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Study of lysozyme and its relation to natural bactericidal phenomena

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A STUDY OF LYSOZYME AND ITS RELATION TO NATURAL BACTERICIDAL PHENOMENA

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INTRODUCTION

Invaluable contributions have developed from a study of natural occurring materials, such as penicillin. Because of this it was thought that it would be worthwhile to investigate such a widely distributed and powerful bacteriolytic agent as lysozyme. In this thesis a concept of lysozyme, perhaps somewhat arbitrarily, will be presented and upon this basis its properties will be evaluated and an attempt will be made to determine its role in the bactericidal properties of such biological materials as tears, mucus, and saliva. Finally, it will be discussed in regard to disease resistance and therapeutic possibilities.

The history of lysozyme research has been relatively recent. In 1909 the microscopic lysis of Bacillus subtilis and related organisms by egg white was observed by Laschtshenko (2). Suzuki (2) in 1911 reported microscopic clearing of emulsions of air cocci by leukocytic extracts. Bloomfield (3) in 1919 while working on the fate of organisms in the upper air passages, discovered a high potency of nasal and mouth secretions in their ability to kill sarcina lutea. He did not, however, attempt to purify the substance responsible for this and to further analyze its properties. It remained for Fleming (1) in 1922 to appreciate the remarkable potency and widespread nature of these lytic substances. This event was the real starting point for the great amount of experimental work that has been done since on this problem.

Fleming's evidence showed that the agents concerned in the lytic action were of enzymatic nature, and he applied to them the term, "lysozyme," or lytic enzyme. The term has since been generally used but has been applied loosely. There has been no agreement as to whether it should be applied to all agents that dissolve the test micrococcus of Fleming or whether agents distinct from lysozyme could dissolve these micrococci; likewise it is uncertain whether the term should be

applied to substances which dissolve other organisms but not Fleming's micrococcus. Many workers have taken for granted that any anti-bacterial activity of complex biological fluids known to dissolve micrococci was due to lysozyme. Since there is clear evidence that several agents, widely different in their properties, are concerned in such anti-bacterial effects, there is need for more precise criteria for the application of this term.

Because of its ready availability and its high lytic content for saprophytic bacteria, hen egg white has been studied intensively and a rather exact knowledge of its chemical and physical nature has been accumulated. Because of this, Thompson (2) believes the concept of lysozyme should be built upon this knowledge. Therefore in this paper the term "lysozyme" will be reserved for only those agents which possess the properties of the lytic agent of egg white.

In his original paper, Fleming (1) reported that egg white lysozyme as tested on his test micrococci was soluble in water and physiological saline. It was precipitated but not harmed by chloroform, ether, acetone, alcohol, and toluene. Later he and Allison (4) found it was not affected by peptic or tryptic digestion. However Epstein later showed an inactivation by

crystalline pepsin but not by crystalline trypsin. Wolff (2) attempted to purify it by removing extraneous matter with colloidal iron and then precipating it with acetone. He obtained a very active material but his chemical analysis of it showed neither nitrogen or sulphur which is in disagreement with the work of all the more recent investigations. Meyer and his co-workers (5) next attempted purification of the lytic agent of egg white. Later contributors, Roberts and co-workers (6) and Abraham and co-workers (7), are essentially in agreement with the work of Meyer.

According to them, lysozyme is of the following nature. It is a basic protein or polypeptide containing about 16% nitrogen and 2 to 3% sulphur. It has a molecular weight between 18,000 and 25,000 and a molecular size of less than 30 millimicrons. Lysozyme is soluble in acidified aqueous mediums and insoluble in alkaline mediums. It is stable in acid. Partially purified solutions retain their original activity after being kept at 100 degrees centigrade for thirty minutes at pH 4. Very pure preparations are less stable but their stability increases down to pH 4. In neutral and especially in alkaline mediums this lysozyme is inactivated readily by heat. In acid solutions it passes

easily through Berkefield filters but in neutral solutions it passes with difficulty and only with the filtration of large volumes. Meyer (5) and co-workers found it was inactivated by oxidizing agents, such as hydrogen peroxide and iodine, and by some heavy metals. Under certain conditions this inactivation was reversed by hydrogen sulfide or by a cyanide, and these workers concluded that one active group contained a sulphydryl radical.

The actions of lysozyme

The lysis of certain susceptible saprophytic bacteria is the property which has attracted most interest and is the one from which the term lysozyme was derived. Fleming (1) used this as a means of determining lysozyme concentration by making serial dilutions and finding the lowest concentration which would clear an opaque suspension of the susceptible bacteria. Later Boasson (8) measured the amount of lysis by measuring the scattered light thermoelectrically. Thompson (2) has used photoelectric methods to measure the same effects.

The bacterial changes which occur before lysis takes place have included marked swelling. This knowledge, however, was acquired from the study of stained preparations. Boasson (8) later studied dark field suspensions

as well and found no swelling there. From this he concluded that there was only a loss of turgor which caused flattening and apparent enlargement of the stained specimen. He attributed the loss of turgor to increased permeability of the cell wall and the escape outward of the soluble cell contents.

It has been shown by Fleming (1) and confirmed by other workers that lysozyme will not only dissolve the susceptible bacteria but will exert also a bactericidal and bacteriostatic effect as well. He made agar pour plates of M. lysodeikticus and placed lysozyme solutions in cups cut out of the agar. On incubation the cocci grew throughout the plates but not in the zones around the area of lysozyme. The zones of inhibition were wide and distinct and thus indicated that the lysozyme diffused quite freely through the agar. He showed that the cleared zones were usually sterile. Thompson (2) and colleagues have generally agreed that the lytic power, bacteriostatic power and bactericidal power closely parallel one another in lysozyme preparations.

The fate of lysozyme during lysis of organisms further suggests an enzymatic nature. Fleming (1) showed that the lytic agent is rapidly drawn from solution and fixed to the cells. Boasson (8) later confirmed this by centrifuging the cells from a mixture before

lysis took place. Furthermore, Fleming and Allison (9) showed that when lysis occurs, lysozyme is released with no demonstrable neutralization occurring. In fact they thought there may be some increase in lysozyme following the lysis. Other workers (Boasson (8); Thompson (2) have not confirmed this however. In this way lysozyme differs from the ordinary bacteriolysin of serum, which is diminished as lysis proceeds (Fleming 10).

The chemical nature behind the action of lysozyme has from the first, as its name implies, been considered that of an enzyme. It has been generally agreed from the microscopic evidence mentioned above that the site of action is on the cell membrane. However it was not until the work of Meyer and his co-workers (11) that any insight as to the nature of this action was gained. They showed that lysozyme preparations contain no protease, kinase, amylase, lipase or phophatase activity. By studying the chemical changes induced by lysozyme on suspensions of susceptible sarcinae, they found a marked increase in reducing sugars and concluded the enzyme had a specif c action on the sugar linkages of certain amino sugar-containing carbohydrates. Increases in nonprotein nitrogen and inorganic phosphorus which occurred o solution of bacterial cells they ascribed to releas of the material enclosed by the bacterial mem-

branes. This work indicated that the primary change produced by the enzyme probably consists in disaggregation of a highly viscous component. Later Epstein and Chain (12) isolated from the dried bodies of M. lysodeikticus (a highly susceptible air cocci isolated first by Fleming (1), the substrate. This was a highly molecular weight polysaccharide forming viscous solutions in water, insoluble in organic solvents but soluble in 3% trichloroacetic acid. It was not dialyzable. However when acted upon by purified lysozyme, about 50% of the material in the purest preparation became dialyzable, the dialyzable split products containing N-acetyl hexosamine and keto-hexose groups. This showed that lysozyme is an enzyme of the carbohydrase class. They too found the purified substrate water soluble but concluded it occurred in the bacteria as an insoluble form possibly either in a higher polymerized state or in combination with a protein, resistant to the action of pepsin. This polysaccharide substrate was found by them in all lysozyme susceptible organisms, even those which were not lysed.

The main factors influencing the action of lysozyme are pH, salt concentration, temperature, and bacterial changes. Fleming (1) found that the rate of ly-

sis was directly related to temperature, increasing to a maximum at about 60 degrees centigrade. Thompson (2) found it to be 55 degrees centigrade. Obviously the point at which increase in temperature ceases to increase the rate of action, depends on the temperature at which destruction of the enzyme begins, which in turn depends on the pH of the material used.

Fleming (1) found that maximum lysis occurred at about the neutral point. Other workers have obtained somewhat different results. However it seems to be definitely established by Thompson (2) that the optimum pH as determined by the cleaning of bacterial suspensions is not actually the most favorable pH for the action of the enzyme but a composite optimum depending on several factors. At some point not far above neutrality an increase in the lability of the lysozyme enters and destruction may occur at the temperature of incubation. Nakumara (cited by Thompson (2) showed further, and it has been confirmed, that acidified cells are not lysed by lysozyme but that if alkali is added to the mixture the cells dissolve immediately. This is explained upon the basis that the cellular contents are not soluble in the more acid solutions. Actually both Meyer (11) and Epstein (12) found the optimum pH for the action of lysozyme on the mucoid substrate to be 3.5. This of

course is not in agreement with those figures obtained by lytic titration methods using bacterial suspension.

Another factor affecting the action is salt concentration. Fleming and Allison (1) working with tear preparations found 0.5% the optimum concentration, higher ones causing inhibition. This has been confirmed for egg white (2). Boasson (8) has shown that the higher the valency the stronger the inhibitory effect of the salt. He thought this might indicate the importance of electrostatic forces in absorption and associate the lysozyme reation with other immunity reactions. He also showed that distilled water inhibits lysis for the same reason that acid does, i.e. because of lack of solubility in it of bacterial cell contents.

Bacterial changes themselves affect the lytic action. Most workers seem to agree that heat killed organisms are definitely less susceptible. Again, as shown by Fleming and Allison (1), this is a matter of producing insolubility of the cell contents. They found that the suspensions could be cleaned by trypsin, which had no effect previous to the lysozyme action. Increase in susceptibility was found by Kopeloff (27) and co-workers to occur when the cocci were cultivated on beef extract agar instead of casein digest agar. Fleming and Allison (13) found that extremely resistant strains

could be developed by culturing in a broth containing progressively higher concentrations of egg white. Hallauer (cited by Fleming (10) also showed this effect by a similar method.

Other sources of agents which act in a similar manner as egg white lysozyme.

Fleming (1) first appreciated the widespread distribution of lysozyme. He has demonstrated its presence in many plants, in tissues and secretions of all types of animal life. Hen's eggs comprise the richest source of lysozyme being followed by human tears which are about one-half as potent as egg white. Of the human secretions and body fluids, all contained lysozyme except urine, sweat and cerebrospinal fluid. Tears, at least as far as M. lysodeikticus is concerned, are the most potent, while nasal mucus and sputum are also very powerful. According to his results blood serum is only 1/000 as potent as tears. Bradford and Roberts (14) maintain that the lysozyme of the serum probably originates from the leucocytes. These were found to be exceptionally rich in lysozyme by both Fleming (10) and Ridley (15).

Many human tissues have been tested for lytic agent content by Fleming (10). His preparations were normal saline extracts of the tissues. These were then

titrated against M. lysodeikticus suspensions. He found human cartilage to be richest in lysozyme. In another publication Fleming (16) stated, somewhat teleologically, that this might compensate for the lack of infection resistance mechanisms, as poor blood supply, of this tissue. The least powerful tissue was the brain. Stomach, intestine, were moderately potent. Florey (17) titrated the lytic agent content of extracts of many tissues of several animals and compared them with human tissues. His results in the main agreed with those of Fleming except in regard to cartilage which he found only moderately potent. Florey used a different test organism than Fleming which may account for the discrepancy. Other points of interest in Florey's work were that he found all cat tissues quite deficient in the lytic agent with the striking exception of the salivary gland. He found that human tissues are almost always stronger than those of the guinea-pig, but that the guinea-pig's lymph gland is eight times stronger than the human's.

What is the similarity of these agents to the egg white lysozyme? It would not be expected that enzymes from such widely varied sources would have an identical constitution. There would be practical proof of this, however, if it were demonstrated that they have similar

chemical properties and a specific action on identical substrate in susceptible organisms. Fleming (10) found that the active agents in a number of substances which dissolved his test coccus, were alike in heat resistance and solubility and in being precipitated and not injured by acetone and similar organic solvents. Roberts (18) and co-workers doing careful studies on cat saliva found it to be essentially the same as egg white lysosyme. In doing this they formed similar salts of similar solubility and sedimentation constants. Meyer (5) and co-workers did, however, found that the agents in egg white and saliva were antigenically distinct. The serum of rabbits immunized with purified enzyme from egg white precipitated and neutralized this enzyme but had no effect on purified enzyme from cat's saliva. A slight neutralization and precipitation of the enzyme in human saliva was effected by the anti-egg white enzyme serum.

Probably even more significant in proving important similarities of these various agents was the work of Epstein and Chain (12). They studied the chemical actions of rabbit leucocytic extracts, human tears, cat and human saliva on the purified carbohydrate substrate obtained from M. lysodeikticus. They found that all these agents acted on the substrate with the liberation

of N-acetyl hexosamine and other reducing substances as did purified egg white preparation.

Fleming and Allison (19) developed resistant strains of cocci by culturing them in media containing dilute amounts of lysozyme preparations. They found that strains made resistant to one agent were similarly resistant to other agents tested from such varied sources as turnip juice, egg white, sputum and tears.

From the foregoing evidence it can probably be reasonably concluded that these various agents found to be lytic for air micrococci resemble each other closely in chemical constitution and act in an identical manner on the carbohydrate substrate in these organisms and thus can all be called by the term "lysozyme."

Factors which influence the lysozyme concentration in secretions and tissues.

Hypersecretion of lysozyme secreting glands definitely seems to cause a decreased concentration in the secretion. This was first shown by Ridley (15) who found that the tears of people suffering from epiphora were below normal in lysozyme concentration. This has since been confirmed by James (10), and Thompson and Gallardo (21). Hallauer (cited by Thompson (22) found that stimulation of tears in a dog by pilocarpine diminished the lysozyme content. Both James (20) and Ridley (15)

demonstrated that the use of atropine in patients suffering from epiphora caused the lysozyme titre of the tears to increase, although it had no such effect when used on a normal person. According to the studies of Hilding (23) lysozyme concentrations are diminished during a cold. However he does not believe this is due to hypersecretion because he showed marked decreases before the hypersecretion phase took place. Along this same line of thought Cohn-Bronner (24) thought the lack of lysozyme in a hypersecreting nose holds true only for the common cold. They further showed that although the noses of hay-fever patients ran profusely, the mucus still showed a high lysozyme content. Exhaustion through hypersecretion has been advanced as the explanation for the low lysozyme content of commercial cow's milk. Sullivan and Manville (25) reported that pilocarpine produced as a result of increased secretion of mucus, a marked lessening of the lysozyme content in the colonic mucosa of rabbits. It is probably safe to conclude then, that hypersecretion is a definite factor in causing diminution of the lysozyme content but that it is probably not the all important factor operating as some workers have seemed to indicate.

Another important factor affecting lysozyme content in secretions is vitamin A. This was first

suggested by the work of Findlay (28), who treat xeropthalmic eyes of rats deficient in vitamin A with human tears and obtained marked improvement. Anderson (cited by Thompson (2) studied human twins suffering from xeropthalmia and found tear lysozyme content to be much lower than normal. On the addition of vitamin A to the diet the xeropthalmia improved and the lysozyme became normal. Paradoxically, tissue concentrations of lysozyme appeared to be increased in the presence of vitamin A deficiency. Sullivan and Manville (25) explained this by an inability of the glands to secrete mucus. Assuming that lysozyme is secreted with the mucus as a vehicle, the enzyme is stored up in the tissues when failure to secrete mucus occurs. They ascribed this failure to a disturbance in uronic acid metabolism and supported their views by showing that menthol poisoning brought about the same train of events and that the feeding of pectin tended to overcome the disturbance.

Some of the earlier workers conjectured that possibly the production of lysozyme is influenced by immunity reactions. With this theory in mind Allison (26) prepared vaccines of lysozyme susceptible organisms and injected animals with it. He found no increase in lyso-

syme content of the secretions and tissues resulting. James (20) found that injections of nonspecific protein had no effect on the lysozyme content of tears.

The next problem to be considered is the role of lysozyme in the antibacterial activity, in regard to pathogens and parasitic bacteria, of various lysozyme containing materials. Thus far lysozyme has been discussed only in relation to saprophytic air cocci. This has been generally assumed as its primary effect. However it has been noticed by many workers that certain secretions which have a significant content of lysozyme also exert a definite antibactericidal effect on pathogens. Several workers, as Ridley (15), Fleming (16), and Hilding (23), apparently assume, on the basis of circumstantial evidence only, that this activity is due to lysozyme. Some of the more intensively studied materials will now be discussed separately in regard to this.

Tears. Early bacteriologists noted that comparatively few organisms could be cultivated from normal conjunctivas. They also noted that organisms placed in the conjunctival sac would rapidly disappear (Thompson (22). Thompson (22) has tabulated the results of all earlier workers on tear antibacterial action. Although there are many contradictory results, his table does

definitely indicate that an anti-staphylcocci aureus action exists. Also there is some indication that enteric gram negative organisms are somewhat less affected. In addition to this Ridley (15) during carefully controlled experiments with tears obtained by irritating the conjunctiva with minute quantities of lemon juice, found that hemolytic streptococci, fecal streptococci, pneumococci were markedly inhibited by the tears. He showed that a slight decrease in tear concentration would cause complete loss of the antibacterial action. Seventy-five per cent concentration allowed the growth of pneumococci which were killed by tears in ninety per cent concentration. These results are not characteristic of lysozyme action seen on susceptible cocci. Thompson and Gallardo (29) during carefully controlled work with pooled tears showed that all 25 of his coagulase-negative white staphylococcic strains were completely inhibited by tear dilutions of 1:2 and 1:4. Sixteen coagulase-positive orange strains were not inhibited by 1:4 dilutions . These results are clear. Lack of nutrition could have played no part, since growth always occurred in the equivalent broth saline mixtures.

What is the part of lysozyme in this activity? Thompson and Gallardo (22) by utilizing the property of

lysozyme, its great resistance to heat in acid solution showed that the anti-staphylococcic factor could be completely destroyed without affecting the lysozyme titre whatsoever. The results were definite and show at least that lysozyme is not the one and only factor involved. However nobody has made a tear preparation, destroying the lysozyme and yet retaining the anti-staphylococcic factor.

The next secretion to be considered will be nasal secretions. Many early workers showed the rapid disappearance of organisms from the nasal mucosa. That this is due partly to mechanical factors is no doubt true. However others have demonstrated antibacterial activity of the isolated nasal secretions themselves (Thompson (2). Fleming (16) demonstrated this on pathogenic bacteria and, assuming the activity to be due to lysozyme, he stressed the fact that while lysozyme is most active against non-pathogens, it can attack pathogenic organisms when allowed to act in the full strength in which it occurs in the secretion itself. This seems to be unsound reasoning, although he did definitely show antibacterial activity of nasal mucus against pathogenic bacteria, especially staphylococci and streptococcus viridans. Later Ignatius (cited by Daly (30) showed activity against the diptheria bacillus, and Bacillus

Anthracis. In accordance with other workers, he found no effect on gram negative organisms. The destruction of activity at 70 degrees and 80 degrees centigrade, which he observed, is not incompatible with the agent's being lysozyme, since the pH of nasal mucus is usually definitely alkaline (as mentioned previously, lysozyme is quite heat labile at an alkaline pH). He also showed that the agent active against diptheria was not as diffusible in the agar as was purified lysozyme. Daly (30) prepared fairly pure extracts from normal nasal secretions by precipitating them with acetone, extracting the precipitate with normal saline and then acidifying and filtering through a Berkefeld filter. He then grew cultures of various organisms on media containing serial dilutions of the lysozyme. He did not find the antibactericidal effect found in untampered nasal secretion but did observe that it caused hemolytic streptococci, Staph. aureus and type III Pneumococcus to grow in a granular manner. Inhibition of growth may have occurred but the reduced number of colonies obtained by pour plate methods from broth culture tubes may have been caused by the clumping of the organisms. Burnet and co-workers (31) found that all types of influenza virus were neutralized by nasal secretions obtained on nasal tampons. Francis (32) confirmed this

by showing that 26 of 48 specimens showed a marked neutralizing action while only 11 had no action on the virus. Neither worker found that lysozyme was concerned in this effect. Burnet showed that egg white and some other materials rich in lysozyme had no antiviral activity. He did not show whether or not the antiviral activity of nasal secretions could be destroyed without changing the lysozyme titre.

The earlier work done on salivary antibacterial activity is contradictory and inconclusive. However in 1934 Dold and Weigmann (cited by Thompson (2) introduced new methods and more adequate controls. They and other German workers have more or less concentrated their work on the diptheria bacillus and Lactobacillus acidopholus, which will now be considered separately.

They found that diptheria bacilli inoculated into fresh saliva died out in a few hours but grew in the same saliva after it had been heated to 56 degrees C. for thirty minutes. Agar plates containing 25% fresh saliva would not grow the organisms. Their findings have been confirmed. They found a great deal of variation in this activity among different people. Immunity had no relation to the activity. These men do not think that lysozyme plays a role in this activity because their agent is more heat labile than lysozyme and is not as

filterable. It is less diffusible and more affected by sunlight and drying. Lysozyme is water soluble and precipitated but not injured by alcohol or acetone. The anti-diptheria agent is insoluble in water and destroyed by these agents. Unfortunately pH was not controlled, and because it has been shown that this has a great affect on the above properties, their statements hold little significance. However, Thompson (2) later showed that it was possible to destroy the anti-diptheria agent of saliva without affecting the lysozyme content. This was done by acidifying and boiling. He also pointed out that tears and egg white which contain more lysozyme than saliva had no effect on the diptheria bacillus. Thus it is fairly well proven that lysozyme activity is not dependent on the anti-diptheria agent. The reverse of this has never yet been shown however.

Because of the relation of L. Acidophilus to dental caries, a number of workers have studied the action of saliva on that organism. Bibby and co-workers (33) report inhibition of colonies in a zone around some saliva placed in a depression on the agar disks. Later Bibby (34) showed that these were actually killed. There is a high index of variation in the amount of activity among different persons tested. Hill (35) thought his results showed more activity in specimens

from caries-resistant people as compared to those of caries-susceptible persons. This has not been confirmed. Again lysozyme has been shown not to be this agent. Bibby (34) obtained, by Berkefeld filtration, the material active against L. Acidophilus but not against M. lysodeikticus. Thompson (2) by heating acidified saliva was able to destroy the L. acidophilus agent and yet retain the original lysozyme content. Bibby (36) also found salivary agents inhibitory for staphylococci, streptococci, and the colon-typhoid group. Thompson (2) found non-pathogenic staphylococci to be quite inhibited and pathogenic staphyloccoccus and hemolytic streptococcus to be less so. Both of these workers found the agent showing the last mentioned action to show exactly the same qualities in regard to lysozyme as did the anti-acidophilus agent.

A summary of these facts regarding saliva seems to indicate that the anti-diptheria agents, anti-acidophilus agents, agents against staphylococci, streptococci and other organisms, are similar and may even be identical. These agents are differentiated from saliva by their heat lability in acid solutions, filterability in neutral solutions. However their true nature is not known and it has not been shown that their action can exist independently of lysozyme.

Egg white is another material known for some time to have antibacterial properties. Although very rich in lysozyme, it has generally been found that the anti-pathogen activity of egg white does not stand much dilution. Gay and Beckwith (37) found that while 50% egg white killed typhoid bacilli and streptococci, these organisms grew in 25% egg white. Fleming and Allison (4) showed that some staphylococci, colon bacilli and enterococci were inhibited by egg white dilutions as high as 1:64 but typhoid and cholera organisms only by concentrations of 1:4 to 1:8. The part of lysozyme in these activities is difficult to determine at the present time. Very little work has been done with purified lysozyme preparations on pathogenic organisms. It should also be attempted to destroy lysozyme and yet retain other antibacterial agents. Perhaps this could be accomplished by heating the egg white which had been alkalinized.

Very extensive work has been done on the antibacterial activity of serum. Since it is relatively poor in lysozyme content, (Fleming (16), it will be considered only briefly here. The heat sensitive fraction (sensitizer-alexin complex) has been shown by Thompson (2) to have absolutely no relation to lysozyme action. However the heat stable components of serum proved to

be that portion of the serum which particularly affects lysozyme susceptible cocci. Mackie and Finklestein (38) found other organisms including staphylococci, streptococci, and pneumococci affected more by the serum components than they are by egg white, which suggests an activity due to some other factor than lysozyme according to the concept accepted in this paper. Again more experimental work must be done before concrete conclusions regarding the relation of lysozyme to the heat stable serum lysins can be drawn. At the present, in the author's opinion, lysozyme probably is not concerned in the action against the pathogenic bacteria.

Leukocytic extracts are well known for antibacterial properties. Barnes (39) has analyzed these extracts into a number of enzymes. One of these, known to older workers as leukin, was found to readily lyse the lysozyme-susceptible air cocci and B. Anthracis. While some workers claim leukin also kills staphylococcus, streptococcus, pneumococcus, and B. typhosus, the results are still very controversial. Barnes further showed that leukin possessed properties similar to lysozyme and was probably identical to it. What relation, if any, it has to the other bactericidins in the leukocytes has not yet been determined.

Relationship of bacteriophage to lysozyme

It was apparent to even early workers such as Fleming (1) that phage and lysozyme were lytic agents of quite different natures. The action of lysozyme was more rapid than phage and occurred over a wider range of temperature. Phage only affected young cultures, while lysozyme dissolved all ages. Phage was not diffusible in agar, while lysozyme was. Phage is able to reproduce itself while lysozyme is not. White (40) has shown a possible relationship between the two. He found that very feeble cholera phages, showing scarcely any lytic power and which on transfer gradually died out, could be made to produce marked lysis and could be kept going indefinitely in series transfers by the addition of egg white to the phage-bacteria mixtures. He did not show that pure lysozyme or lysozyme from other sources had the same action.

Does lysozyme bear any relation to resistance to disease? This question cannot be answered until more work has been done with purified lysozyme and on the distribution of the substrate. Thompson (22) suggests that the first step in the successful evolution from susceptible saprophyte to parasite could certainly require the development of a mechanism of resistance to the lysozyme, whether or not this were the only re-

quirement for such a step. It alsc certainly seems suggestive that individuals suffering during the early stages of a cold have lowered lysozyme content of their nasal secretions and do become.infected easily with secondary invaders. On the other hand, hay fever patients, who have a high nasal lysozyme content, seldom become secondarily infected (Cohn-Bronner (24).

Other workers, Fleming (13), Ridley (15), Mackie and Finklestein (38), have tried to make the point that there is a relation between the degree of pathogenicity and resistance to lysozyme. However they experimented with complex fluids and there is no proof that the agent concerned was lysozyme.

A group of workers have associated the high lysozyme content of breast milk with the fact that breast fed babies suffer less from diarrheas (Rosenthal and Lieberman (41), Pricket and co-workers (42). Again their work was not done with purified lysozyme and perhaps other factors were involved. Melnik (cited by Thompson (21) showed that lysozyme was lacking in the feces of nurslings suffering from gastrointestinal disturbances much more frequently than in those from healthy nurslings.

One fact which would tend to discount the importance of lysozyme in disease resistance is the fact

tha cats, whose tear lysozyme is very low (Flarez (17) are not any more subject to ocular disease than humans whose tear lysozyme is very high.

The evaluation of lysozyme as a therapeutic agent would be as yet quite premature. However a number of workers have used egg white with seemingly good results. Russian workers (cited by Thompson (22) used it in eye infections with favorable results but not at all conclusive. Barondes (43) used it in chronic ulcerative colitis with apparent improvement resulting. His series was small, criteria for cure indefinite and all in all his work appears very inconclusive.

SUMMARY

Lysozyme is a ubiquitous powerful lytic agent for certain saprophytic air cocci. It is an enzyme which attacks the polysaccharide material in the bacteria cell membrane. It is heat stabile in acid solution and labile in alkaline. It may kill bacteria without lysis but when lysis occurs it is released for further action. Substances which contain lysozyme in significant amounts have been found to be bactericidal for pathogens. It has been definitely shown that other factors are involved here, but it has never been shown that the action could occur in the absence of lysozyme. There is

suggestive but inconclusive evidence that lysozyme is a factor in disease resistance. Lysozyme therapy is as yet in an infantile stage and more fundamental work must be done on lysozyme and the lysozyme-containing substances before it can possibly be put on a solid basis.

CONCLUSIONS

1. Lysozyme is a powerful lytic agent for certain saprophytic cocci and is found in many tissues and secretions.
2. Evidence is suggestive that lysozyme may play a part in the antibacterial activity of certain substances against pathogens. It has been proven that other factors are involved.
3. Further work, on the above problem, with purified lysozyme preparation and a further analysis of the other factors involved must be done to determine the relation of lysozyme to disease resistance.
4. Therapeutic results using lysozyme are as yet inconclusive.

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