BACTERIAL ANTIFERMENTS.

STUDIES ON FERMENT ACTION.. XVII.*[1]

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The resistance of bacteria to enzyme action, because of its importance as a factor in the defense of the invading organism against the destructive agencies of the host, has interested several observers, among them Kruse (1), Fermi (2), Kantorowicz (3), and Weinkopff (4). Influenced possibly by the epochal advances in protein chemistry and by the fact that bacterial antigens are probably purely protein in character, workers in immunology have naturally investigated with great care the bacterial proteins, and the effect of proteolytic enzymes upon them.

The older conception that bacteria resisted digestion because of some vital property had to be discarded when it was found that organisms killed by chloroform, toluol, carbolic acid, etc., seemed to resist digestion as well as viable bacteria. It has so far been the implied idea that since bacteria contain protein, such protein should be an available substrate for enzyme action, as casein or edestin might be used; in other words, that the bacterial cell represented a naked and a freely exposed mass of protein. In dealing with an intact cell there are, however, certain factors which would indicate that this position is untenable.

Were we to assume that bacteria have a limiting membrane, such a membrane would, if an analogy to plant cells is permissible, contain no protein (Czapek (5)); if similar to animal cells they would probably be protected by a predominatingly lipoidal membrane (Meyer, Overton). There is, of course, no direct evidence, although with the exception of Hamm (6) there has been a uniformity in the reports of the absence of nitrogenous material even from the capsular material derived from the lower organisms. The absence of cellulose in bacteria is of no great import, for many of the true fungi have no cellulose wall and are protected

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only by a delicate membrane; unfortunately the evidence as to the presence or absence of chitin, which might have some bearing on the subject, is conflicting.

Bacteria do, however, contain fats and lipoids in varying amounts, which, because of their marked effect on surface tension, would for purely physical reasons tend to become concentrated at the periphery of a colloidal system such as the bacterial protoplasm. With or without a morphologically distinct limiting membrane we can reasonably assume that the external surface of the bacterial cell is potentially lipoidal. Upon such a substrate we should expect lipolytic rather than proteolytic action. Immunologists have recognized that in cytolysis there are other factors than such distinctly protein manifestations of immunity as are exemplified by the precipitin and agglutinin reactions. Zinsser (7) has recently emphasized this fact. In this connection it is interesting to recall again an observation of Metchnikoff (8). Metchnikoff noted that the intestinal tract of the larva of certain insects (moths) was free from bacteria, and reasoned that this absence of intestinal flora must have some connection with the nature of the enzymes secreted into the gastro-intestinal tract. He calls attention to the fact that these insects, utilizing waxes and fats to a large extent, must secrete powerful lipases.

Oppenheimer (9), reviewing the subject of bacteriolysis from an entirely different standpoint, reaches a similar conclusion. He says: "I have formerly classified these substances (cytolysins) among the ferments because the view was prevalent that the destruction of the cell membrane was due to a specific proteolytic ferment." This view must be discarded, but it is probable that we may later place them among the lipases (10). In a recent paper (11) we have shown by microchemical analysis that bacteriolysis is not necessarily associated with proteolysis.

As briefly outlined, the failure of proteolysis by the action of proteolytic ferments on the intact cell offers then no problem in itself, for there is probably no exposed protein substrate upon which the enzyme may act.

Kantorowicz (3) has probably studied the subject most carefully. He confirmed the older observations of the absence of digestion of the intact cell whether living or dead, and showed that heating Gram-negative organisms to 70° C. made them lose their power of resistance, while Gram-positive organisms would resist digestion even after boiling. He concluded that the resistance was due to an antiferment. He found that dried organisms were as resistant as fresh bacteria, but noted that after drying, grinding in a mortar, and extracting with acetone, the organisms lost their resistance. He assumed two factors to be concerned in bacteriolysis, one of which had to do with the overcoming of the antiferment. Kruse (1) did not, however, agree with the conclusion of his pupil, and doubted that an antiferment as such was involved in the resistance of the organisms. He inclined to the view that differences in resistance among bacteria were due to differences in permeability of the organisms.

Fermi (2) working with cultures, introducing ferments into culture media, reached conclusions similar to those of Kantorowicz. He noted, however, that dried organisms were less resistant than those freshly killed.

The foregoing observations were made by using suspensions of organisms either living or killed in various ways, to which the enzyme was added and the resulting digestive effect observed by the relative clearing of the suspensions. This method is open to several objections. The interpretation of the results is inexact and arbitrary, depending on the observer's judgment, and varying under different conditions. Probably of more importance is the fact that solution of the organisms is interpreted as proteolysis. Solution of a bacterial cell may be largely a physical process related most intimately to changes in its lipoidal constituents. The actual proteolytic cleavage is a purely chemical phenomenon and cannot be determined by ocular observation. This distinction, to which Jobling and Strouse (10) have previously called attention, should be clearly kept in mind, for the careless use of these terms has led to considerable confusion in the interpretation of some immunological experiments. There is, furthermore, a technical error in that it is not possible by the means so far employed to present an approximately equal amount of substrate for the enzyme action, which is important when the comparative rate of digestion of different organisms is sought.

In order to obtain results which would be free from the objections above referred to, our observations have been based exclusively on the determination of non-coagulable nitrogen before and after digestion. These were made by means of the Folin method. Only by such a method can we determine the exact percentage of digestion. We have used organisms grown on agar in large flat bottles. The bacteria were washed, dried *in vacuo* at a low temperature, and then ground in an agate mortar. During the process of drying changes must occur in the colloidal state of the external limiting membrane, so that the uniform resistance of various bacteria to proteolytic action while intact is overcome, and the degree of resistance remaining should be an index of the actual antiferment power of the different organisms.

One other physical factor, the intimacy of the lipoid-protein combination, must be taken into consideration. Numerous workers have pointed out that the lipoids occurring in cells are so closely combined with the proteins that it is impossible by the ordinary

extraction methods to break up their union. This can be accomplished only by a complete hydrolysis of the protein. Thus the determination of bacterial lipoids by means of extraction in the Soxhlet apparatus will give results which are much too low, the real ratio of the lipoidal substances being obtained by extraction after saponification. We have noticed during the course of the work that the lipoids from the Gram-positive bacteria are proportionately extracted more slowly by means of lipoid solvents than from the Gram-negative organisms.

The bacterial emulsions were, as a rule, made up in a 1 per cent. suspension. When fresh organisms were used an effort was made to have the bacterial concentration about one milligram of nitrogen per cubic centimeter. The total non-coagulable nitrogen both before and after digestion was determined by precipitating the coagulable protein with acetic acid and salt, boiling for ten minutes, filtering through kaolinized hard filter paper, and making the usual Folin determination on the filtrate. The trypsin used was freshly prepared before each experiment by dissolving a dried preparation in physiological salt solution.

The lipoid content of the bacteria was determined by saponifying on the water-bath for two hours with alcoholic potash, acidifying, and extracting thoroughly with ether. The iodin value of the lipoids was obtained by the usual Wijs method. It should be noted, however, that the iodin determinations, while often repeated, were of necessity carried out on very small amounts of lipoids, and some caution must be observed in basing conclusions on single observations. When it was not possible to repeat the determinations, we have placed a question mark with the figure to denote the fact. Further observations which we hope to make on larger amounts of material will no doubt give more accurate values. It is reasonable to assume that the lipoid extract and the degree of unsaturation will show marked fluctuations under the varying conditions of growth, nutrition, age, and oxygenation, and, as will be noted, the variations in the total lipoid content of different growths of the same organism were at times marked. Lyons (12) has studied the influence of sugars on the fat content of bacteria, and Cramer (13) has carried out similar experiments.

The amount of lipoids (including fats) has been determined by several observers, as shown in the following table (table I). Inasmuch as the majority of these observations were made by simple ether extraction, the values given are in some instances probably much too low.

TABLE I.

Organism.	Lipoids (including fats).	Observer.
Tubercle bacilli	36–44 per cent.	Baudran (14).
Tubercle bacilli	40 per cent.	Kresling (15).
Tubercle bacilli	31.56 per cent.	Levene (16).
Tubercle bacilli	37.57 per cent.	de Schweinitz and Dorset (17).
Tubercle bacilli	26.2–28 per cent.	Hammerschlag (18).
Bacillus mallei	7.91 per cent.	de Schweinitz and Dorset (19).
Diphtheria bacillus	1.62 per cent.	Dzierzgowski and Rekowski (20).
Meningococci	5.94 per cent.	Ditthorn and Woerner (21).
Pneumobacillus	1.7 per cent.	Brieger (22).
Anthrax bacillus	7.8 per cent.	Dyrmont (23).
Bacillus prodigiosus	4.83 per cent.	Kappes (24).
Bacillus xerosis	8.06 per cent.	Kappes (24).

PROTOCOL I.

Effect of Lipoidal Extraction on the Rate of Digestion of Tubercle Bacilli.

Bacterial suspension 1 c.c.	Trypsin.	Total nitrogen.	Gross digestion.	Digestion, per cent.	Lipoid content, per cent.
Tubercle bacilli[2] (dried)	0.2 c.c.	0.55 mg.	0.13	23	31.2
Tubercle bacilli (extracted with ether, chloroform, or alcohol)	0.2 c.c.	0.74 mg.	0.33	44	9
Tubercle bacilli (extracted in Soxhlet apparatus) (ether 120 hrs.; alcohol 100 hrs.; benzol 50 hrs.)	0.2 c.c.	0.80 mg.	0.46	57	7

The suspensions were made up with an equal amount of sodium carbonate solution.

To these we can add the following determinations:

Tubercle bacilli	32.7 per cent.
Staphylococci	4.51–8.5 per cent.
Diphtheria bacilli	5.5 –7.5 per cent.
Typhoid bacilli	7.0 –8.2 per cent.
Colon bacilli	4.2 –8.15 per cent.
Subtilis bacilli	1.7 per cent.

There are no available determinations of the iodin values of

[2] Previously killed by boiling.

these lipoids with the exception of those made upon the waxes and fats of the tubercle bacillus.

The values which we have found are as follows:

Tubercle bacilli	20 for total lipoids; 24.2 for fatty acids.
Staphylococci	60–80
Diphtheria bacilli	80–110
Typhoid bacilli	33–38
Colon bacilli	32–40
Subtilis bacilli	44 (?)
Tetanus bacilli	44 (?)

Having previously shown that the unsaturated fatty acid radical is antitryptic and its inhibition proportional to the degree of unsaturation (25); that such unsaturated lipoids can be isolated from tubercle bacilli (26) and from tuberculous caseous material (27); that serum antitrypsin consists of similar lipoids, either as free fatty acids, as esters of cholesterol, or combined with lecithin (28), we considered it reasonable to assume that bacteria might resist digestion in a degree proportional to the amount of the unsaturated lipoids contained. It will be remembered that Kantorowicz found that the antiferment was removed from the bacteria when they were ground up and extracted with acetone.

INFLUENCE OF LIPOIDAL EXTRACTION ON DIGESTIBILITY.

We first determined the influence of lipoidal extraction on the tryptic digestion of tubercle bacilli. 1 per cent. suspensions of (1) freshly dried organisms, of (2) extracted bacilli (with 9 per cent. of lipoids), and of (3) extracted bacilli (with 7 per cent. of lipoids) were used. The rate of digestion is shown in protocol I. The organisms used in this work were obtained from Dr. Hitchens and had been killed by heating at high temperatures. In addition they were old, and so some of the unsaturated fatty acids may have become oxidized.

A similar result was obtained with other organisms, as is shown in the following experiment with typhoid and colon bacilli (protocol II). Müller (29) noted that typhoid bacilli from which the lipoids had been extracted were more easily digested by leucocytes.

PROTOCOL II.

Effect of Lipoidal Extraction on the Rate of Digestion of Colon and Typhoid Bacilli.

Bacterial suspensions 1 c.c.	Total nitrogen.	Non-coagulable nitrogen.	Substrate.	Gross digestion.	Increase in non-coagulable nitrogen.	Digestion, per cent.
Colon bacilli.....	1.77 mg.	0.17 mg.	1.60 mg.	0.545 (−0.17)	0.375 mg.	23
Ether-extracted colon bacilli.....	1.87 mg.	0.23 mg.	1.64 mg.	0.95 (−0.23)	0.72 mg.	44
Typhoid baciili.....	1.08 mg.	0.14 mg.	0.94 mg.	0.66 (−0.14)	0.52 mg.	56
Ether-extracted typhoid bacilli.....	1.0 mg.	0.2 mg.	0.8 mg.	0.87 (−0.2)	0.67 mg.	84

THE LIPOIDS AS ANTIFERMENTS.

We have previously noted that the lipoids extracted from tubercle bacilli were antitryptic when saponified (26). That the same is true of the lipoids from other organisms is shown in the following experiment.

Pure cultures of staphylococci and of colon bacilli were removed from bottles, washed, and saponified. After acidification, the lipoids were extracted and again saponified. The soaps were then dissolved in physiological salt solution. Various amounts were mixed with one unit of trypsin, incubated for thirty minutes, and casein was then added for digestion. In another set of tubes the unsaponified bacterial lipoids, suspended in salt solution, were used in equal amounts. The relative inhibition will be noted in protocol III.

PROTOCOL III.

Effect of Bacterial Lipoids, Saponified and Emulsified, on Tryptic Digestion.

Tube No.	Casein.	Trypsin.	Staphylococci lipoids saponified.	Colon lipoids saponified.	Staphylococci lipoids emulsified.	Colon lipoids emulsified.	Gross casein digestion.	Digestion, per cent.
1	2 c.c.	0	0	0	0	0	0.0 mg.	0
2	2 c.c.	0.1 c.c.	0	0	0	0	1.66 mg.	100
3	2 c.c.	0.1 c.c.	2 c.c.	0	0	0	0.217 mg.	13
4	2 c.c.	0.1 c.c.	0	2 c.c.	0	0	0.715 mg.	43
5	2 c.c.	0.1 c.c.	0	0	2 c.c.	0	1.33 mg.	80
6	2 c.c.	0.1 c.c.	0	0	0	2 c.c.	1.54 mg.	92

The iodin value of the lipoids was 97 for those from the staphylococci, and 32 from the colon bacilli.

THE COMPARATIVE RATE OF DIGESTION.

We next studied the comparative rate of digestion of bacteria, and the relation to the lipoids contained in the bacteria. Such an experiment is shown in protocol IV, and has for convenience been charted (text-figure 1). As a lipoidal antitryptic index we may multiply the percentage of lipoids by the iodin value, as determined for the organisms used in this experiment.

PROTOCOL IV.

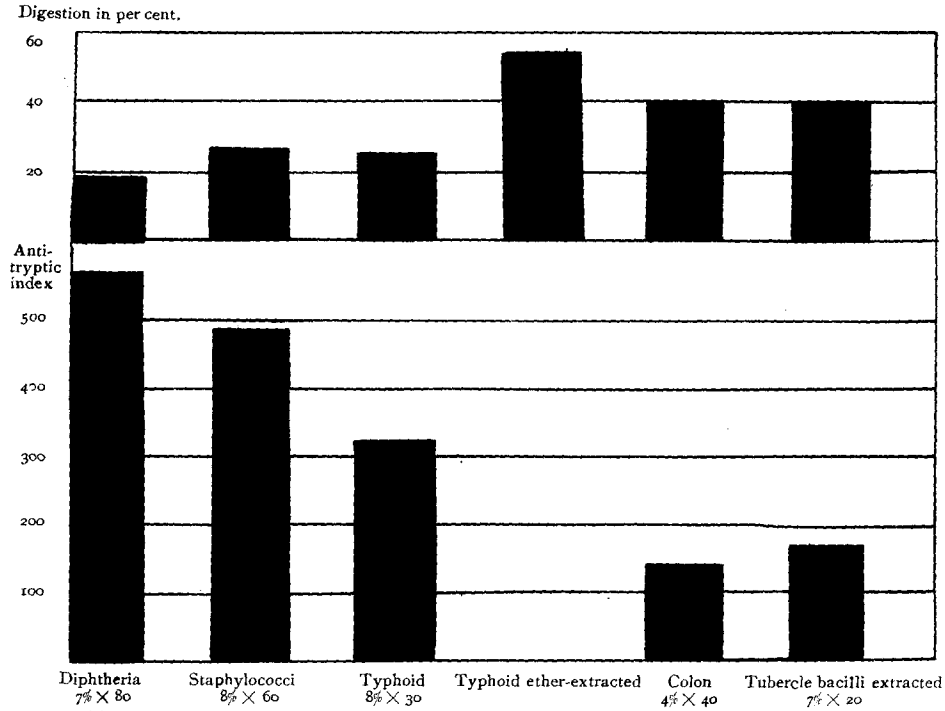
Comparative Rate of Tryptic Digestion of Bacteria.

Bacterial suspension 1 c.c.	Total nitrogen.	Non-coagulable nitrogen.	Substrate.	Gross digestion.	Increase in non-coagulable nitrogen.	Digestion, per cent.
Diphtheria bacilli	1.0 mg.	0.12 mg.	0.88 mg.	0.28(−0.12)	0.16 mg.	18
Staphylococci	0.83 mg.	0.15 mg.	0.68 mg.	0.31(−0.15)	0.16 mg.	23
Typhoid bacilli	1.42 mg.	0.21 mg.	1.21 mg.	0.5 (−0.21)	0.29 mg.	24
Typhoid bacilli (ether-extracted)	0.9 mg.	0.35 mg.	0.55 mg.	0.66(−0.35)	0.31 mg.	56
Tubercle bacilli (ether-extracted)	0.83 mg.	0.12 mg.	0.71 mg.	0.4 (−0.12)	0.28 mg.	40
Colon bacilli	0.8 mg.	0.12 mg.	0.68 mg.	0.39(−0.12)	0.27 mg.	40

Diphtheria bacilli ..... 7 per cent. × 80 = 560
Staphylococci ..... 8 per cent. × 60 = 480
Typhoid bacilli ..... 8 per cent. × 30 = 240
Typhoid bacilli (extracted) ..... ? ? ?
Colon bacilli ..... 4 per cent. × 40 = 160
Tubercle bacilli (extracted) ..... 7 per cent. × 20 = 140

This offers, of course, only a general approximation, and cannot take into consideration the fact that the lipoids vary no doubt in the intimacy of their connection with the proteins, a factor which must influence their ability to protect the protein. The digestion as determined was as follows:

Diphtheria bacilli ..... 18 per cent.
Staphylococci ..... 23 per cent.
Typhoid bacilli ..... 24 per cent.
Typhoid bacilli (extracted) ..... 56 per cent.
Colon bacilli ..... 40 per cent.
Tubercle bacilli (extracted) ..... 40 per cent.



TEXT-FIG. 1. Relation of the antiferment index to the rate of digestion of various organisms.

These figures show a striking parallelism between the antitryptic property and the lipoidal protecting bodies.

A similar experiment can be shown if we compare the rate of digestion of the Gram-positive organisms, the lipoids being as follows:

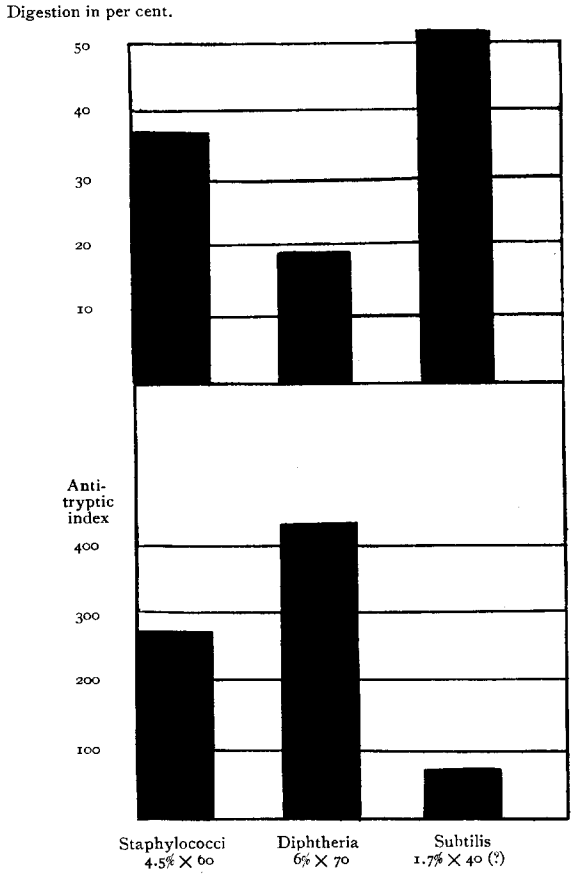
Diphtheria bacilli ..... 6.0 per cent. × 70 = 420
Staphylococci ..... 4.5 per cent. × 60 = 270
Subtilis bacilli ..... 1.7 per cent. × 40 = 68

PROTOCOL V.

Comparative Rate of Digestion of Staphylococci and Diphtheria and Subtilis Bacilli.

Bacterial suspension 2 c.c.	Total nitrogen.	Non-coagulable nitrogen.	Substrate.	Gross digestion.	Increase in non-coagulable nitrogen.	Digestion, per cent.
Staphylococci.....	1.54 mg.	0.375 mg.	1.17 mg.	0.8 (−0.375)	0.425	36
Diphtheria bacilli.....	1.66 mg.	0.19 mg.	1.47 mg.	0.47 (−0.19)	0.27	19
Subtilis bacilli.....	1.3 mg.	0.48 mg.	0.82 mg.	0.909 (−0.48)	0.429	52

The organisms digested in the order indicated, the digestion being least with the diphtheria bacilli. The results are shown in protocol V and in text-figure 2.



TEXT-FIG. 2. Relation of the antiferment index to the rate of digestion of Gram-positive organisms.

THE EFFECT OF HEAT.

It has been mentioned that a difference existed in the resistance to heat of the Gram-positive and the Gram-negative organisms. This is shown in the following experiments.

A suspension of typhoid bacilli was heated at 50°, 60°, 70°, and 100° C. The

tubes were then subjected to the action of trypsin. The percentage of digestion was as follows:

50° C. = 31 per cent.
60° C. = 31 per cent.
70° C. = 36 per cent.
100° C. = 41 per cent.

As Kantorowicz (3) has found, there is a reduction of the resistance after heating to 70° C., but the effect is not so striking as one might expect if a complete destruction of the antiferment had resulted. The Gram-positive organisms, staphylococci for example, show no change after heating, unless it is a slight increase in the resistance. The percentage of digestion was as follows:

Unheated = 55 per cent.
50° C. = 54 per cent.
60° C. = 35 per cent.
70° C. = 41 per cent.
100° C. = 38 per cent.

In a second experiment the results were as follows:

Unheated organisms = 16 per cent.
70° C. = 18 per cent.
100° C. = 14 per cent.

LIPOIDAL ADSORPTION BY BACTERIA.

We have previously mentioned that bacteria suspended in normal serum are able to adsorb the unsaturated lipoids from the serum, and by virtue thereof to become more resistant to the action of trypsin (30). Such an experiment follows.

A suspension of typhoid bacilli was incubated with 10 c.c. of normal guinea pig serum for six hours. The bacteria were then centrifuged, washed, and dried at a low temperature. Untreated bacteria were used as a control. Emulsions were prepared containing 1 mg. of nitrogen per cubic centimeter, and to each was added 0.1 c.c. of trypsin solution and 2 c.c. of a 0.5 per cent. solution of sodium carbonate. Digestion was permitted for six hours. The digestion of the untreated bacteria was 75 per cent., of the serum-treated bacteria 23 per cent.

The lipoidal adsorption can be shown in a more direct manner, as is illustrated in the following experiment.

Dried subtilis bacilli were added to 25 c.c. of horse serum and incubated for three hours. The bacteria were then washed after being centrifuged from the serum. The lipoid content of the bacteria and of the supernatant serum was now determined.

Total lipoids in 25 c.c. of original serum		0.083 gm.	
Total lipoids in 25 c.c. after incubating with bacteria		0.060 gm.	
		0.023 gm.	loss.
Weight of bacteria recovered	0.6 gm.		
Lipoid content originally present, 1.7 per cent	0.0102 gm.		
Total lipoids recovered from bacteria		0.036 gm.	
Less lipoids originally present		0.0102 gm.	
		0.0258 gm.	gain.

While there has been a loss of 0.023 of a gram in the ether-soluble substances from the serum, the bacteria show a corresponding gain. The absorption of lipoids is made especially evident in this case because of the small lipoid content of the organisms used. The lipoid determinations were made after thorough saponification of both bacteria and serum on the water-bath for three hours.

DISCUSSION.

Inasmuch as the bacterial lipoids may show more marked fluctuations than the other constituents, the quantity depending largely on the composition of the nutrient media and to some extent on the age of the culture, and the chemical quality, unsaturation, being subject to changing oxidative conditions, we are of the opinion that the demonstration that the antiferment property of bacteria depends on definite quantitative as well as qualitative differences in the lipoidal constituents of the bacteria may offer the means of solution of some still obscure problems in immunity. Thus in the study of virulence, depending on the invasive power of the organisms and on their resistance against the defensive mechanism of the host a partial solution might be found in a comparative study of the lipoids under different conditions of bacterial existence. We have experiments under way to elucidate this point. So too, a further study of the adsorption of the lipoids from the serum offers an attractive field. While in this process the resistance of the intact bacteria to proteolytic enzymes is not increased, for on such organisms the

enzymes probably find no substrate upon which they may become fixed and active, nevertheless the metabolic processes of the cell, depending wholly upon the rate of exchange of nutritive and excretory products through the cell membrane, must be profoundly altered. Indeed it might be conceived that such a disturbance of the proper functioning of the membrane would under some conditions lead to the death of the cell. On the other hand, an absorption of serum lipoids, by preventing the excretion of toxic metabolic products, such as leucocydins, virulins, etc., might bring about phagocytosis of organisms otherwise resistant. That the so called opsonins are closely related to the lipoids seems to have been established by the work of Stuber (31).

That the bacterial lipoids influence the intracellular proteolytic activity becomes apparent when we compare the amount of non-coagulable nitrogen contained in various organisms. Thus diphtheria bacilli, with a high antiferment index, have only from 5 to 7 per cent. of the nitrogenous material in a non-coagulable form, whereas in subtilis bacilli, with a low antiferment index, a correspondingly greater protease activity is indicated by the presence of from 33 to 35 per cent. of the total nitrogen in a non-coagulable form. Staphylococci, typhoid bacilli, and colon bacilli occupy an intermediate position. This influence is analogous to the effect of the antiferment on the protein metabolism of higher organisms, as we shall show in a subsequent paper.

The antiferment, being non-specific, is able not only to inhibit the action of the digestive ferments, but is probably the agent concerned in preventing excessive protease action, or autolysis, in the cells. It is easy to understand that with the death of the cells and subsequent development of an acidity because of the failure in the removal of acid waste products, the antiferment property is greatly lessened by changes in the state of dispersion brought about by the increase in acidity. This lowering of the antiferment property of the unsaturated lipoids by a change in their dispersion is most easily demonstrated with serum antitrypsin. The morphological expression of the process is possibly to be found in the development of the so called myelin figures found in cells stained during the early stages of autolysis. It is probable that the increase in the rate of

autolysis following the addition of certain inorganic colloids to liver emulsions, noted by Ascoli and Izar (32), depends on an absorption of the lipoidal antiferments.

Kantorowicz (3), in view of the difference in behavior of the antiferment in Gram-negative and Gram-positive organisms, is inclined to the view that the antiferments are in this case different. It seems more reasonable to assume that the difference noted is due to a purely physical condition. It is possible that in the Gram-positive organisms the lipoids are in more intimate association with the protein molecule, so that when dispersion changes are brought about, as by heating, the relative protection by these lipoids remained unaltered. This idea would find support in view of the fact that the lipoids from Gram-positive organisms resist extraction by lipoid solvents to a greater extent than do those from Gram-negative bacteria.

In view of the work of Tamura (33), definitely showing that the bacterial constituent responsible for the Gram stain is lipoidal in that it can be found in the lipoid extract, and as the unsaturated fatty acids and their salts take the Gram stain easily, because the iodin can be bound to the unsaturated carbon atoms, it would seem rational to seek some connection between the degree of unsaturation of the bacterial lipoids and the Gram staining ability. From this point of view the high iodin values of the lipoids from diphtheria bacilli and staphylococci, as compared with those of typhoid or colon bacilli, might seem significant, were it not for the fact that in the case of the tubercle and subtilis bacilli, the results are apparently the reverse. Knudson (34), working in Gies's laboratory, finds, however, that the lipoids from tubercle bacilli, which have a low iodin value (24), contain several of the higher unsaturated fatty acids.

As a result of our study we are inclined to emphasize the importance of the unsaturated lipoids as representing probably a most important factor in the regulation of the proteolytic activity of the cellular metabolism, due to the fact that the protein lipoid combination resists the action of the proteolytic enzymes.

CONCLUSIONS.

1. Intact bacteria probably resist tryptic digestion because of the absence of an exposed protein substrate.

2. Dried organisms resist digestion in a degree proportional to their content of unsaturated lipoids.

3. Lipoidal extractives reduce the resistance to tryptic digestion.

4. The extracted lipoids (saponified) are antitryptic in a degree proportional to their unsaturation.

5. The inactivation of the antiferment in Gram-negative organisms is probably due to changes in the degree of lipoidal dispersion.

6. Bacteria adsorb lipoids from the serum when incubated at 37° C. Such organisms when dried are found to be more resistant to tryptic digestion than untreated organisms.

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