STUDIES IN BACTERIAL ASSOCIATION

by

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Holman has recently reviewed the literature on bacterial association in a most thorough manner and included an introduction which is very inclusive. "Bacterial association has taken in recent years a much more important place in bacteriological studies than formerly. This increased interest is largely due to the greater attention which is being given to the finer metabolism of bacteria and the interactions which occur between the bacteria and their environment. The idea long held by many that bacteria represent the lowest forms of life and are therefore comparatively simple in their metabolic activity has been replaced by a realization that we are dealing with just as highly specialized and complicated functional activities as in any of the so-called "higher" plants or animals. It is true that we are dealing with unicellular forms of very small size and that these as suggested by Kendall should be considered as similar to living colloids in which surface phenomena are so important. Many of the activities in the life of the bacteria can be appreciated better if this complicated metabolism is kept in mind, and the study of mixed or double cultures helps in an understanding of the actual life processes." It is possible that some of the changes in the fermentative and putrifactive world are due to a combination of metabolic events of various species of bacteria and without this combination or combinations, said changes

would not occur. In human physiology and pathology, bacterial association may play an undreamed of part. The possibilities of treatment of infection by associative processes have been suspected by many and actual therapeutic measures involving bacterial association have been demonstrated.

"It is generally recognized but often fogotten that under natural conditions mixed cultures are the rule, and the earliest work on bacterial associations is to be found largely in the studies which attempted to analyze such natural phenomena. The most variable results may be obtained in these mixed cultures, and there are many factors taking part which determine the final outcome. There may be simple mixtures with no demonstrable effect of one bacterium on another but this is uncommon since one or the other usually dominates the picture. One microbe may favor the growth and activity of another or both may be benefited by the combination. The latter condition is usually spoken of as symbiosis, but true examples of this relationship are rare. The term metabiosis is sometimes used where one action follows another, and is well illustrated in innumerable examples in nature. Antagonism or antibiosis is often combined with the foregoing but is mostly employed for the occurrences where there is a clearly demonstrable harmful effect of one micro-organism on another or when a characteristic product fails to be formed or disappears in the mixed culture. Because of the impossibility in many cases of determining the actual

processes at work these terms must be used with reservations." Holman has also suggested the general word association for all of these phenomena and synergism, which was introduced into bacteriological nomenclature for the first time by Kammerer, for those in which definite changes are demonstrable which indicate or suggest the combined work of two or more micro-organisms. Zoeller is quoted as using cumulative cultures to express the results obtained by him in certain biological combinations. As has been suggested, syngerism may be readily divided when one or the other result dominates, into an antagonistic synergism and a beneficent synergism.

"Among the early observations we find the recognition by Pasteur of the harmful effect of wild yeast on the normal fermentation processes in the beer and wine industries. He further noted the beneficial effect of aerobic forms which developing a scum on the surface, and using up the oxygen, favored anaerobic growth. Winogradsky isolated an aerobe which only fixed nitrogen from the air in the presence of other bacteria. Burri and Stutzer demonstrated that horse feces split nitrate with the production of free nitrogen. He isolated from the feces B.coli communis and a strict aerobe, and these two in combination gave the same result. The B. coli could be replaced by B. typhosus, and therefore it was the strict aerobe which gave the actual gas production. Previous

to this, Marshall Ward described a yeast and a bacterium which together formed a ginger beer-like product in a saccarine fluid."

ANAEROBES

Holman quotes Nencki as reporting, with a double culture of B. paralactici and B. chauvoei the formation from glucose of normal butyl alcohol, a substance not produced by either culture alone. Roger is quoted as being the first to show that B. prodigiosus added to the bacillus of malignant edema renders sublethal doses of this anaerobe fatal for rabbits. Novy according to Holman found that the injection into guinea pigs of B. proteus and his new anaerobe (B. oedematiens) resulted in rapid death and an enormous growth of the anaerobe in the animal body. The overgrowth was absent in the animal when pure cultures were used. Also, by adding B. proteus and other aerobes to his anaerobe, he was able to grow the latter in the presence of air. Sturges devised a method, based on bacterial association, for the isolation and cultivation of B. putrificus and other obligate sporebearing anaerobes. By growing B. coli and B. putrificus in a Novy jar, he obtained colonies composed of both types of bacteria and by heating these colonies, the coli were killed leaving a pure culture of putrificus. By growing Staphylococcus aureus and B. putrificus on aerobic plates, he ob-

tained colonies composed of both types and, after killing the staphylococci by heat, he had a pure culture of the anaerobe. Barrieu noted that B. proteus and certain non-pathogenic spore-bearing aerobes found in wounds exalted, by their proteolytic activity, the virulence of pathogenic bacteria. Holman notes the following: "Pringsheim grew Frankel's bacillus (B. welchii) with B. fecalis alcaligenes for ten transfers on agar slants and could see in the growth of the latter the opaque colonies of the anaerobe. A liquefying sarcina allowed B. welchii and B. butyricus to grow in open tubes. After six days' growth the sarcina had disappeared from the B. butyricus culture, and he suggested this as an easy method to obtain a pure culture. Weinbery and Otelesco considered that many war wound infections, looked upon as of pure anaerobic origin, may be due to an association with B. proteus since this latter organism increased the virulence of B. perfringens, V. septique, and others. Animals injected with B. sporogenes and B. proteus did not develop putrid lesions." In studying the bacterial flora of war wounds, Holman observed the combined growth of aerobes and anaerobes on surface cultures and he points to the frequency with which anaerobes were found in mixed colonies on aerobic plates.

There has been quite a bit of work done on the associative effects of various bacteria on B. botulinus and its toxin. Hall and Peterson found that certain acid-producing aerobes in-

hibit toxin production in glucose but not in non-carbohydrate media, and some of these aerobes actually destroyed toxin in glucose broth. Acid itself was ineffective and apparently the the nascent state was necessary according to Holman. Hall and Peterson, however, describe it as "something more" than acid. Dack in some experiments on Cl. botulinum, its toxin, and the effect of various anaerobes, especially Cl. sporogenes, demonstrated another important phase of bacterial association. He showed that the filtered toxin of Cl. botulinum added to beef heart medium is gradually destroyed by the growth of Cl. sporogenes and also by the growth of several other anaerobes with proteolytic and non-proteolytic properties. The destruction of the toxin apparently did not depend on a change in the hydrogen ion concentration. They found Cl. botulinum type A. to grow scantily in a filtrate of Cl. sporogenes; it grew more vigorously in a filtrate which had been boiled, and Cl. botulinum type B. did not grow at all in the unheated sporogenes filtrate nor did it produce toxin. No reduction in potency of toxin of Cl. botulinum type A. was noted when an equal amount of sporogenes filtrate was added to it. Francillon is quoted as studying the same problem and finding that Staphylococcus aureus, B. proteus vulgaris and other bacteria inducing the growth of Cl. botulinum in open tubes of plain and glucose bouillon; however the growth was never as good as under other anaerobic conditions. A moist-meat medium gave somewhat better

growth. Toxin was found in the mixed cultures in bouillon and meat, the amount varying with the aerobe. The B. pyocyaneus mixture gave no toxin in the bouillon but a strong one from the meat. There was but little effect on the toxin by two weeks' contact with B. proteus, B. coli, or B. pyocyaneus.

Holman continues, "Passini found that a putrefactive anaerobe B. putrificus verrucosus destroyed B. tuberculosis in nine days. Omeliansky studied the fixation of atmospheric nitrogen as Winogradsky had done years before. He noted that in the surface layers of the soil numerous organisms used the oxygen and created anaerobic conditions for the B. clostridium pasteurianum, but in addition some of these accompanying forms also supplied carbon compounds for the anaerobe. The Azotobacter being alkaligenic used up such products from the anaerobe as butyric acid and thus favored the synergistic process. The other aerobes may at times do harm by depriving the Azotobacter of oxygen. These two nitrogen-fixing forms, one aerobic; the other anaerobic, worked very well together. The work of Kammerer and his associates gave interesting examples of synergistic action. They observed that emulsions of human feces reduced pure bilirubin and mesobilirubin to urobilin but had no action on biliverdin and that the feces of herbivora did not have this action because of its active fermentation. Filtrates had no effect. These changes they believed were due to a synergism between B. putrificus and certain aerobes. B. coli either helped the bilirubin

production or hindered it, depending on the presence or absence of fermentable material. They further demonstrated the development of hematoporphyrin from blood by a similar synergism and that sugar or bile inhibited it." Speakman and Phillips report a commercially important example of bacterial association. During the war, acetone and butyl alcohol were produced on a large scale by fermentation of cereals and carbohydrates. Serious difficulties developed in the plants, owing to the contamination of the cultures of B. granulobacter-pectinovorum by the aerobic bacillus B. volutans. The acetone yield, as a result of the mixed culture, dropped or disappeared and the development of the lactic acid increased. They considered this associative process to be due to a partial inhibition of the physiological processes of B. granulobacter-pectinovorum by some factor produced in the B. volutans cultures. This inhibitory factor was not characteristic of cultures of B. volutans grown for several generations in artificial media containing sugars. They adopt the tentative hypothesis that this substance is a product of the nitrogen metabolism of B. volutans growing in media containing vegetable protein with traces of carbohydrate material.

Khouvine is reported as discovering a strict anaerobe, B. celulosae dissolvens, which distroyed five times as much cellulose when in association as when alone.

Donaldson describes an anaerobic spore bearer closely resembling Cl. sporogenes, which he calls the "reading" bacillus, and which is probably present in the majority of gun shot wounds. This

organism acts by virtue of its proteoclastic enzymes as an organic catalyst which hydrolyzes the substrate of dead protein. It disintegrates the protein base from which pathogenic organisms operate and while so doing does not itself give rise to fresh toxic substances. Donaldson says, "Not only so, but it is probably able to hydrolyze also the toxic degradation products of other organisms." And he supports this statement with a resume of experiments on tetanus and other toxins which show that the "Reading" bacillus, out of a series of organisms investigated is alone able to reduce the toxicity of these toxins. There is one exception, namely Cl. sporogenes which however, does not appear to be so potent in this direction as is the "Reading" bacillus. The ability to disintegrate necrotic tissue does not necessarily imply an equal power of hydrolyzing toxin as is illustrated by the experiment with (B) histolyticus which may be highly useful in museum work but would probably be dangerous in wounds. Peterson and Mulvania studied an experiment on corn mash cultures. In the mash inoculated with granulobacter-pectinovorum, the common type of cereal lactic acid bacteria grow rapidly producing large amounts of lactic acid. The formation of the lactic acid is dependent upon the associated action of the two groups (the lactic acid and butyl organisms). The presence of the lactic acid bacteria alone is not sufficient. The growth of granulobacter-pectinovorum favors the development of the lactic acid bacteria in several ways; 1. by hydrolysis of the

starch to fermentable carbohydrates, 2. by proteolysis of the nitrogen compounds to amino acids -- the production of buffer substances. Although this association is beneficial to the lactic acid bacteria, it is extremely harmful to the solvent forming organism. The higher acid forming organisms are the most injurious, and if the acidity is removed, the injurious property is lost.

THE ACID-LOVING OR ACIDOPHILIC GROUP

This group and the effect of its members on other bacteria have been closely studied. They are usually facultative aerobes and their action is considered as chiefly antagonistic. It was thought by several that this group had quite a therapeutic possibility and numerous experiments have been devised in an attempt for such a demonstration. Zinsser reviews them, "On the basis of the mutual antagonism existing in culture between many acid-producing bacteria and those of putrefaction -- a phenomenon recognized by some of the earliest workers in this field, many investigators have suggested the possibility of combating intestinal putrefaction by adding acid-forming bacteria together with carbohydrates to the diet of patients suffering from this condition. The first to suggest this therapy was Escherich, who proposed the use, in this, of Bacillus lactic aerogenes; with the same end in view, Quincke, a little later, suggested the use of yeasts -- Oidium lactis. The reasoning underlying these attempts was meanwhile upheld by experiments carried out both in

vitro and upon the living patient. Thus Brudzinski was able to demonstrate that Bacillus lactis aerogenes, in culture, inhibited the development of certain races of the proteus species and succeeded in obtaining markedly favorable results by feeding pure cultures of Bacillus lactis aerogenes to infants suffering from fetid diarrhea. Similar experiments carried out with the Welch bacillus (aerogenes capsulatus) and Bacillus coli, however, had no such corroboratory results since this anaerobe possesses a considerable resistance against an acid reaction. In considering the difficulties of the problems involved in this question, it occurred to Metchnikoff that much of the practical failure of therapy based on the principles stated might be referred to insufficient powers of acid production on the part of Bacillus coli, Bacillus lactis aerogenes, and other germs previously used. The administration of the Bacillus bulgaricus to patients suffering from intestinal putrefaction, first suggested by Metchnikoff in 1906 has since that time, been extensively practiced and often with remarkable success." B. bulgaricus could not be implanted in the intestinal tract however and B. acidophilus, a normal inhabitant, was substituted. B. bifidus found in the intestines of breast fed infants, is responsible for inhibition of the growth of other bacteria, and its acid products are thought to be mild stimulants to the bowel walls. B. doederlaini, in the vagina, is believed to keep the reaction acid, thus inhibiting the growth of contaminating bacteria.

Thomas, however, finds Doederlein's vaginal bacillus is Lactobacillus. This organism which is present in less than ten per cent of normal vaginas of children gets into the vagina by exterior passage from the intestinal tract and can be introduced into the vagina by feeding a culture by mouth. It lives and develops acid either from a secretion of the vagina or in conveyed fermentable intestinal material or both. The organism is not present in the vagina in cases of gonococcal vaginitis. It has an inhibiting effect on the growth of the gonoccocus in vitro and Lactobacillus aciddphilus vaginal implantation may present a rational cure for gonococcal vulvovaginitis and other gonococcal infections.

According to Holman, Landau was the first to use fresh beer yeast in the treatment of leucorrhea and considered the anti-catarrhal action was due to mechanical overgrowth, the using up of food material and the action of metabolic products in injuring or destroying other bacteria, neutralizing the toxins and changing the reaction to acid. He suggested injecting cultures of this yeast into the bladder in cases of cystitis with alkaline urine. Holman continues, "Schiller, with a strain of B. acidophilus from a dog, found that it rapidly destroyed and dissolved many strains of streptococci in fluid media and suggested this as a useful way to obtain bacteriolysis of cocci. He showed that this action was not due to lactic acid since it occurred in alkaline media and filtrates from a glucose broth culture living or killed (by heat or age) allowed a good growth of the streptococcus. The harmful substance

was only formed by the B. acidophilus in the presence of the streptococcus or its products. Filtrates of the mixed culture, after thirty-six hours at 37°C. (when the streptococci are killed) were as toxic as when living cultures of the bacilli were used. Streptococcus cultures killed by heat or age had no toxic effect on living streptococci. He considered the phenomenon an example of induced antagonism and reported other examples in a series of four articles. In the first he used B. mesentericus and forced on it an antagonistic action against streptococci by growing it with the latter in a medium of poor-food value. It secreted a bacteriolytic substance which digested the living bacteria as it would any other insoluble albuminous material, and the amount depended on the number of sensitive streptococci present. It also acted when the B. mesentericus had been removed by centrifugation and after evaporation and drying. It was not completely specific. Schiller further showed that yeasts can be made antagonistic against bacteria including B. tuberculosis if the medium contained sugars but lacked nitrogenous materials. Then acted in the same way as the foregoing, and a more active bacteriolytic substance was secreted in the presence of more resistant forms so that the enzyme induced by B. tuberculosis was even capable of attacking beeswax. The reverse was also found. Bacteria (staphylococcus, B. typhosus, B. paratyphosus) became antagonistic to yeasts in nitrogen-free media, and the secreted cytolytic substance was similar to the

foregoing but had no effect on coagulated serum or egg albumin. This method of dissolving the yeast membrane he thought might be of interest in the study of zymase."

Rogers in some experiments on buttermilk arrived at some interesting conclusions. If a bulgaricus culture is used to increase the acidity of ordinary buttermilk, the action is slow and may even fail entirely. If milk is inoculated with equal quantities of Streptococcus lactis, and B. Bulgaricus cultures, or only slightly predominating quantities of the Streptococcus lactis, a typical bulgaricus fermentation will ensue. If, however, the lactic inoculation predominates, the bulgaricus culture will be suppressed and the fermentation will be of the Streptococcus lactis type. The inhibiting effect does not seem to be due to acidity. Heating to boiling does not destroy the inhibiting property but it is partly removed by filtering through an earthenware filter. Dead cells do not have the inhibiting property.

Bachrach in some experiments on the effects of poisons on bacteria demonstrated a possible explanation of some of the bacterial associative phenomena. He applied different poisons to lactic acid bacilli and estimated their action by the production of lactic acid, thereafter. He found that it was thus possible to modify the cell so that it became at the same time more resistant to some drugs and more susceptible to others.

Holman quotes Gratia and Dath as discovering an aerobic streptothrix which had a powerful destructive action on a variety of

bacteria. It did not act on B. tuberculosis and showed no lipolytic enzyme. Filtrates were equally effective, and could act without free oxygen, but were somewhat variable. The dissolved bacteria caused specific response when used for vaccination. "Rosenthal worked with an organism apparently very much like the foregoing and found it was antagonistic to many bacteria including B. diptheria. He referred to the report of Gasperini of 1890 on a similar form acting against bacteria. Rosenthal and his associates found that it could be implanted in the intestines of guinea pigs and that when injected parenterally it was enterotropic. A very interesting study by Gratia and Rhodes proved that staphylococci could live on killed suspensions of staphylococci in saline or in saline agar made cloudy with killed staphylococci. Thus we see that bacteria can and do remetabolize their own substances or that of other bacteria, and this helps in the understanding of the antagonistic action of many forms of bacteria."

THE THEOBALD AND D.E. SMITH PHENOMENON

"One of the most interesting examples of bacterial association ever reported was that by Theobald Smith and D.E. Smith in 1920. They found that B. paratyphosus B, after it had grown in lactose bouillon for four to six days, prevented the development of gas by B. coli when this was added. Members of the closely related hog cholera group had no such action in the given time, but

after eighteen days' growth they also inhibited the gas production for the B. coli added at this time." Holman and Meekison in reporting some experiments of a similar nature, described some very interesting results. They found that if a lactose fermenting streptococcus were added to a culture of B. paratyphosus in a tube of lactose broth, gas was produced, altho no gas was ever formed in this medium by either organism in pure culture. The same results were obtained by using a saccharose fermenting streptococcus and coli communis. Staphylococcus aureus could be substituted for the streptococcus with similar results and a number of combinations were studied. In such mixed cultures, the presence of the streptococci was not always evident since the gram negative bacilli outgrow it in these serum free mediums.

They say that the mother substance of thegas is an intermediary substance or product rather than an end product of the acid fermentation of the sugar and that this intermediary product, even in pure cultures of the acid former, is decomposed as fast as formed. They then have the situation in which one organism of a symbiotic pair gives rise to a substance which both organisms can decompose but with the formation of different end products. In the one case, acids are the end products; in the other, gas and presumably acids also. Castellani describes a very similar experiment, using as the media mannitol, sorbite, and maltose. According to Holman, Brutsaert found the phenomenon most inconstant and variable even in repeated tests of the same strain of bacillus. A hog-cholera type-agglutinating culture

ORGANISMS		CARBOHYDRATES	GAS
Streptococcus fecalis	B. cholera suis	lactose	"
Streptococcus fecalis	B. paratyphosus B.	lactose	"
Staphylococcus aureus	B. paratyphosus B.	lactose	"
Staphylococcus aureus	B. proteus vulgaris	lactose	"
Streptococcus fecalis	B. proteus vulgaris	lactose	"
Streptococcus fecalis	B. paratyphosus A	lactose	"
Staphylococcus aureus	B. coli communis	saccharose	"
Streptococcus fecalis	B. coli communis	saccharose	"
Streptococcus fecalis	B. acidi lactici	saccharose	"
Streptococcus ignavus	B. paratyphosus B.	saccharose	"
Streptococcus ignavus	B. paratyphosus A.	saccharose	"
Staphylococcus aureus	B. paratyphosus A.	saccharose	"
Staphylococcus aureus	B. proteus vulgaris	mannite	"
Streptococcus fecalis	" "	"	"
Streptococcus pyogenes	" "	"	"
B. dysentery flexner	" "	"	"
B. typhosus	" "	"	"

Sears and Putnam describe experiments somewhat similar to the above.

B. prodigiosus	B. cholerae suis	lactose	"
Staphylococcus aureus	B. cholera suis	"	"
Streptococcus fecalis	B. cholera suis	"	"
Streptococcus fecalis	B. paratyphosus B.	"	"
Streptococcus fecalis	B. pneumoniae	"	"

ORGANISMS			CARBOHYDRATES	GAS
Staphylococcus aureus	B. proteus vulgaris		lactose	"
Streptococcus fecalis	B. Morgani		"	"
B. butyricus	B. proteus vulgaris		"	"
Vibrio proteus	"	"	"	"
B. Lautus	"	"	"	"
B. Prausnitzer	"	"	"	"
Staphylococcus aureus	B. coli communis		saccharose	"
B. butyricus	B. coli communis		saccharose	"
Streptococcus fecalis	B. acidi lactici		"	"
Streptococcus fecalis	B. coli communis		"	"
Spirillum Metchnikovi	B. paratyphosus B.		"	"
Spirillum cholera	"	B.	"	"
Staphylococcus aureus	B. icteroides		"	"
B. viscosum	B. cholerą suis		"	"
B. prodigiosus	"	"	"	"
Staphylococcus aureus	B. proteus vulgaris		mannite	"
Streptococcus fecalis	"	"	"	"
Streptococcus hemolyticus	"	"	"	"
Streptococcus viridans	"	"	"	"
B. dysentery Flexner	"	"	"	"
B. dysentery "	Y.	"	"	"
B. typhosus	"	"	"	"
B. centrosporus	"	"	"	"

inhibited, but as a rule members of this group did not. Moreover, the phenomenon failed if, instead of lactose bouillon, a lactose peptone water were used. He found it too irregular for classification. Von Jeny is quoted as investigating the subject very fully. He found five strains of B. paratyphosus B among twenty-six studied which increased the B. coli gas production. Also plates from the mixtures at times gave pure B. coli. The strains of the B. paratyphosus, isolated from the mixture did not always give the same results on retest. B. typhosus also inhibited. Some workers have gotten fairly regular results with the combinations mentioned above, while others haven't. In summing up these results which seem quite variable, it seems that by the combinations definite changes are brought about which neither organism can accomplish alone.

DIPHTHERIA GROUP

The associative properties of the diphtheria group have been quite extensively studied both from the standpoint of carriers and their treatment and also purely from bacteriological standpoint. Holman considers, "Streptococci and B. diphtheria have long been considered mutually helpful in producing severe infections in the throat. Roux and Yersin in studying the problem of the return of virulence in attenuated cultures of B. diphtheriae, were successful in accomplishing this by injecting the attenuated culture along with a non-fatal dose of an erysipelas strain of streptococcus. The virulence returned, and it was retained on successive cultures.

They, therefore, warned against the use of Streptococcus erysipelatus to combat diphtheria as had been suggested by Babtchinski. Similar results were obtained by Barbier and Schreider. Funck found the fact to be true but did not consider it as striking as had previous workers and showed the presence of the streptococci in no way affected the specific action of the diphtheria toxin. Klein also showed that streptococci enhanced the effect of B. diphtheriae." Arnold found a marked increase in numbers of hemolytic streptococci in throats of patients during diphtheria. The streptococci in the throats at this time have a limiting hydrogen ion concentration differing from that of the normal throat flora; this is accompanied by an overgrowth of the types fermenting lactose and salacin. The strains that produce the limiting hydrogen ion concentration between 4.3 and 4.5 are considered by most investigators, as of bovine origin or non-pathogenic strains and those reaching the lesser hydrogen ion concentrations limits of 5.0 and 5.5 of human or pathogenic origin. If this is true, the streptococcic flora of the human throat can under normal conditions be considered as non-pathogenic and the flora of the diphtheria throats as human or pathogenic. Gate, Papacostas, and Billa are quoted as finding the filtrates of avirulent streptococci stimulating diphtheria toxin production. The increased virulence was not retained on further cultures. Holman quotes Zoeller as showing "it was possible to produce in his cumulative cultures a diphtheria-streptococcus altero-toxin by growing a scarlet fever strep-

tococcus in a diphtheria toxin to which had been added a little horse serum." Stovall, Scheid, and Nichols did some work which indicated that there is a definite effect on the part of cultures of Staphylococcus aureus for cultures of B. diphtheriae, and that the staphylococci will completely eradicate the diphtheria bacillus from the culture if present in large enough numbers. The effect does not seem to be a matter of overgrowth because when staphylococci are planted with B. diphtheriae in such amounts as to allow for abundant growth of the latter, the morphology and staining of it are markedly changed. This influence is marked and constant. The result of this work indicates that when diphtheria-like bacilli are found in throat cultures in which staphylococci are growing it is necessary to isolate and study the bacillus in pure culture even though it seems quite remote from B. diphtheriae in throat cultures. The effect on the morphology of the diphtheria bacillus by growth in mixed culture with staphylococci indicates that the variation of the staining characteristics of these organisms is not so much dependent upon the special staining method used as upon the influence on the morphology and staining reactions by growth in mixed culture. It appears to the authors that this change in morphology and staining reaction is due to some change in the composition and structure of the organisms; that some change in the physiology of the organisms has been brought about. If they are correct in the above supposition, then

it is easy to explain why stains cannot be relied upon to differentiate toxin from non-toxin producers. If changes have been brought about which interfere with granule formation, then, of course, no stain will be able to stain granules. It is certain that in mixed cultures with staphylococci, diphtheria bacilli will stain as solid bacilli while in pure culture they are distinctly beaded. Of course, toxin producers cannot be distinguished from non-toxin producers. This is in brief the findings and theories of the workers mentioned immediately above, and apparently they do not fall short in advancing theories. Lorenz and Ravenel quote Schiotz as introducing, in 1909, the practice of over-riding the throats of chronic diphtheria carriers with suspensions of Staphylococcus aureus. He was led to the use of Staphylococcus aureus by the observation that a patient with a sore throat due to the staphylococci did not contract diphtheria, when exposed and also that several convalescents failed to show positive diphtheria cultures after an attack of staphylococcic sore throat. He treated six cases with prompt disappearance of the diphtheria bacilli in all of them. In their excellent review of this particular type of bacterial association they also quote Page as reporting, in 1911, the successful use of staphylococci in one case in which the bacilli had persisted in the throat for three months after clinical recovery; also Catlin, Scott and Day in the same year as reporting the successful use of staphylococci in eight cases; and Dewitt in

a series of experiments in animals as finding out of thirty-two animals inoculated with B. diphtheriae and afterward treated with staphylococcus cultures, nine were apparently not influenced, fourteen were worse and nine were better, and, concluding that there is no rational basis for treating diphtheria or carriers with Staphylococcus aureus. DeWitt admits, however, that the animal experiments indicate that a certain percentage of acute cases may clear up more quickly under staphylococcic treatment than under orginary antiseptic treatment. Lorenz and Ravenel, themselves, however, in work on diphtheria carriers had good results with the staphylococcus spray. Two of three carriers were reported negative after six applications. Later one of these patients again became a carrier and the reinfection cured in the same manner. The third carrier had a negative throat after a nasal and throat application. In some work on convalescents who still retained the diphtheria organisms in spite of treatment, they found that out of six cases, four cleared up on one week after treatment with the staphylococcus spray. In other series of patients, it seems that better results were obtained in those patients treated with the staphylococcus spray than those treated by the ordinary antiseptic method and they conclude that pure cultures of Staphylococcus aureus sprayed into the throat and nasal cavity will cause a disappearance of diphtheria bacilli with best results in carriers with no clinical manifestations of the disease. Holman says, "There were also a number of unfavorable reports such as that of C. M. Davis

who reported the development of tonsillitis following the use of the staphylococcus spray. Nicholson and Hogan were encouraged by the results on nine acute cases, using sprays of B. bulgaricus and sour milk. Papacostas and Cate studied the question of the antagonism between the pneumobacillus of Friedlander and B. diphtheriae following the observation that clinical cases of such mixed infections were usually mild. Mixed cultures of these two bacteria showed a progressive predominance of the former on serial transfers and morphology of the latter also changed toward a more homogeneous form on staining. By the use of filtrates of each culture they could not discover any evidence that the toxin of the former was able to neutralize diphtheria toxin in vivo or in vitro. If the two are grown together, however, no toxin is formed, nor is there any if the filtrate of the pneumobacillus growth is used to grow the B. diphtheriae. They suggested the therapeutic use of filtrates."

"Van der Reis, having demonstrated an antagonistic action of B. coli to B. diphtheria showed that it was possible, by spraying B. coli into the mouth, to have it colonize there. In nine cases it was still present after fifty-four days. A careful study of the antagonistic activity of B. coli led him to conclude that there is formed a thermolabile, volatile, non-dialyzable, non-filterable, inhibitory substance not absorbed by charcoal, not identical with the normal metabolic products of the colon bacillus, but that it may be a special toxic product. It was tried in acute cases of

diphtheria by means of sprays of B. coli and particles of B. coli agar with the result that the B. diphtheriae disappeared more quickly than in controls. Carriers could also be rapidly freed of their bacilli. On the other hand, Pesch and Zschocke, although confirming the crowding out of the B. diphtheriae by B. coli in cultures, were unsuccessful in treating nasal carriers because the B. coli would not grow in the nose. Bloomfield failed in his attempts to implant Friedlander's bacillus from carriers to non-carriers, and even a foreign strain of the bacillus failed to establish itself in the throat of a carrier of another strain. Pringsheim studied the inhibiting effect of a strain of B. mesentericus vulgatus against a variety of bacteria but particularly against B. diphtheriae. He found that B. typhosus, B. paratyphosus A and B., B. fecalis alcaligenes, B. coli and streptococcus were strongly inhibitive. Staphylococcus aureus was mildly stimulating as seen in larger colonies as was also a weakly sporulating B. subtilis strain. On agar plates the effect of his B. mesentericus was to produce a circular zone of inhibition and just beyond this a ring of larger colonies. Filtered or heated cultures had no effect. Other proteolytic bacteria had no such action. It was tried on patients but the results were inconclusive. The findings of Zukerman and Minkewitsch with B. mesentericus vulgatus were somewhat different. The antagonism was inherent in the bacillus and was not increased by serial passage. It acted only on diphtheria and pseudo-diphtheria forms and not against a long list of other bacteria."

COLON-TYPHOID GROUP

Frost has done some of the earlier work on this group and his paper includes the literature to this date. V. Freudenreich, in 1888 showed that when certain bacteria were grown in flasks of broth for some time and then filtered, the typhoid bacillus failed to grow in the filtrate in some cases and in others it grew only feebly.

Staphylococcus aureus Bacillus typhosus Bacillus of chicken cholera Microspira comma Spirillum of Miller Spirillum of Denecke	Scant growth.
Staphylococcus albus Staphylococcus pyogenes Bacillus pyocyaneus Bacillus phosphorescens	No growth.

Garre, in 1888 showed that Pseudomonas fluorescens putida produces in its growth on artificial media substances which are antagonistic to the typhoid bacillus. He grew this organism on gelatin and then scraped it off and seeded the typhoid bacillus and found it would not grow although Pseudomonas fluorescens putida would grow on media from which a growth of Bacillus typhosus had been scraped. He also demonstrated the existance of an antagonism by means of alternate streaks of the two organisms arranged radially on a gelatin plate. Where the streaks were near together the typhoid bacillus did not grow but near the circumference, where the distance between the streaks was greater, both organisms developed normally. Olitzky in

1891 worked on the antagonism which is exerted by Bacillus fluorescens liquefaciens, employing methods slightly modified from those of Garre.

This observer demonstrated a marked antagonism and laid stress on the hygienic importance of this fact. Laws and Andrews, in 1894, working on the duration of the life of Bacillus typhosus in sewage, appeared to show that the presence in the sewage of Bacillus fluorescens liquefaciens, and especially Bacillus fluorescens steroralis, shortened the life of the typhoid bacillus. Sidney Martin in 1898-1900, while studying the growth of the typhoid bacillus, in soil, determined that, while some of the soils furnished conditions favorable for the prolonged existance of the bacillus -- in one case as long as 456 days -- other soils presented conditions which were inimical to the growth of the typhoid bacillus. This antagonism of certain soils he was able to trace to the effect of definite bacteria which he isolated and grew in pure culture. The identification of these bacteria was not established but they were described as Chichester 1, 2, 3, 4, and 5. The antagonism was tested by growing the organisms separately with the typhoid bacillus in 200 c.c. of sterile water to which 10 c.c. of sterile broth had been added. With number 1, the typhoid bacillus died in less than 12 days at 8--12 C., and 6 days at 37 C. When these organisms were grown with the typhoid bacillus in sterile soil, number 1 had gained the upper hand in 12 days at 8°--12°C. and in six days at 37°C, Number 3 had gained the upper hand in 15 days at 37°C. and number 5 in 3 days at 37°C.

Remy in 1901 writes on the antagonism exhibited by Bacillus coli for Bacillus typhosus. He was however unable to show any antagonism but maintained that the specific characters of both germs were changed. This change was in respect to their agglutination reaction as well as to their cultural characters.

Horrocks in 1901, working with Pseudomonas fluorescens, found that Bacillus typhosus would not grow on gelatin which had already yielded a growth of Pseudomonas fluorescens, but that gelatin which had served as a medium for Bacillus typhosus would still permit the development of Pseudomonas flurescens. In sterile sewage he was unable to obtain Bacillus typhosus after it had grown seven days with Pseudomonas fluorescens. Working also on the effect on Bacillus typhosus of its association with Bacillus coli, with what he considered improved methods, namely, alkaline glucose litmus agar surface plates, he found that when these two organisms were grown together in sterile tap water, it could not be discovered after 7 days. Frost himself found that a marked antagonism was exerted by mixed cultures of bacteria obtained from the soil and water on Bacillus typhosus when these mixed cultures are grown in broth and a colloidian sac containing the typhoid bacillus is immersed therein. The antagonism results in not merely checking the growth but in actually killing the typhoid bacilli. In many cases, the killing off amounts to extinction. The death rate of Bacillus typhosus is more or less rapid depending on the period of preliminary cultivation of

the antibionts. The antagonistic substance does not seem to exist ready formed in the soil or water, but rather the antagonism depends on the rapid development of the organisms in the immediate presence of Bacillus typhosus. Apparently, there was a wide spread activity of these antagonistic bacteria in nature for they were found in various types of soils and waters. An antagonism was definitely associated with several different species of bacteria--Bacillus vulgatus, Bacillus vulgaris, Pseudomonas, fluorescens, and Pseudomonas putida. Changes in the environment of these organisms such as temperature, oxygen supply, reaction of the medium, amount of dextrose seemed to have little or no influence on the production of the antagonistic substances. In other words, whenever the environment was such that a good growth of the organism occurred, the antagonistic substances were apparently always produced. The energy with which the antagonistic substances acted, depended on the temperature. At 38°C. the action was very pronounced. At the temperature of the ice chest, 10° - 12°C. the typhoid bacillus sometimes grew in the by-products of the other germs which at higher temperatures were quickly fatal. The antagonistic substances were theremostable, being able to withstand a temperature of 120°C. for at least 10 minutes. Frost asserts that the antagonism was not due, in the cases studied, to the exhaustion of the food supply, the action of proteolytic enzymes, poisons or the production of hydroxyl ions alone.

Smith in 1922, reported the following experiment. Mixtures were made of approximately similar suspensions of twenty-four

hours growth of a stock culture of Bacillus typhosus with the various cultures of Bacillus coli from carriers, and with a stock culture of Bacillus coli respectively, in the proportion of one of Bacillus typhosus to four of Bacillus coli. The mixtures were then used to inoculate a series of tubes of fluid medium containing brilliant green in concentration ranging from 1:1,000,000 to 1:143,000, which were incubated and subcultured thereafter as usual. The result was that Bacillus typhosus could not be recovered from any of the growths in the mixtures containing Bacillus coli derived from the carriers, although it was found in practically pure culture from the mixture with the stock Bacillus coli in suitable amounts of the dye. The experiment was then carried out in a different form; thus, varying volumes of Bacillus coli suspensions were added to one volume of a suspension of a Bacillus typhosus culture and after the mixtures had stood overnight, at room temperature, each was used to inoculate peptone water containing brilliant green 1:286,000. The result was that Bacillus typhosus was recovered from the mixture containing 800 volumes of the stock Bacillus coli culture to 1 volume of Bacillus typhosus, whereas in the case of the carrier Bacillus coli, Bacillus typhosus was not recovered from a mixture in which the relative proportions were 50--1. Thus it is apparent that the strains of Bacillus coli present in these cases possessed in marked degree the property of inhibiting the growth of Bacillus typhosus. Direct plating of mixtures of Bacillus typhosus

with the stock Bacillus coli, showed that the latter also had an inhibitory effect on Bacillus typhosus. This was demonstrated in the following way--from a mixture of Bacillus typhosus (one volume of a saline suspension of a young agar culture) with Bacillus coli (four volumes of a suspension of similar opacity to that of the typhoid culture) a series of decimal dilutions was prepared. In a control series, the typhosis suspension without Bacillus coli was diluted similarly with saline. Then successive stroke cultures on plates of MacConkeys medium were made with a constant loop. On incubation at 37°C. it was found that proportion of typhoid colonies which developed in the series containing Bacillus coli was much smaller than in the controls without Bacillus coli. By the higher dilutions, where widely isolated colonies developed, it was shown that the Bacillus typhosus was inhibited and not merely masked by the abundant growth of colonies of Bacillus coli.

B. INFLUENZA GROUP

In 1903, Neisser while making cultures on blood agar from a measles conjunctivitis, observed colonies of B. xerosis which were at first round and later became indented and irregular at the edges, thanks to the presence of minute colonies of the influenza bacillus. Subinoculation of the two organisms on ordinary agar free from blood produced the same results, and cultures were carried successfully through twenty generations. It was often impossible to

identify the colonies of B. influenza with the naked eye, but this organism could always be demonstrated by subcultures on blood agar or by microscopic examination. In the mixed cultures on agar, the influenza bacillus frequently lived as long as ten, sixteen and even twenty days. B. diphtheria was successfully substituted for B. xerosis, but with less satisfactory results. Neisser endeavored to grow the Pfeiffer bacillus with cultures of B. xerosis, which had been killed by heat, with extracts of this organism, and with the dried bacilli. The results were negative, except for an occasional culture which could be carried to the fourth generation. Neisser concluded that living cultures of B. xerosis rather than the killed organisms are necessary for the growth of B. influenza.

Cantani reported scanty growth of the Pfeiffer bacillus on plain agar in association with living cultures of the diphtheria bacillus, the gonococcus, and some unidentified cocci. Such mixed cultures gave much better results on ascitic agar, though no control is reported to establish that the ascitic agar was free from hemoglobin. Luxuriant growth of Pfeiffer bacillus was obtained on ordinary agar mixed with killed cultures of the gonoccus and of the diphtheria bacillus. However, Gohn, von Preyss, Lueissen, and Putnam and Gay couldn't confirm the above results.

The experiments of Jacobson, Roos, Yanagisawa, Albert and Kelman, and Wolf have shown that the simultaneous injection of Pfeiffer's bacillus with other bacteria raises the virulence of the Pfeiffer's

bacillus. Yanagisawa measured the increase and showed that the other bacteria (streptococci, staphylococci, Dip. pneumoniae, and Dip.catarrhalis) injected with the Pfeiffer bacillus were relatively more influenced the symbiotic relation. Albert and Kelman concluded that the injection of the Pfeiffer bacillus favors the invasion of tissue by other bacteria as well as conversely. Wasserman and Keysser writing on mixed and secondary infection refer repeatedly to clinical and laboratory findings of Pfeiffer's bacillus as having important pathological significance because of relations with other bacteria, especially the pneumococcus. Huntoon and Hannum report that guinea pigs inoculated with Pfeiffer's bacillus or its extracts succumbed spontaneously and experimentally to subsequent exposures to the inoculations with various organisms, with the appearance of secondary lesions.

Hudson in working on white mice, found that a freshly isolated strain of Pfeiffer's bacillus was increased in virulence when injected with recently isolated strains of Streptococcus hemolyticus or with a stock strain of the pemmococcus type 1. The same strains of streptococcus showed increased virulence with this bacillus, but this property of the pneumococcus was not so markedly increased. When the Pfeiffer's bacillus was injected two hours before or simultaneously with Streptococcus hemolyticus, a higher proportion of early mouse deaths occurred than when the streptococci preceded the bacillus or was injected alone.

B. ANTHRACIS

Holman has reported that guinea pigs did not die after large infections of washings from soil contaminated months before by B. anthracis in the slaughtering of a diseased cow, but gave typical results after injection of the cultures isolated from the soil. He explains the results by the fact that the anthrax spores are phagocyted by the leukocytes attracted to the site of injection by the other bacteria before they start developing and then are destroyed or eliminated. It is a well known fact that one of the best known and most easily demonstrated experiments on bacterial antibiosis is the one in which Ps. pyocyaneus inhibits the growth of B. anthracis on plain agar plates. Gundel is quoted as finding that coli not only inhibited the growth of B. anthracis but under certain conditions completely destroyed it.

A further valuable contribution to our knowledge of these mutual relationships of bacteria is to be found in the work of Gordon and M'leod on the inhibition of growth by some amino acids. They found that the effect differed markedly as tested on different bacteria. B. coli and staphylococcus were not at all affected while other more delicate bacteria were. Tryptophane, as an example is most toxic and affects the widest variety of bacteria. Indol, a deaminization product of tryptophane, may account for the toxicity of tryptophane since it is more toxic than carbolic acid. Serum prevents to a degree these inhibitory actions. Others are beneficial,

such as taurine, aspartic acid, and alanine. It is readily seen that we have here an additional explanation for certain of the phenomena being considered. Various bacteria will produce amino acids harmful or beneficial to others and on these products will depend the effect on the associated bacteria in proportion to the relative sensitivity of the latter.

EXPERIMENTAL METHODS

Introduction

The possibility that many more bacterial associative phenomena exist unrecognized in nature combined with a curiosity to understand more about them served as a stimulus whereby a better understanding of these phenomena might be obtained. Accordingly a series of experiments was devised.

Organisms

For the more detailed study in this work various strains of streptococci, designated as 1, 2, 4, 5, 9, 10, 11, 12, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32 and 33, were obtained from human throats and from stock cultures. They were all classified according to the Smith Brown classification. Other organisms used in this work were Staphylococcus aureus, Staphylococcus albus, Staphylococcus tetragenus and Neisseria catarrhalis, all stock culture strains. The above cultures were all purified by streaking and restreaking on blood agar plates as described below.

Media

The most constantly used media, that which was used for the actual experimental studies was calcium carbonate broth, containing single strength meat infusion broth, 1% dextrose and a crystal of calcium carbonate weighing approximately 1 gram was placed in

test tube. About 10 c.c. of the broth was placed in each test tube. Accessory media were also used as follows; Agar slants containing 1% peptone, and 1/2% meat extract; blood agar composed of meat extract agar, 1% peptone and 3% human blood. Blood agar slants were made containing 5 c.c. blood agar and blood agar plates were made with an approximate depth of 5 m.m. of blood agar; Loeffler's alkaline methylene blue, Saturated, alcoholic methylene blue -- 30 c.c., 1-10,000 potassium hydrate in water -- 100 c.c.

Methods of Study

At this point, only a general resume of the various methods used will be stated, a more detailed account will be found before each table of results. The various strains of streptococci were grown with staphylococcus aureus, staphylococcus albus, staphylococcus tetragenus and Neisseria catarrhalis in calcium carbonate broth. Inoculations were made from suspensions of similar opacity of 24 hours growths on plain agar in the case of the above four organisms and on blood agar in the case of the streptococci into the calcium carbonate broth and the tubes incubated at 37°C. At the end of 15 hours, 40 hours, 64 hours, 88 hours and 7 days, the growths were streaked out on blood agar plates and observations made and recorded. The streaking was done with a platinum wire inoculating needle as follows: Three loopfuls of the mixed culture were placed on one side of a blood agar plate. This plate and two others were inoculated by streaking, without reinoculating the needle. Also at the above intervals, morphological studies of the mixtures were made. Three loopfuls were spread

on a glass slide and stained with methylene blue for thirty seconds. The slides were then studied for morphological variations. The mixtures were differentiated by the fact that the staphylococci and the catarrhalis stain a pale blue, while the streptococci take a much deeper stain and their cell membranes stain much thicker, giving the appearance of a dark blue halo around a lighter blue center. The chain formation is also easily recognizable.

At the intervals mentioned above observations were made on the hydrogen ion concentration of the various mixtures of organisms. The hydrogen ion concentrations were determined by the quinhydrone method as outlined by Lang. It is a gas chain method using instead of the hydrogen electrode the quinhydrone electrode.

EXPERIMENTAL RESULTS

Normals

First of all, all of the organisms used in this work were grown in calcium carbonate broth and observations made at the intervals mentioned above. All of the observations intended on the mixtures of organisms were made on the pure cultures. The following table shows the changes in growth, morphology and hydrogen ion concentration which were observed.

	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
Streptococcus # 1 (beta)	Ph	6.7	5.0	5.3	5.0	5.4
	Morphology	L.C.S. 15-50 +growth	Same	Same	Same	Same
Streptococcus # 2 (alphaprime)	Ph	5.6	5.0	5.0	5.0	4.9
	Morphology	L.C.S. +growth	Same	Same	Same	Same
Streptococcus # 4 (gamma)	Ph	5.7	4.8	4.8	4.9	5.0
	Morphology	L.C.S. +growth	Same	Same	-growth	-growth
Streptococcus # 5 (beta)	Ph	6.7	6.2	5.2	5.3	5.5
	Morphology	L.C.S. +growth	Same	Same	Same	Same
Streptococcus # 9 (beta)	Ph	6.7	5.3	5.0	5.3	5.4
	Morphology	L.C.S. +growth	Same	Same	Same	Same
Streptococcus # 10 (alphaprime)	Ph	6.3	4.9	5.0	5.0	5.1
	Morphology	L.C.S. +growth	Same	Same	Same	Same
Streptococcus # 11 (beta)	Ph	6.6	5.1	5.4	5.2	5.3
	Morphology	L.C.S. +growth	Same	Same	Same	Same
Streptococcus #12 (beta)	Ph	6.6	5.4	5.5	5.2	5.2
	Morphology	L.C.S. +growth	Same	Same	Same	Same
Streptococcus # 21 (alpha)	Ph	6.6	5.1	5.0	5.0	5.1
	Morphology	L.C.S. +growth	Same	Same	Same	Same

Streptococcus # 22 (beta)	Ph	6.7	5.7	5.5	5.4	5.2
	Morphology	L.C.S. +growth	Same	Same	Same	Same
Streptococcus # 23 (alphaprime)	Ph	6.6	5.4	5.3	5.4	5.4
	Morphology	L.C.S. +growth	Same	Same	Same	Same
Streptococcus # 24 (alphaprime)	Ph	5.2	4.8	4.8	4.7	4.8
	Morphology	L.C.S. +growth	Same	Same	Same	Same
Streptococcus # 25 (alphaprime)	Ph	6.6	5.5	5.5	5.6	5.5
	Morphology	L.C.S. +growth	Same	Same	-growth	-growth
Streptococcus # 26 (alphaprime)	Ph	6.8	5.2	5.4	5.5	5.5
	Morphology	L.C.S. +growth	Same	Same	-growth	-growth
Streptococcus # 27 (alphaprime)	Ph	6.8	5.5	5.7	5.2	5.3
	Morphology	L.C.S. +growth	Same	Same	Same	Same
Streptococcus # 28 (alpha)	Ph	6.6	5.4	5.4	5.3	5.2
	Morphology	L.C.S. +growth	Same	Same	Same	Same
Streptococcus # 29 (gamma)	Ph	6.4	4.4	4.9	4.7	5.0
	Morphology	L.C.S. +growth	Same	Same	Same	Same
Streptococcus # 30 (alphaprime)	Ph	6.6	5.4	5.5	5.2	5.3
	Morphology	L.C.S. +growth	Same	Same	Same	Same
Streptococcus # 31 (alphaprime)	Ph	6.7	5.8	5.6	5.5	5.3
	Morphology	L.C.S. +growth	Same	Same	Same	Same
Streptococcus #32 (alphaprime)	Ph	6.6	5.6	5.5	5.5	5.4
	Morphology	L.C.S. +growth	Same	Same	Same	Same

Streptococcus # 33 (alpha)	Ph Morphology	5.9 L.C.S. +growth	5.1 Same	5.1 Same	5.3 Same	5.3 Same
Streptococcus # 34 (alpha)	Ph Morphology	5.2 L.C.S. +growth	5.0 Same	4.8 Same	4.9 Same	5.2 Same
Streptococcus # 35 (alpha)	Ph Morphology	6.6 L.C.S. +growth	5.4 Same	5.2 Same	5.1 Same	5.1 Same
Staphylococcus Aureus	Ph Morphology	4.7 Masses, clusters, +growth	4.7 Same	4.6 Same	4.6 Same	4.7 Same
Staphylococcus Albus	Ph Morphology	4.7 Masses, clusters, +growth	4.7 Same	4.7 Same	4.7 Same	4.7 Same
Staphyloccus Tetragenus	Ph Morphology	6.7 Masses, of tetrads, +growth	6.2 Same	6.2 Same	6.2 Same	6.2 Same
Neisseria Catarrhalis	Ph Morphology	7.1 Masses, pairs +growth	7.1 Same	7.4 Same	7.5 Same	7.6 Same

L.C.S. - long chained streptococcus.
S.C.S. - short chained streptococcus.

Above is a table of the organisms used in these experiments, and of the results of inoculating these organisms in pure culture into calcium carbonate meat infusion broth. The various strains of streptococci were numbered and classified as described above. They were then incubated and observed at 15 hours, 40 hours, 64 hours, 88 hours and 7 days. Observations were also carried at 10 days and 14 days, at first, but it was later found unnecessary for the purpose of this experiment. The hydrogen ion concentration of each was observed at each of these intervals, and also observations on growth and morphology were noted.

The above observations were also noted on Staphylococcus aureus, Staphylococcus albus, Staphylococcus tetragenus, and Neisseria catarrhalis.

In the first experiment the strains of streptococci were grown with Staphylococcus aureus and at the intervals mentioned above, observations were made as to changes in growth of either organism, changes in morphology of either organism, and changes in the hydrogen ion concentration of the mixture. If changes in growth characteristics did occur, it was decided to determine the cause of such changes if possible, whether it be due to toxic by-products or to changes in hydrogen ion concentration. The following table shows the results obtained in the experiment and following the table is an explanation of the results.

Organism	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
Staphylococcus aureus and Streptococcus # 1 (beta)	Character of plates	Few strep. Many aureus	Few strep. Many aureus	No strep. Many aureus	Same	Same
	Ph	4.7	4.7	4.6	4.6	4.7
	Morphology	Normal	Normal	Normal	Normal	Normal
Staphylococcus aureus and Streptococcus # 2 (alpha prime)	Character of plates	Few strep. Many aureus	Few strep. Many aureus	Few strep. Many aureus	No strep. Many aureus	Same
	Ph	4.7	4.7	4.8	4.8	4.8
	Morphology	Normal	Normal	Normal	Normal	Normal
Staphylococcus aureus and Streptococcus # 4 (gemma)	Character of plates	Few strep. Many aureus	Few strep. Many aureus	Few strep. Many aureus	Few strep. Many aureus	No strep. Many aureus
	Ph	4.5	4.7	4.7	4.7	4.7
	Morphology	Normal	Normal	Normal	Normal	Normal
Staphylococcus aureus and Streptococcus # 5 (beta)	Character of plates	Few strep. Many aureus	No strep. Many aureus	Same	Same	Same
	Ph	4.7	4.7	4.7	4.8	4.8
	Morphology	Normal	Normal	Normal	Normal	Normal

	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
Staphylococcus aureus and Streptococcus # 9 (beta)	Character of plates	Few strep. Many aureus	Few strep. Many aureus	No strep. Many aureus	Same	Same
	Ph	4.7	4.7	4.7	4.7	4.7
	Morphology	Normal	Normal	Normal	Normal	Normal
Staphylococcus aureus and Streptococcus # 10 (alpha prime)	Character of plates	Few strep. Many aureus	Few strep. Many aureus	Few strep. Many aureus	No strep. Many aureus	Same
	Ph	4.7	4.7	4.8	4.8	4.8
	Morphology	Normal	Normal	Normal	Normal	Normal
Staphylococcus aureus and Streptococcus # 11 (beta)	Character of plates	Few strep. Many aureus	Few strep. Many aureus	No strep. Many aureus	Same	Same
	Ph	4.6	4.7	4.7	4.8	4.7
	Morphology	Normal	Normal	Normal	Normal	Normal
Staphylococcus aureus and Streptococcus # 12 (beta)	Character of plates	Few strep. Many aureus	Few strep Many aureus	No strep. Many aureus	Same	Same
	Ph	4.7	4.7	4.8	4.8	4.8
	Morphology	Normal	Normal	Normal	Normal	Normal

	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
Staphylococcus aureus and Streptococcus #21 (alpha)	Character of plates	Few Strep. Many aureus	Few strep. Many aureus	No strep. Many aureus	Same	Same
	Ph	4.7	4.7	4.7	4.8	4.8
	Morphology	Normal	Normal	Normal	Normal	Normal
Staphylococcus aureus and Streptococcus #22 (beta)	Character of plates	Few strep. Many aureus	Few strep. Many aureus	Few strep. Many aureus	No strep. Many aureus	Same
	Ph	4.7	4.7	4.6	4.6	4.7
	Morphology	Normal	Normal	Normal	Normal	Normal
Staphylococcus aureus and Steptococcus #23 (alpha prime)	Character of plates	Few strep. Many aureus	Few strep. Many aureus	No strep. Many aureus	Same	Same
	Ph	4.7	4.7	4.7	4.8	4.8
	Morphology	Normal	Normal	Normal	Normal	Normal
Staphylococcus aureus and Streptococcus #24 (alpha)	Character of plates	Few strep Many aureus	Few strep. Many aureus	No strep. Many aureus	Same	Same
	Ph.	4.6	4.7	4.7	4.7	4.8
	Morphology	Normal	Normal	Normal	Normal	Normal

	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
Staphylococcus aureus and Streptococcus #25 (alpha prime)	Character of plates	Few strep. Many aureus	Few strep. Many aureus	No strep. Many aureus	Same	Same
	Ph	4.7	4.7	4.8	4.8	4.7
	Morphology	Normal	Normal	Normal	Normal	Normal
Staphylococcus aureus and Streptococcus #26(alpha prime)	Character of plates	Few strep. Many aureus	Few strep. Many aureus	No strep. Many aureus	Same	Same
	Ph	4.7	4.7	4.6	4.6	4.7
	Morphology	Normal	Normal	Normal	Normal	Normal
Staphylococcus aureus and Streptococcus #27(alpha prime)	Character of plates	Few strep. Many aureus	Few strep. Many aureus	No strep. Many aureus	Same	Same
	Ph	4.6	4.7	4.7	4.7	4.7
	Morphology	Normal	Normal	Normal	Normal	Normal
Staphylococcus aureus and Streptococcus #28 (alpha)	Character of plates	Few strep. Many aureus	No strep. Many aureus	Same	Same	Same
	Ph	4.7	4.7	4.6	4.6	4.7
	Morphology	Normal	Normal	Normal	Normal	Normal

	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
Staphylococcus aureus and Streptococcus # 29 (gamma)	Character of plates	Few strep. Many aureus	Few strep. Many aureus	No strep. Many aureus	Same	Same
	Ph	4.5	4.7	4.7	4.7	4.7
	Morphology	Normal	Normal	Normal	Normal	Normal
Staphylococcus aureus and Streptococcus #30(alpha prime)	Character of plates	Few strep. Many aureus	Few strep. Many aureus	No strep. Many aureus	Same	Same
	Ph	4.7	4.7	4.7	4.7	4.7
	Morphology	Normal	Normal	Normal	Normal	Normal
Staphylococcus aureus and Streptococcus #31(alpha prime)	Character of plates	Few strep. Many aureus	Few strep. Many aureus	No strep. Many aureus	Same	Same
	Ph	4.7	4.6	4.6	4.7	4.7
	Morphology	Normal	Normal	Normal	Normal	Normal
Staphylococcus aureus and Streptococcus #32(alpha prime)	Character of plates	Few strep. Many aureus	Few strep. Many aureus	Few strep. Many aureus	No strep. Many aureus	Same
	Ph	4.7	4.6	4.6	4.7	4.8
	Morphology	Normal	Normal	Normal	Normal	Normal

	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
Staphylococcus aureus and Streptococcus #33 (alpha)	Character of plates	Few strep. Many aureus	Few strep. Many aureus	Few strep. Many aureus	No strep. Many aureus	Same
	Ph	4.6	4.7	4.7	4.8	4.8
	Morphology	Normal	Normal	Normal	Normal	Normal
Staphylococcus aureus and Streptococcus #34 (alpha)	Character of plates	Few strep. Many aureus	No strep. Many aureus	Same	Same	Same
	Ph	4.6	4.7	4.7	4.8	4.8
	Morphology	Normal	Normal	Normal	Normal	Normal
Staphylococcus aureus and Streptococcus #35 (alpha)	Character of plates	Few strep. Many aureus	Few strep. Many aureus	No strep. Many aruesus	Same	Same
	Ph	4.7	4.7	4.6	4.6	4.7
	Morphology	Normal	Normal	Normal	Normal	Normal

Above is a table of the results of inoculation of Staphylococcus aureus and the various strains of streptococci as outlined above. For purposes of clarity it is possibly best to note the result of inoculating Staphylococcus aureus with each of the various strains of streptococci separately. But in this case the results are for the most part similar that an individual analysis would be superfluous. In practically every case the streptococci grew but only scantily in comparison to the staphylococci which as would be expected completely outgrew their co-habitors, the streptococci disappearing in 64 hours to 88 hours. When I say disappear, I mean only that they were not demonstrable when three loops of the mixed broth culture were streaked without reinoculating on blood agar plates as described above. The results obtained did not vary with the type of streptococci used. The staphylococci seemed to be bothered very little by the presence of the streptococci and acted as though they were in pure culture. They grew rapidly, the hydrogen ion concentration being about the same as it was in pure culture. The morphology as seen by a smear stained by methylene blue was normal as was that of the streptococci which in pure culture grew in long chains ranging in some cases to lengths of over one hundred organisms.

The cause of the disappearance of the streptococci lies probably in three factors; acid production, other metabolic wastes,

and mechanical overgrowth. The first undoubtedly is the most important, because as can be seen in the table there is a rapid rise in the hydrogen ion concentration, sufficient at the end of 15 hours to limit most strains of streptococci.

The second study was similar to the first, except that instead of Staphylococcus aureus, Staphylococcus albus was used. Observations were made as in the preceding experiment. The explanation of the results, follows the table.

	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
Staphylococcus albus and Streptococcus # 1	Plates	Few strep. Many albus	Few strep. Many albus	Few strep. Many albus	No strep. Many albus	Same
	Ph	5.1	4.7	4.7	4.8	5.0
	Morphology	S.C.S.+growth	Same	Same	-growth	-growth
Staphylococcus albus and Streptococcus # 2	Plates	Few strep. Many albus	Few strep. Many albus	Few strep. Many albus	No strep. Many albus	Same
	Ph	4.9	5.0	5.0	5.0	5.0
	Morphology	S.C.S.+growth	Same	Same	-growth	-growth
Staphylococcus albus and Streptococcus # 4	Plates	Few strep. Many albus	Few strep. Many albus	Few strep. Many albus	No strep. Many albus	No strep. Many abus
	Ph	4.5	4.7	4.7	4.7	5.0
	Morphology	L.C.S.+growth	Same	Same	-growth	-growth
Staphylococcus albus and Streptococcus # 5	Plates	Few strep. Many albus	Few strep. Many albus	No step. Many albus	Same	Same
	Ph	4.9	4.6	4.7	4.7	4.9
	Morphology	S.C.S.+growth	Same	-growth	-growth	-growth

	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
Staphylococcus albus and Streptococcus # 9	Plates	Few strep. Many al bus	Few strep. Many al bus	No strep. Many al bus	Same	Same
	Ph	4.8	4.7	4.7	4.7	4.7
	Morphology	S.C.S.+growth	Same	-growth	-growth	-growth
Staphylococcus albus and Streptococcus #10	Plates	Few strep. Many albus	Few strep. Many albus	No strep. Many al bus	Same	Same
	Ph	4.9	4.9	4.9	5.0	5.0
	Morphology	S.C.S.+growth	Same	-growth	-growth	-growth
Staphylococcus albus and Streptococcus #11	Plates	Few strep Many al bus	Same	No strep. Many al bus	Same	Same
	Ph	5.0	4.7	4.7	4.8	4.8
	Morphology	S.C.S.+growth	Same	-growth	-growth	-growth
Staphylococcus albus and Streptococcus #12	Plates	Few strep. Many albus	No strep. Many albus	Same	Same	Same
	Ph	5.1	4.7	4.7	4.8	5.0
		S.C.S.+growth	-growth	-growth	-growth	-growth

	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
Staphylococcus albus and Streptococcus #21	Plates	Few strep. Many albus	Few strep. Many albus	No strep. Many albus	Same	Same
	Ph	5.0	4.7	4.7	4.8	4.8
	Morphology	S.C.S.+growth	Same	-growth	-growth	-growth
Staphylococcus albus and Streptococcus #22	Plates	Few strep. Many albus	Few strep. Many albus	No strep. Many albus	Same	Same
	Ph	5.0	4.6	4.7	4.7	4.7
	Morphology	S.C.S.+growth	Same	-growth	-growth	-growth
Staphylococcus albus and Streptococcus #23	Plates	Few strep. Many albus	Few strep. Many albus	Few strep. Many albus	No strep. Many albus	Same
	Ph	5.2	4.7	4.7	4.7	4.9
	Morphology	L.C.S.+growth	Same	Same	Growth	Growth
Staphylococcus albus and Streptococcus #24	Plates	Few strep. Many albus	Few strep. Many albus	No strep. Many albus	Same	Same
	Ph	5.2	4.7	4.8	4.8	4.8
	Morphology	L.C.S.+growth	Same	growth	growth	growth

	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
Staphylococcus albus and Streptococcus #25	Plates	Few strep. Many albus	Few strep. Many albus	No strep. Many albus	Same	Same
	Ph	5.0	4.7	4.7	4.7	4.8
	Morphology	S.C.S.+growth	Same	growth	growth	growth
Staphylococcus albus and Streptococcus #26	Plates	Few strep. Many albus	No strep. Many albus	Same	Same	Same
	Ph	5.1	4.7	4.8	4.9	4.9
	Morphology	S.C.S.+growth	-growth	-growth	-growth	-growth
Staphylococcus albus and Streptococcus #27	Plates	Few strep. Many albus	Same	No strep. Many albus	Same	Same
	Ph	5.2	4.6	4.7	4.7	4.7
	Morphology	S.C.S.+growth	Same	-growth	-growth	-growth
Staphylococcus albus and Streptococcus #28	Plates	Few strep. Many albus	Few strep. Many albus	No strep. Many albus	Same	Same
	Ph	5.0	4.7	4.7	4.8	4.8
	Morphology	L.C.S.+growth	Same	-growth	-growth	-growth

	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
Staphylococcus albus and Streptococcus #29	Plates	Few strep. Many albus	Few strep. Many albus	Few strep. Many albus	No strep. Many albus	Same
	Ph	5.2	4.6	4.6	4.7	4.8
	Morphology	S.C.S.+growth	Same	Same	-growth	-growth
Staphylococcus albus and Streptococcus #30	Plates	Few strep. Many albus	Few strep. Many albus	No strep. Many albus	Same	Same
	Ph	5.1	4.7	4.8	4.9	5.0
	Morphology	S.C.S.+growth	Same	-growth	-growth	-growth
Staphylococcus albus and Streptococcus #31	Plates	Few strep. Many albus	No strep. Many albus	Same	Same	Same
	Ph	4.9	4.7	4.7	4.9	5.0
	Morphology	S.C.S.+growth	-growth	-growth	-growth	-growth
Staphylococcus albus and Streptococcus #32	Plates	Few strep. Many albus	Few strep. Many albus	No strep. Many albus	Same	Same
	Ph	4.6	4.7	4.7	4.9	5.0
	Morphology	L.C.S.+growth	Same	-growth	-growth	-growth

	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
Staphylococcus albus and Streptococcus #33	Plates	Few strep. Many albus	No strep. Many albus	Same	Same	Same
	Ph	4.6	4.6	4.7	4.8	4.9
	Morphology	S.C.S.+growth	-growth	-growth-	-growth	-growth
Staphylococcus albus and Streptococcus #34	Plates	Few strep. Many albus	No strep. Many albus	Same	Same	Same
	Ph	4.5	4.6	4.6	4.8	4.9
	Morphology	S.C.S.+growth	-growth	-growth	-growth	-growth
Staphylococcus albus and Streptococcus #35	Plates	Few strep. Many albus	No strep. Many albus	Same	Same	Same
	Ph	4.7	4.7	4.8	4.9	5.0
	Morphology	L.C.S.+growth	-growth	-growth	-growth	-growth

The above table shows the results of inoculating Staphylococcus albus and the various strains of streptococci. We find that the results of this experiment differ somewhat from those of the preceding experiment with the exception of the growth on plates which shows the same relationship exists between the various strains of streptococci and albus as existed between them and aureus. The hydrogen ion concentration did not increase as rapidly as in the preceding experiment, nor as rapdily as in the case of albus in pure culture. In the experiment with aureus and the streptococci, the hydrogen ion concentration at the end of 15 hrs. was in every case above Ph 5.0, and in the case of albus alone in pure culture, the concentration of hydrogen at the end of 15 hrs. was Ph 4.7. However, the hydrogen ion concentration arrives, within the ensuing 24 hours at a level very comparable to that of albus in pure culture. A marked alteration is noticed in the morphological characteristics of the normally long chained streptococci, at least some of them. After growing in calcium carbonate broth with the albus, strains 1,2,5,9,10,11,12,21, 22,25,26,27,29,30,31,33, and 34 of the streptococci apparently to a great extent have lost their former property of forming long chains. The remaining strains continue to form long chains. In the case of the strains mentioned above, the missing property is nothing absolute, for here and there on a slide it may be possible to demonstrate two or three fairly long chains. But when this is compared with the normally 25--50 organism chains, it can be seen that there is some alteration.

In this study Staphylococcus tetragenus is grown with the various strains of streptococci and observations made as above. On the page following the tables an explanation of the results is found.

Staphylococcus tegragenus and-	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
	Plates	Strep.++++ Tetra 1/2+	Strep.++++ Same	Same	Same	Same
Streptococcus # 1	Ph	5.5	5.1	5.3	5.2	5.2
	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.+++ Tetra 1/2+	Strep.+++++ Same	- -	- -	- -
Streptococcus # 2	Ph	4.8	4.7	4.7	4.6	5.0
	Morphology	L.C.S.	L.C.S.			
	Plates	Strep.++ Tetra 1/2+	Strep.++++ +	++++ +	- -	- -
Streptococcus # 4	Ph	4.6	4.7	4.7	4.7	4.8
	Morphology	L.C.S.	L.C.S.	L.C.S.		
	Plates	Strep.+++ Tetra 1/2+	++++ 1/2+	++++ +	- -	- -
Streptococcus # 5	Ph	4.7	4.8	4.7	4.8	4.9
	Morphology	L.C.S.	L.C.S.	L.C.S.		
	Plates	Strep.++ Tetra 1/2+	+++ +	+++ +	- -	- -
Streptococcus # 9	Ph	5.2	4.7	4.8	4.8	5.0
	Morphology	L.C.S.	L.C.S.	L.C.S.		

Staphylococcus tegragenus and-	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
	Plates	Strep.+++	++++	++++	-	-
		Tetra 1/2+	+	+	-	-
Streptococcus	Ph	4.9	4.7	4.6	4.8	5.0
# 10	Morphology	L.C.S.	L.C.S.	L.C.S.		
	Plates	Strep.++	+++	++++		
		Tetra 1/2+	+	+	Same	Same
Streptococcus	Ph	6.0	5.0	5.1	5.1	5.2
# 12	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.+++	++++			
		Tetra 1/2+	+	Same	Same	Same
Streptococcus	Ph	5.5	5.3	5.2	5.2	5.3
# 21	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.++	++++	-	-	-
		Tetra +	+	-	-	-
Streptococcus	Ph	5.0	4.8	4.9	5.0	5.0
# 22	Morphology	L.C.S.	L.C.S.			
	Plates	Strep.+++	++++	++++		
		Tetra 1/2+	1/2+	+	Same	Same
Streptococcus	Ph	6.3	5.3	5.0	4.8	5.3
# 23	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.

Staphylococcus tetragenus and-	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
	Plates	Strep.++	++++	++++		
		Tetra 1/2+	+	+	Same	Same
Streptococcus	Ph	6.0	5.2	5.0	5.2	5.3
# 24	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.+++	++++			
		Tetra 1/2+	+	Same	Same	Same
Streptococcus	Ph	4.9	4.8	4.9	4.9	5.0
# 25	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.+++	++++	++++	-	-
		Tetra 1/2+	+	+	-	-
Streptococcus	Ph	4.8	4.7	4.6	4.6	4.8
#26	Morphology	L.C.S.	L.C.S.	L.C.S.		
	Plates	Strep.+++	++++	-	-	-
		Tetra 1/2+	+	-	-	-
Streptococcus	Ph	4.9	4.7	4.7	4.8	4.8
# 27	Morphology	L.C.S.	L.C.S.			
	Plates	Strep.++	++++			
		Tetra 1/2+	+	Same	Same	Same
Streptococcus	Ph	5.2	4.9	4.9	5.0	5.1
# 28	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.

Staphylococcus tetragenus and-	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
	Plates	Strep.++	++++			
		Tetra 1/2+	+	Same	Same	Same
Streptococcus	Ph	6.0	5.2	5.0	5.1	5.2
# 29	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.++	++++	-	-	-
		Tetra 1/2+	+	-	-	-
Streptococcus	Ph	4.8	4.7	4.6	4.7	5.0
#30	Morphology	L.C.S.	L.C.S.			
	Plates	Strep.+++	++++			
		Tetra 1/2+	+	Same	Same	Same
Streptococcus	Ph	5.0	5.0	4.9	4.9	5.1
# 31	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.+++	++++	-	-	-
		Tetra 1/2+	+	-	-	-
Streptococcus	Ph	4.8	4.7	4.7	4.6	5.0
# 32	Morphology	L.C.S.	L.C.S.			
	Plates	Strep.++	+++	-	-	-
		Tetra 1/2+	+	-	-	-
Streptococcus	Ph	4.8	4.7	4.6	4.8	5.0
# 33	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.

Staphylococcus tetragemus and-	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
	Plates	Strep.+++	++++	++++	-	-
		Tetra 1/2+	+	+	-	-
Streptococcus	Ph	5.0	4.9	4.8	4.8	5.0
#34	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.+++	++++	++++	-	-
		Tetra 1/2+	+	+	-	-
Streptococcus	Ph	5.2	4.8	4.8	4.9	5.1
#35	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.

The above table shows the results of inoculating Staphylococcus tetragenus and the various strains of streptococci as done with aureus and albus in the two preceding experiments. With streptococcus #1, the tetragenus was marked inhibited in its growing characteristics as noted by the comparison of 1/2+ for the tetragenus and ++++ for the streptococci. This system of comparison extends from 1/2+ to+++++ , and represents a count of from 1 to innumberable. As can be seen it is purely relative. The comparative growth at the end of 15 hours was in the same ratio at the end of 40 hrs., 64 hrs., 88 hrs., and 7 days. The hydrogen ion concentration was somewhat lower than that of the tetragenus in pure culture throughout the periodical observations. The Ph corresponded to that of the streptococci in pure culture, except at the end of 15 hrs., where the Ph of the mixture was 5.5 as compared to 6.7 of streptococcus #1.

In the mixture with streptococcus #2, the plates showed much the same ratio as those mentioned above, except that between 40 hrs., and 64 hrs., both of the organisms were destroyed. An explanation is found in the hydrogen ion concentration of the mixture. It is much lower than that of the tetragemus in pure culture and somewhat lower than that of the streptococcus in pure culture. With the above two cases explained, the rest of the table may be followed and it can be seen that the growth characteristics of the

organisms when mixed are somewhat different from those of the organisms in pure culture, and also that this difference may be partially traced to acid production, which in itself is altered by the mixing.

There were no morphological changes observed.

This experiment which later proved to be the most interesting of the group was carried out in a somewhat similar manner as the above three. The organism grown with the strains of streptococci was Neisseria catarrhalis. Due to the fact that several unlooked for changes did occur, the results were checked and rechecked very closely. Observations were made as above on the changes in growth morphology and hydrogen ion concentration at the intervals shown in the table. If growth changes followed, could they be explained on the basis of changes in hydrogen ion concentration? That is the question that occurred to me. The tables of the experiment and explanation of the results follow.

Neisseria Catarrhalis	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
	Plates	Strep.++	+++	++++	++++	++++
		Cat.++++	++++	+++	-	-
Streptococcus	Ph	6.7	5.0	5.3	5.0	5.4
# 1	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.++	+++	-	-	-
		Cat.++++	-	-	-	-
Streptococcus	Ph	5.5	4.8	5.0	5.0	5.1
# 2	Morphology	L.C.S.	L.C.S.	L.C.S.		
	Plates	Strep.++	+++	+++	-	-
		Cat.++++	-	-	-	-
Streptococcus	Ph	5.2	4.6	4.8	4.8	5.0
# 4	Morphology	L.C.S.	L.C.S.	L.C.S.		
	Plates	Strep.++	+++	+++	+++	+++
		Cat.++++	++++	+++	++	++
Streptococcus	Ph	7.0	5.2	5.4	5.0	5.4
# 5	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.+++	+++	+++	+++	+++
		Cat.++++	+++	+++	+++	-
Streptococcus	Ph	6.9	5.7	5.3	5.3	5.4
# 9	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.

Neisseria Catarrhalis and	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
	Plates	Strep.++	+++	+++	++	++
		Cat.++++	+++	-	-	-
Streptococcus	Ph	5.8	5.0	5.1	5.2	5.0
# 10	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep++	+++	+++	+++	+++
		Cat.++++	++++	+++	++	-
Streptococcus	Ph	6.8	6.0	5.3	5.3	5.3
# 11	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.++	+++	+++	+++	+++
		Cat.++++	++++	++++	+++	+++
Streptococcus	Ph	6.8	5.3	5.5	5.4	5.3
# 12	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	
	Plates	Strep.++	+++	+++	+++	+++
		Cat.+++	+++	+++	-	-
Streptococcus	Ph	6.5	5.4	5.1	5.1	5.1
# 21	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.++	+++	+++	+++	+++
		Cat.++++	+++	+++	+++	+++
Streptococcus	Ph	6.5	5.0	5.4	5.5	5.6
# 22	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.

Neisseria Catarrhalis and	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
	Plates	Strep.++	+++	+++	+++	+++
		Cat.++++	+++	+++	+++	+++
Streptococcus	Ph	6.3	5.3	5.3	5.4	5.4
# 23	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.++	+++	+++	+++	+++
		Cat.++++	-	-	-	-
Streptococcus	Ph	4.6	4.5	4.8	4.9	5.1
# 24	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.++	+++	+++	+++	+++
		Cat.++++	+++	++	+	-
Streptococcus	Ph	6.7	5.2	5.2	5.2	5.4
# 25	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.++	+++	+++	++	++
		Cat.++++	+++	-	-	-
Streptococcus	Ph	6.9	5.0	5.0	5.2	5.1
# 26	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.++	+++	+++	+++	++
		Cat.++++	+++	+++	+++	-
Streptococcus	Ph	6.8	5.4	5.5	5.0	5.6
# 27	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.

Neisseria Catarrhalis and	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
	Plates	Strep.++	+++	++	++	++
		Cat.++++	-	-	-	-
Streptococcus	Ph	5.0	4.9	5.0	5.0	5.2
# 28	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.++	++	++	++	++
		Cat.++++	-	-	-	-
Streptococcus	Ph	5.4	4.5	4.8	4.7	5.0
# 29	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.++	+++	+++	+++	+++
		Cat.++++	+++	++	-	-
Streptococcus	Ph	6.8	5.3	5.1	5.1	5.3
# 30	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.++	+++	+++	++	++
		Cat.++++	+++	-	-	-
Streptococcus	Ph	6.6	5.1	5.0	5.1	5.3
# 31	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.++	+++	+++	+++	+++
		Cat.++++	+++	-	-	-
Streptococcus	Ph	6.5	5.3	5.0	5.0	5.3
# 32	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.

Neisseria Catarrhalis and	Time	15 hrs.	40 hrs.	64 hrs.	88 hrs.	7 days
	Plates	Strep.++	+++	++	-	-
		Cat.++++	++	-	-	-
Streptococcus	Ph	5.9	5.1	5.1	5.3	5.3
# 33	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.++	+++	++	++	++
		Cat.++++	-	-	-	-
Streptococcus	Ph	5.2	5.0	4.8	4.9	5.2
# 34	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.
	Plates	Strep.++	+++	++	++	++
		Cat.++++	-	-	-	-
Streptococcus	Ph	5.5	5.0	4.8	4.9	5.1
# 35	Morphology	L.C.S.	L.C.S.	L.C.S.	L.C.S.	L.C.S.

Above is a table of results obtained when Neisseria catarrhalis and each of the various strains of streptococci were inoculated together into calcium carbonate broth and observations made as in the above three experiments. When Neisseria catarrhalis was mixed with streptococcus #1, on observing the plates after an interval of 15 hrs., it was seen that the catarrhalis greatly outgrew the streptococcus. At the end of 40 hrs., it was still outgrowing the streptococcus. However, at the end of 64 hrs., there came a reversal for at this time the streptococci were in the majority. And, at the end of 88 hrs., there was no catarrhalis remaining alive in the mixture. The streptococci remained alive. In the mixture with streptococcus #2, the ratio of the two organisms on the plates was the same as with streptococcus #1, except that the catarrhalis disappeared at the end of 40 hrs. In the mixture with streptococcus #5, the ratio of the number of organisms on the plates was similar to the above two, except that the catarrhalis did not disappear. Instead, it merely diminished at the end of the 88 hrs., but never did it disappear as it had done in the previous two cases. It was a question whether the acid produced by the much slower growing streptococci was the explanation of the destruction of the prolific catarrhalis. Consequently the hydrogen ion concentrations of all of the mixtures were run and recorded. And it can be seen that the increase in hydrogen ions corresponds closely with the death of the

catarrhalis. With streptococcus #1, the lowest Ph reached is 5.0, and with #5, the lowest Ph also is 5.0. Yet, in the former case the catarrhalis was destroyed, while in the latter, it remained alive.

In those cases where the catarrhalis was destroyed, the mixtures were filtered thru a sterile Berkfeld filter and tested for sterility. They were then neutralized with N/10 Na OH, retested for sterility and inoculated with catarrhalis and incubated at 37°C. In 15 hrs., there was a good growth of the organisms. To complete the experiment, it was repeated and the neutralization omitted. This time, the catarrhalis did not grow, nor did it remain alive. The inoculating was done by transfering 2.c.c. of a cloudy broth culture to 10 c.c. of the filtrate. According to this experiment, the acid was playing an important part in the destruction of the catarrhalis.

To rule out the possibility of a thermolabile toxin being the explanation of the refusal of the Neisseria catarrhalis to grow, the following experiments were done.

In those cases, again, where the catarrhalis was destroyed, the broths were filtered through a sterile Berkfeld filter and tested for sterility. The broths were then divided into five equal parts each and boiled respectively ½ minute, 1 minute, 2 minutes, 5 minutes and 10 minutes. They were then all reinoculated with Neisseria Catarrhalis and incubated at 37°C. for 24 hrs. At the end of that time no growth appeared. But upon neutralization and subsequent

inoculation and incubation, profuse growth obtained at the end of 24 hours.

The next question that came into my mind was, "Is 4.8--5.0 a sufficiently low Ph to actually kill the catarrhalis organisms?" Consequently, calcium carbonate broth ranging from Ph 4.4 at Ph 0.2 intervals to Ph 7.0 was prepared in the respective tubes. It was inoculated as above and incubated for 24 hours. The organisms were destroyed somewhere near Ph 5.0. More broth was prepared in tubes from Ph 4.6 to 5.2 with an interval of Ph. 0.5. This served as a double check and determined the acid death point more accurately. At the end of 24 hrs. the tubes after being inoculated and incubated as above, were tested for the presence of catarrhalis organisms and also their hydrogen ion concentration redetermined. This was necessary because 2.c.c. of neutral inoculum alters the Ph of the various tubes. This time, the acid death point of catarrhalis was 5.05. In most of the cases then, the hydrogen ion concentration was sufficient to destroy the catarrhalis. But in the case with streptococcus #9, the lowest Ph reaches was 5.3. Similarly, with streptococci #11, 21, 25, 30, 33, the lowest Ph reached is above the acid death point of catarrhalis. There is then the suggestion that another factor might be responsible in a small way for the destruction of the catarrhalis organisms.

No alteration in the morphology of either was observed.

SUMMARY

Staphylococcus aureus

1. Outgrew all of the strains of streptococci.
2. Streptococci disappeared 64 hrs.--88 hrs.
3. The type of streptococci used altered the results very little.
4. Staphylococci grew as readily as in pure culture.
5. Hydrogen ion concentration was about the same as in pure culture.
6. No alteration in the morphology of either was observed.

Staphylococcus albus

1. Outgrew all of the strains of streptococci.
2. Streptococci disappeared 64 hrs.--88 hrs.
3. The type of streptococcus used altered the results very little.
4. The hydrogen ion concentration increased less rapidly than with aureus.
5. Hydrogen ion concentration after about 40 hrs. was about the same as in pure culture.
6. Morphological characteristics of some of the strains of streptococci altered by the association.

Staphylococcus tetragenus

1. Was outgrown by all strains of streptococci.
2. In some cases there was more acid production by the mixture than either of the organisms produced in pure culture.
3. In some cases, where the hydrogen ion concentration was high, there was subsequent death of both organisms.
4. The hydrogen ion concentration for the most part corresponded more nearly to that of the streptococci than to that of the tetragenus.
5. The growth characteristics of each of the organisms when in the mixtures are altered by the other.
6. In some cases, the hydrogen ion concentration is altered by the association.
7. No morphological changes were observed.

Neisseria catarrhalis

1. Grew rapidly at first only to be destroyed later.
2. Destruction of catarrhalis corresponded very closely to the increase in the concentration of hydrogen ions.

3. In cases where there was no destruction of catarrhalis, the concentration of hydrogen ions was not as low as in cases where there was destruction of catarrhalis.
4. When the filtrates of the mixtures were neutralized and reinoculated with catarrhalis, there was very good growth.
5. When the filtrates of the mixtures were inoculated without neutralization, there was no growth, but destruction.
6. The acid death point of the strain of catarrhalis used in the experiment was Ph 5.05, for 24 hrs.
7. In certain cases an additional factor besides the acid aid in the destruction of the catarrhalis.
8. No morphological changes were observed.
9. Association alters the growth and acid producing characteristics of an organism.
10. Heating did not increase the growth qualities of the filtrates of organisms which in mixture killed catarrhalis.

It is suggested that when mentioning the acid death point of an organism, the time element be not emitted. Organisms which do not succumb to a Ph 5.0, in 24 hrs. may do so in 48 hrs.

CONCLUSIONS

1. Staphylococcus aureus and staphylococcus albus both greatly outgrow strains of streptococci in calcium carbonate broth. The changes in hydrogen ion concentration are adequate to explain the slow growth of the streptococci and their death later.

2. Staphylococcus tetragenus is outgrown by most strains of streptococci.

3. In mixtures of staphylococcus aureus and various strains of streptococci, the former is the dominant organism.

4. In mixtures of staphylococcus albus and various strains of streptococci, the former is the dominant organism.

5. In mixtures of Staphylococcus tetragenus and various strains of streptococci the latter are the more dominant.

6. In mixtures of Neisseria catarrhalis and various strains of streptococci, the former are apparently dominant at first and the latter dominant later. In most of the cases, changes in hydrogen ion concentration do explain the changes in growth characteristics but not in all of the cases. Something besides acid is present which with the acid destroys the catarrhalis.

7. Changes may be induced by association of organisms which are not present when the organisms are grown in pure culture.

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