STUDIES ON OXIDATION AND REDUCTION BY PNEUMOCOCCUS.

VII. Enzyme Activity of Sterile Filtrates of Aerobic and Anaerobic Cultures of Pneumococcus.

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In the preceding paper (1) it has been shown that the oxidation products formed during the oxidation of broth extracts of unwashed cells exert a deleterious action on certain enzymes of Pneumococcus. These enzymes are endocellular and are actual constituents of the pneumococcus cell. If cultures of Pneumococcus are filtered during the period of active growth before autolysis occurs, the enzymes are retained in the cells and are not demonstrable in the cell-free filtrate.

During cell autolysis which accompanies senescence of Pneumococcus the intracellular enzymes are liberated into the culture medium almost as completely as when the cells are dissolved by bile or are ruptured by freezing and thawing. Hence, it would seem that all of the intracellular enzymes which are demonstrable in bile solutions or frozen and thawed suspensions would likewise be present in the sterile culture filtrates. Avery and Cullen (2) have, in fact, demonstrated an active lipase and peptonase in the filtrates of autolyzed cultures. However, in view of the results of the preceding paper, it is evident that the enzyme activity of sterile filtrates of pneumococcus cultures will depend not only upon whether the endocellular enzymes are liberated into the culture medium, but also upon whether the enzymes have been protected from products of oxidation. Accordingly, one might expect to find much the same relations between the enzyme activity of the filtrates of anaerobic and of aerobic cultures as those already demonstrated between "reduced" and "oxidized" broth extracts of unwashed cells. The most obvious difference between the broth extracts of unwashed cells and the filtrates of autolyzed

cultures consists in the concentration of the cellular substances. However, an equally important difference exists in the concentration of the peroxide which is formed in aerobic cultures in comparison with that which accumulates in "oxidized" cell extracts. Although peroxide is formed in relatively large amounts during the oxidation of a sterile pneumococcus extract, it gradually decreases in concentration and frequently disappears within 24 hours. On the other hand, peroxide persists for long periods in aerobic broth cultures of Pneumococcus (Avery and Morgan (3)) since apparently the cells continue to respire with the production of peroxide long after active cell growth ceases (Avery and Neill (4)).

A more complete destruction of enzymes, therefore, might be expected in an aerobic, autolyzed culture than in an "oxidized" sterile cell extract, since the initial concentration of the enzymes is smaller in the unconcentrated culture fluids and since the period of exposure of the enzymes to high concentrations of peroxide is considerably longer in the case of the aerobic cultures. Accordingly, it is possible that some of the pneumococcus enzymes that are unaffected during the oxidation of the sterile cell extracts might be destroyed or at least seriously impaired by prolonged exposure to the oxidation products which accumulate in the aerobic cultures. To test these relations, comparisons have been made of the activity of various intracellular enzymes in filtrates of autolyzed cultures of pneumococci grown under aerobic and anaerobic conditions.

In unagitated culture flasks containing large volumes of broth with a small surface exposed to the air the medium has a low oxygen tension and presents reducing conditions not unlike those prevailing in anaerobic cultures. Under these circumstances the enzymes are afforded a considerable degree of protection from oxidizing agents. Therefore, in order to facilitate the ready diffusion of oxygen into the culture fluid, the aerobic cultures used in the following experiments were grown in broth with a large surface area exposed to air.

Aerobic and anaerobic cultures of four strains of pneumococci (Type I, I 93; Type II, G and D 39; Type III, A 66) were prepared by adding equal inocula of each strain to a series of flasks of plain broth. For the aerobic series, 50 cc. of broth were exposed to the air in 300 cc. Erlenmeyer flasks; in the case of the anaerobic series, 100 cc. Florence flasks were filled to the neck with broth and

sealed with vaseline after inoculation. The inoculated flasks were incubated at 37°C. for 7 days, after which time autolysis was practically complete in each culture of both series.

To control any effect of oxygen tension upon the actual elaboration of the enzymes, one strain (G), was grown in a 200 cc. anaerobic culture. At the end of 36 hours one portion of this culture was exposed to air under the above conditions and the other portion was allowed to autolyze under anaerobic conditions.

The autolyzed cultures of all the strains were then filtered through Berkefeld V candles; the anaerobic cultures were filtered in a nitrogen atmosphere by the method employed for the filtration of sterile "reduced" extracts. The filtrates were subcultured in blood media as sterility controls.

TABLE I.

Comparison of Activity of Various Enzymes in the Filtrates of Aerobic and Anaerobic Cultures of Pneumococcus.

Strain.	Lipase. N/14 acid in 10 cc.		Sucrase. Reducing sugars.		Bacteriolytic enzymes. Bacteriolysis.		Hemotoxin. Hemolysis.	
	Aerobic filtrate.	Anaerobic filtrate.	Aerobic filtrate.	Anaerobic filtrate.	Aerobic filtrate.	Anaerobic filtrate.	Aerobic filtrate.	Anaerobic filtrate.
	cc.	*cc.*						
G	0.8	5.2	−*	++†	−	+++	−	+++
I 193	1.0	4.9	−	++	‡	‡	‡	‡
D 39	0.6	4.8	−	++	−	+++	−	+++
A 66	0.8	5.0	−	++	−	+++	−	++++

* Negative test; less than 0.03 per cent glucose.

† Between 0.2 and 0.3 per cent glucose.

‡ Not determined.

The sterile filtrates of the aerobic and anaerobic cultures were tested for enzyme activity by the substrate-enzyme mixtures indicated below.

Lipase.—10 cc. filtrate + 0.2 cc. tributyrin.

Sucrase.—10 cc. filtrate + 1.0 cc. 20 per cent solution of sucrase.

Bacteriolytic Enzyme.—0.1, 0.5, and 1.0 cc. of filtrate were added to a series of tubes containing 0.5 cc. suspension of heat-killed pneumococci (Type II) in 0.10 M phosphate solution (pH 7.6); the volume was made up to the 1.5 cc. in each case with salt solution.

Hemotoxin.—0.5 cc. filtrate + 2.5 cc. of 1 per cent solution of red blood cells.

The activity of the various enzymes in the filtrates was determined by the methods used in analogous experiments in the preceding paper (1). The results are given in Table I.

The results of the type experiment given in Table I show that the enzyme activity of the filtrates of anaerobic cultures of Pneumococcus is much greater than that of the filtrates of aerobic cultures. The hemotoxin, sucrase, and bacteriolytic enzymes were not demonstrable in any of the aerobic filtrates, although all of these substances were active in the filtrates of the anaerobic cultures.

The bacteriolytic enzyme, which was destroyed only to a small extent during the oxidation of the sterile cell extracts, was completely inactivated in the autolyzed cultures which were exposed to air. Similarly, although lipase was apparently unaffected by oxidation of the extracts, this enzyme was almost completely destroyed in all of the aerobic cultures. It is evident, then, that the exposure of the enzymes to the oxidizing agents present in the aerobic cultures results in a more complete loss of activity than that which occurs during the oxidation of the sterile extracts. The destruction of the relatively resistant enzymes in the aerobic cultures is no doubt due to the longer exposure to higher concentrations of peroxide.

Differences in enzyme activity of the filtrates of aerobic and anaerobic cultures would seem to be due to three possible factors: (1) the possible influence of different oxygen tensions during cell growth upon the actual elaboration of the various enzymes and other cell constituents; (2) the possible influence of oxygen tension upon the liberation of the elaborated enzymes by autolysis; and (3) the destruction of the enzymes, after their elaboration, by oxidizing agents in a manner analogous to that occurring during the oxidation of sterile pneumococcus extracts. The first of the above factors seems to be eliminated since anaerobic cultures show a similar loss in enzyme activity when later allowed to autolyze under aerobic conditions. The influence of oxygen concentration upon autolysis and hence upon the liberation of the enzymes seems also to be eliminated, as there was no significant difference in the degree of autolysis finally reached in the aerobic and anaerobic cultures. Hence, the observed differences in the enzyme activity of the filtrates of aerobic and anaerobic cultures of Pneumococcus may be attributed to an actual destruction of the formed enzymes by oxidation reactions analogous to that which occurs during the oxidation of sterile broth extracts of unwashed pneumococci.

SUMMARY.

Sterile filtrates of autolyzed anaerobic cultures of Pneumococcus contain much higher concentrations of active endocellular enzymes than do the filtrates of autolyzed aerobic cultures. These differences in the enzyme activity of the filtrates of aerobic and anaerobic cultures may be explained by a destruction of the formed enzymes by oxidation reactions analogous to the destruction previously observed during the oxidation of sterile broth extracts of unwashed pneumococcus cells. The occurrence of more complete destruction of enzyme activity in autolyzed aerobic cultures than in "oxidized" sterile cell extracts is probably due to the longer exposure to oxidation products.

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