes 3 to 6); with immune colon serum and fresh complement (tubes 7 to 10); with complement alone (tubes 11 and 12); and with immune colon serum alone (tubes 13 and 14). The total non-coagulable nitrogen in the sera used is shown in tubes 14 and 15, and these amounts, viz., $0.417 + 0.207 = 0.624$ of a milligram, $0.417 + 0.02 = 0.437$ of a milligram are, of course, to be deducted from the digestion mixtures. A parallel series of tubes was prepared, serum action permitted for an equal length of time, the bacteria washed and subjected to tryptic digestion for six hours.

It is apparent in observing the results obtained that the greatest

loss of bacterial nitrogen has occurred in the mixtures with colon bacilli, colon immune serum, and complement, indicating a marked lysis of these bacteria. There is, however, no corresponding increase in non-coagulable nitrogen obtained in the supernatant fluid, the amount so recovered in the 1.0 and 0.1 cubic centimeter dilutions of immune serum being respectively 0.375 of a milligram and 0.363 of a milligram, corresponding very well with the amount of non-coagulable nitrogen introduced originally with the bacteria, 0.38 of a milligram, and representing only a trace more than the amount recovered in the salt solution control (tube 4). There is obviously no relation here between the amount of lysis and the actual proteolysis. It is interesting to note that the greatest lysis of typhoid bacilli occurred in the mixture containing complement alone, although here, too, there is no corresponding increase in non-coagulable nitrogen. When the bacterial residues are digested by trypsin the percentage of digestion is found to be considerably less in all the tubes treated with serum than in the untreated controls (tubes 5 and 6), with the exception of the bacteria treated with immune serum (tubes 9 and 14). We have previously shown (6) that bacteria treated with normal serum become more resistant to tryptic digestion, because of their adsorption of serum antitrypsin.

In protocol I-A is shown the rate of solution and of autolysis of

PROTOCOL II.

Action of Typhoid Immune Serum and Complement on Typhoid Bacilli.
No Demonstrable Proteolysis.

Tube No.	Ty- phoid bacilli.	Immune serum.	Comple- ment.	Bacterial nitrogen recovered.	Loss.	Total nitrogen in supernatant fluid.	Gain in total nitrogen.	Total non-coagulable nitrogen in supernatant fluid.	Gain in non-coag-ulable nitrogen.
1	1.0 c.c.	Total nitrogen = 1.1 mg., total non-coagulable nitrogen = 0.16 mg. per c.c.							
2	1.0 c.c.	Autolysis in sodium car- bonate solution		0.66 mg.	0.44 mg.	0.43 mg.	....	0.17 mg.	....
3	1.0 c.c.	0.1 c.c.	1.0 c.c.	0.83 mg.	0.27 mg.	7 (−6.7) mg.	0.3 mg.	....	....
4	1.0 c.c.	0.1 c.c.	1.0 c.c.	0.83 mg.	0.27 mg.	....	....	0.55 (−0.39) mg.	0.16 mg.
5	......	0.1 c.c.	1.0 c.c.	......	....	6.7 mg.	....	....	....
6	......	0.1 c.c.	1.0 c.c.	......	....	....	....	0.39 mg.	....

these organisms when kept in salt solution at 37° C. under toluol. While solution has occurred in both cases, the autolysis is not equally rapid; indeed it did not occur at all in the typhoid emulsion in this case.

In protocol II a typhoid system was used, with fresh typhoid bacilli and typhoid immune serum. The total nitrogen per cubic centimeter was 1.1 milligrams; the total non-coagulable nitrogen was 0.16 of a milligram per cubic centimeter. Mixtures were made as usual, the reaction in this case being made distinctly alkaline to phenolphthalein, the control being placed in a sodium carbonate solution of equal alkalinity. The control lost 0.44 of a milligram of bacterial nitrogen, 0.43 of a milligram being recovered from the supernatant fluid, of which 0.17 of a milligram was non-coagulable, indicating a lysis without proteolysis in the control. It will be recalled that autolytic enzymes in general act best in a slightly acid medium. In the immune serum (tubes 3 and 4) there has been less lysis than in the alkaline control (loss of 0.27 of a milligram), and while this amount was recovered in the increase in total nitrogen in the supernatant fluid (0.3 of a milligram), the non-coagulable nitrogen had not increased (0.16 of a milligram). It is quite apparent that the solution of the bacteria, due in one case to the alkalinity of the fluid (tube 2), in the other to the lytic effect of the serum complement and immune body (tubes 3 and 4), is not accompanied by proteolysis.

If we permit the action of the immune serum and complement on the bacteria for a shorter period of time (4 hours at 37° C.),

PROTOCOL III.

*Action of Typhoid Immune Serum and Complement on Typhoid Bacteria.
Absence of Proteolysis after 4 Hours' Incubation.*

Tube No.	Typhoid bacilli.	Typhoid immune serum.	Complement.		Bacterial nitrogen recovered.	Loss.	Gain in non-coagulable nitrogen.
1	1.0 c.c.	Total nitrogen = 1.42 mg., total non-coagulable nitrogen = 0.2 mg. per c.c.					
2	1.0 c.c.	0.0 c.c.	0.0 c.c.	Autolysis	1.2 mg.	0.22 mg.	0.15 mg.
3	1.0 c.c.	0.0 c.c.	1.0 c.c.	....	1.2 mg.	0.22 mg.	0.135 mg.
4	1.0 c.c.	1.0 c.c.	1.0 c.c.	....	1.28 mg.	0.14 mg.	0.07 mg.
5	1.0 c.c.	0.1 c.c.	1.0 c.c.	....	1.25 mg.	0.17 mg.	0.13 mg.
6	1.0 c.c.	0.01 c.c.	1.0 c.c.	....	1.28 mg.	0.14 mg.	0.125 mg.

which is ample for the bactericidal effect of the serum even if not for actual bacteriolysis, there is practically no loss in bacterial nitrogen and no increase in non-coagulable nitrogen. As a matter of fact, there is much less splitting in the tube containing the greatest amount of antibody (tube 4, protocol III).

The fact that complement alone has no appreciable proteolytic effect even when bactericidal is shown clearly in protocol IV, in

PROTOCOL IV.

Effect of Complement on Colon Bacilli. No Resulting Proteolysis.

Tube No.	Colon bacilli.	Complement.	Reaction.	Bacterial nitrogen recovered.	Loss	Total nitrogen recovered in supernatant fluid.	Total non-coagulable nitrogen in supernatant fluid.	Gain in non-coagulable nitrogen.
1	1.0 c.c.	Total nitrogen	= 0.9 mg. per c.c.			....	....	....
2	1.0 c.c.	......	Acid	0.39 mg.	0.51 mg.	0.5 mg.	....	0.17 mg.
3	1.0 c.c.	......	Alkaline	0.39 mg.	0.51 mg.	0.5 mg.	....	0.15 mg.
4	1.0 c.c.	1.0 c.c.	Acid	0.56 mg.	0.34 mg.	....	0.45(−0.23) mg.	0.22 mg.
5	1.0 c.c.	1.0 c.c.	Alkaline	0.44 mg.	0.44 mg.	....	0.3 (−0.23) mg.	0.07 mg.
6	......	1.0 c.c.	....	....	....	....	0.23 mg.	....

which dried colon bacilli were used. Dried organisms are, as a rule, more easily digested by trypsin than freshly killed organisms, possibly due to alterations in the physical state of the external limiting membrane of the organisms. The bacillary emulsion contained 0.9 of a milligram of total nitrogen per cubic centimeter. When permitted to autolyze in salt solution, both acid and alkaline in reaction, the solution of the organisms was quite marked, only 0.39 of a milligram remaining after twenty-four hours. The total dissolved nitrogen recovered from the supernatant fluid was 0.5 of a milligram, of which 0.17 of a milligram was non-coagulable. When treated with fresh guinea pig complement the lysis was not so marked, being greatest in the tubes with an alkaline reaction; the proteolysis, on the other hand, was greatest in an acid reaction. The increase of 0.05 of a milligram over the amount in the control has, however, no significance, for such conditions never occur in the living animal.

In studying the effect of varying amounts of the antibody on the bacteria and their consequent resistance to tryptic digestion, we have at various times observed that bacteria treated with the largest amount of antibody became more resistant to the action of trypsin,

thus in a measure simulating the Neisser-Wechsberg phenomenon. Such an experiment is shown in protocol V.

PROTOCOL V.

Effect of Tryptic Digestion on Organisms Treated with Varying Amounts of Specific and Non-Specific Immune Serum and Complement.

Tube No.	Typhoid bacilli.	Staphylococci.	Typhoid immune serum.	Complement.	Trypsin.	Tryptic digestion.	Increase.
1	1.0 c.c.	....	Total nitrogen = 1.4 mg.,				
2	....	1.0 c.c.	total non-coagulable nitrogen = 0.07 mg. per c.c. Total nitrogen = 0.715 mg., total non-coagulable nitrogen = 0.16 mg. per c.c.				
3	1.0 c.c.	....	....	....	0.1 c.c.	0.625 mg.	....
4	....	1.0 c.c.	....	....	0.1 c.c.	0.15 mg.	....
5	1.0 c.c.	....	3.0 c.c.	0.3 c.c.	0.1 c.c.	0.54 mg.	−6%
6	1.0 c.c.	....	1.0 c.c.	0.1 c.c.	0.1 c.c.	0.625 mg.	....
7	1.0 c.c.	....	0.1 c.c.	0.1 c.c.	0.1 c.c.	0.65 mg.	+2%
8	1.0 c.c.	....	0.01 c.c.	0.1 c.c.	0.1 c.c.	0.55 mg.	−5%
9	....	1.0 c.c.	3.0 c.c.	0.3 c.c.	0.1 c.c.	0.13 mg.	−3%
10	....	1.0 c.c.	1.0 c.c.	0.1 c.c.	0.1 c.c.	0.20 mg.	+7%
11	....	1.0 c.c.	0.1 c.c.	0.1 c.c.	0.1 c.c.	0.24 mg.	+12%
12	....	1.0 c.c.	0.01 c.c.	0.1 c.c.	0.1 c.c.	0.16 mg.	+1%

In this experiment an emulsion of fresh typhoid bacilli, total nitrogen 1.4 milligrams, and fresh staphylococci, total nitrogen 0.715 of a milligram, were used, permitted to remain with typhoid immune serum and complement for sixteen hours at 37° C., and then washed and digested with 0.1 of a cubic centimeter of trypsin solution. In the controls the typhoid digestion was 0.625 of a milligram, staphylococci digestion 0.15 of a milligram. It will be noted that with three cubic centimeters of the immune serum there was 6 per cent. less digestion than in the control; with one cubic centimeter an equal amount; with 0.1 of a cubic centimeter, a slight increase; while with 0.01 of a cubic centimeter, there was a drop to an amount less than the control. This action was not specific, for when staphylococci and the typhoid immune serum were used a similar and even more pronounced result was obtained. We are inclined to attribute this result to the adsorption by the bacteria of lipoids from the serum, which, if sufficient, as when larger amounts of serum are permitted to act, will protect the bacteria from

tryptic digestion, and overcome the alteration produced by the immune body which under ordinary circumstances seems to render the bacterial body less resistant to trypsin.

In order to avoid the presence of an excess of antibody we have sensitized bacteria with immune serum. They were then washed, treated with complement, washed again, and tryptic digestion of the sensitized and unsensitized bacteria was permitted.

PROTOCOL VI.

Effect of Sensitization of Typhoid Bacilli on Their Tryptic Digestibility.

Tube No.	Typhoid bacteria.	Sensitized typhoid bacteria.	Complement.	Trypsin.	Non-coagulable nitrogen in supernatant fluid.	Tryptic digestion.
1	1.0 c.c.					
2	....	1.0 c.c.				
3	1.0 c.c.	....	....	0.1 c.c.	....	0.162 mg.
4	....	1.0 c.c.	....	0.1 c.c.	....	0.172 mg.
5	1.0 c.c.	....	1.0 c.c.	....	0.31 mg.	....
6	1.0 c.c.	....	0.1 c.c.	....	0.12 mg.	....
7	....	1.0 c.c.	1.0 c.c.	....	0.35 mg.	....
8	....	1.0 c.c.	0.1 c.c.	....	Lost	....
9	....	....	1.0 c.c.	....	0.305 mg.	....
10	....	....	0.1 c.c.	....	0.06 mg.	....
11	1.0 c.c.	....	1.0 c.c.	0.1 c.c.	....	0.172 mg.
12	1.0 c.c.	....	0.1 c.c.	0.1 c.c.	....	0.15 mg.
13	....	1.0 c.c.	1.0 c.c.	0.1 c.c.	....	0.316 mg.
14	....	1.0 c.c.	0.1 c.c.	0.1 c.c.	....	0.25 mg.

Row 1 (Complement/Trypsin/Non-coagulable columns): Total nitrogen = 0.55 mg., total non-coagulable nitrogen = 0.05 mg. per c.c.

Row 2 (Complement/Trypsin/Non-coagulable columns): Total nitrogen = 0.575 mg., total non-coagulable nitrogen = 0.00 mg. per c.c.

In protocol VI there was no increase in autolysis of the two series of bacteria. This will be seen from tubes 5 to 8, the corresponding serum controls being tubes 9 and 10. The sensitized bacteria are much more easily digested by trypsin (tubes 13 and 14) than the unsensitized bacteria (tubes 11 and 12).

DISCUSSION.

In view of the established fact that the serum of practically all animals contains a markedly active antiferment against trypsin, leucoprotease, and the autolytic ferments,—a non-specific substance which in a neutral or slightly alkaline reaction inhibits protease

action of any kind and is essential to life by preventing the splitting of native proteins in the serum to toxic products,—we can hardly expect a result other than that noted in the above experiments; *i. e.,* the fact that bacteriolysis is not associated with a proteolysis. Jobling and Strouse (7) have called attention to the fact that the mere solution of bacteria should not be confused with proteolysis; the former might be due to purely physical factors, as when organisms are kept under lipoid solvents as preservatives. This distinction should be made with equal emphasis for the immunity reactions.

If bacteriolysis is not associated with proteolysis we must find some other explanation. This, we believe, should be sought in the relation of the lipoids of the bacteria and the lipolytic effect of the serum (Jobling and Bull (8)). That a physical change of some sort is induced in corpuscles treated with immune serum and complement has been shown by Eisner and Friedemann (9), and is indicated by the fact that bacterial organisms are rendered more digestible for trypsin. This might be due to (a) an alteration in the lipoidal surface membranes, rendering the organism more permeable for trypsin; (b) an oxidation, rendering the antiferments of the bacteria less active; or (c) the death of the organism (expressing, of course, a physical change) with resulting alteration of the balance of the bacterial protein and lipoids and consequent loss of antitryptic activity.

Our results might be considered to contradict directly the results obtained by several workers with the Abderhalden dialysis method, who have observed an increase in protein split products when bacteria were allowed to digest with immune serum (Voelkel (10)). These workers have ignored the presence of an excess of antiferment in the blood, assuming that proteolysis might take place under normal conditions with resulting splitting of bacteria. Inasmuch as Plaut (11) has recently shown that such absorbing substances as kaolin, starch, etc., give an equally positive reaction, and since de Waele (12) has just demonstrated the same fact by the simple means of precipitating the globulins of the serum, there can be no question that the idea of a specific protease action is unfounded. This view has also been taken by Kirschbaum and Köhler (13), who have been unable to obtain results by means of the dialysis method which

could in any way be interpreted as indicating a specific protease action upon bacteria. This, of course, does not hold true for the peptases, there being no antiferments in the serum against these ferments. Reasoning from a different line of experiments, based on the demonstration of the lipoidal nature of the antiferments (14), we have shown that serum from which the antiferment has been removed will autolyze (15) and that the antiferment can be removed by adsorption (16), after which there is, of course, no further impediment to either protease or peptase action. It is, therefore, more than probable that the dialyzable split products obtained by various workers from the bacterial digestion by immune serum represent digestion products brought about by an alteration in the balance of the ferment and antiferment when the latter is adsorbed by the bacteria. It is more easy to conceive that the soluble serum proteins are split when their antiferment is adsorbed than that the bacterial protein, protected by its lipoids and membranes, should be attacked.

CONCLUSIONS.

1. There is no demonstrable increase in non-coagulable nitrogen during bacteriolysis.

2. Bacteria treated with immune serum and complement are so altered that they are more readily digested by trypsin.

3. Bacteria treated with complement alone become more resistant to proteolysis.

4. Bacteria treated with an excess of immune serum and complement become more resistant to proteolysis.

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