CHANGES IN THE ORAL FLORA OF THE ALBINO RAT DURING CARIES ACTIVITY

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CHANGES IN THE ORAL FLORA OF THE ALBINO RAT DURING CARIES ACTIVITY

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Introduction

The subject of dental caries has engaged the attention of research men for many years. Through the years, experimental procedures, cultural technics, and the system of classification have been modified by advances in the science of bacteriology. Many methods have been devised to attack the problem and while each has its merits, no one method seems preferred. Countless examinations on human as well as animal subjects have yielded conflicting results. Animal subjects were preferred because a more rigid control of the nature of the food supply could be maintained. In most experiments with animals, the presence or absence of caries was determined, the animal killed, and a bacteriological examination of the teeth and surrounding tissues was made. The bacteriological examination of the teeth and surrounding tissues was made. The bacteriological activity preceeding the development of the dental lesion, in this type of procedure, was never observed.

The development of resistant and susceptible strains of Albino rats by H. R. Hunt and C. A. Hoppert at Michigan State College offered an opportunity to observe changes in the oral flora of these rats as correlated with the development of caries. The cariogenic diet developed by C. A. Hoppert was fed to both groups. The difference in the rate of caries development was thus due to a difference in genetic complement and oral flora. The change in oral flora as caries developed and the difference in the oral flora of the susceptible and resistant strains was investigated.

Review of the Literature

The pathological condition, dental decay, distinguished by a gradual dissolution and disintegration of the enamel, dentin, and eventually the pulp of the tooth has been recognized and described in medical literature for many years. Today, it is still one of the most important diseases, both from a public health and an economic standpoint. People from all social and economic levels are its victims. It is world wide in distribution with no known barriers limiting its activity. While many of the more dramatic diseases have yielded to the new drug and antibiotic treatment, the slower acting, more ubiquitous condition of dental decay has remained immune to prophylaxis. Dental decay, besides creating serious physiological disturbances, has been shown to act as a predisposing cause of more severe infections. Because of its great importance, the problem of dental decay has been investigated not only by bacteriologists, but by workers in the fields of nutrition, physiology, genetics, biochemistry and other allied fields.

Although many theories have been proposed concerning the development of dental caries, the first one to go beyond mere speculation and actually base its conclusions on data obtained from the study of diseased human teeth was the bacterial theory, proposed by Miller (1) in 1884. He showed that the bacteria found in the mouths of human subjects were able to ferment the carbohydrate food substances adhering to the teeth. The acid produced by this fermentation was then shown to be capable of decalcifying the tooth enamel.

Miller's theory had certain weaknesses which caused it to be rejected in part by many investigators. Most controversy centered about four points, unexplained by the bacterial theory. These points are: 1. If bacterially produced acids were the sole cause of dental decay, why were only certain areas of the tooth affected? 2. Why was the caries activity sometimes a sporadic process with decay halted? 3. Why was decay activity increased during pregnancy, malnutrition, and illness? 4. Why did not teeth exposed to lactic acid develop typical carious lesions? As further data in the field of dental research accumulated, two new theories were evolved--the theories of nutritional deficiency and hereditary predisposition.

Early investigators observed that certain people had some resistance to dental decay while others, often in the same family, were susceptible to dental decay to a considerable degree (2,3,4). This was explained by the geneticists who said that the people who were resistant to caries had inherited a genetic complement which rendered them relatively immune to dental decay. Similarly, those people who lacked this particular genetic complement were more likely to develop great numbers of carious lesions. What the difference is between the resistant and susceptible strains has not been determined. In 1937, Hunt and Hoppert (5) began a study of the inheritance of susceptibility and resistance to caries in albino rats. Hoppert et al (6) developed a diet which was satisfactory for promoting growth, health, and ability to reproduce but which was effective in producing caries of the lower molar teeth of the rat. These investigators have noted a constant occurrence of highly

resistant individuals in whom the development of caries is delayed until they have consumed the cariogenic diet for 600 to 700 days or die at an advanced age with no indications of caries present. The authors believe that the development of a caries immune strain may be a possibility.

Nutrition as related to caries development has been the subject of much debate. It was believed that during periods of malnutrition the incidence of caries increased. However, health surveys in the Scandanavian countries during and immediately after the second World War showed that instead of the caries incidence increasing during a widespread shortage of certain foodstuffs, it actually decreased. It was thought that there was a direct correlation between the amount of sugar available for consumption and the incidence of caries. Many authors in this country (7,8) believed that a carbohydrate free diet will decrease caries activity in the human mouth, by decreasing the lactobacilli count. Frobisher (9) believed that the concentration of sugar has no effect on the numbers of lactobacilli present; they are always present to a lesser extent than the acidogenic cocci. Belding and Belding (10) believed that the ingestion of refined cereals and starches that adhere to the teeth furnish an environment in which the acidogenic cocci can flourish and produce dental lesions. There is a general agreement among the investigators that acids produced by the oral flora are responsible for caries. There is no agreement, however, as to what the organism is, nor what part the type of food consumed plays in this action. Experiments now being conducted at the bacteriological laboratories of the University of Notre Dame (Lobund) have shown that rats fed a cariogenic diet in a germ free environment do not

get caries, while 95% to 100% of the rats fed the same diet in an ordinary environment develop caries (11). Kite et al (12) found that, if rats are fed a cariogenic diet by means of a stomach tube, they do not get caries even if they are of a normally susceptible strain. Spies et al (13) in a recent study of the fermentative properties of the Lactobacillus acidophilus (oral) observed that in general the more refined the cereal, the greater the amount of acid produced provided that amylase and the required B complex vitamins were present in the medium. These findings suggest that, in the absence of nicotinic acid and thiamin or substances that act similarly in human saliva, theingestion of large amounts of refined carbohydrates may not be, in themselves, necessarily conducive to caries. If wartime diets in certain countries were deficient in these necessary vitamins, could this be a possible explanation for a decrease in the caries incidence?

Bunting (14) stated, "It is obvious that the acids of caries are not generally distributed in the saliva as some earlier observers believed, for if they were the entire tooth would be affected rather than certain definite areas as is the case. It is obvious then that the presence of aciduric bacteria is not the whole answer."

The dental plaque, a thin felt like mass of material closely adherent to the tooth surface and containing filamentous bacteria which may enmesh other bacterial forms, has been suspected of playing some part in the formation caries. Black (15), Bibby (16), Kligler (17) and Wherry and Oliver (18) have all reported that Leptotrichia and Actinomyces form the plaque found on carious teeth. Van Kesteren (19) showed that the streptococci and Actinomyces produced acid most rapidly and that lactobacilli

were relatively slow in this respect. Bibby (20) found that Leptotrichia was capable of producing a high acidity in media containing carbohydrates. Frisbee et al (21) has shown that hamster molars infected with an actinomycete in broth culture developed lesions similar to those that occur in situ.

Plaques have been found on healthy teeth and Bibby (20) stated, "The plaque so often overlooked in the investigation of caries may be of prime importance. A better understanding of the significance of plaques can be obtained by recognizing different types and appreciating that the presence of some accumulations about the teeth may result in damage while others may give an added measure of protection."

He found that those teeth which showed characteristic brown plaques had only half as much decay as those which lack plaques. His observations of brown plaques and plaque bearing membranes from the surfaces of old sound teeth have revealed a predominance of thread forming bacteria consisting essentially of gram positive filaments and gram negative cocci, the latter on the outside of the plaque. Perhaps this will partly explain the findings of Hoppert and Hunt (22), who found in their experiments with albino rats, that those animals of the susceptible line who were 100 to 150 days old were significantly more resistant to decay than the 35-day-old rats.

The initial lesion, therefore, is dependent partly on a variety of local factors and partly on the nature of the plaque. Although a complete knowledge of the bacterial flora of the plaque is still incomplete,

it is agreed that it is composed chiefly of Leptotrichia, Actonomyces, and perhaps certain types of lactobacilli, vigorous in acid production and highly acid tolerant. Although the flora may vary, apparently the filamentous forms are always present (23,24,25). Stephan (26) has found the reaction of plaques over carious areas to be pH 5.0 or less immediately following a glucose rinse.

The Leptotrichia presents a problem to anyone investigating caries at the present time. As has been stated before, this organism has been reported as being part of the oral flora. Rosebury (27) states that Leptotrichia is a characteristic parasite of the mouth and may simulate fusiform bacilli since bacillary forms are rapidly decolorized by alcohol in all but very young cultures. The Leptotrichia, according to Rosebury, can be distinguished by serological reactions and by virtue of their failure to produce indol or hydrogen sulfide, and by their intermediate acidogenic capacity. However, Bergey's Manual, Sixth Edition (28) states that the genus Leptotrichia is no longer a valid genus. The Manual states that Leptotrichia may be a separate genus and it may be a variant of a Lactobacillus form.

The exact etiological agent of dental decay is still unknown. Lactobacilli and streptococci, isolated most often from carious lesions are considered by many workers to be the chief organisms responsible for dental decay. Bodecker (26) and Oakamura (10) believed that two organisms were necessary to produce dental caries, one, an acid producer, which decalcified the enamel, while the other organism destroyed the organic

material producing a cavity. Oakamura believed the gram positive acid producing rod was Lactobacillus acidophilus, while the gram negative rod which liquified the organic matter was Aerobacter cloacae. Gottlieb (30) believed that it is not an acid action but rather a proteolytic process spreading along and affecting the organic parts of the tooth. Organisms which produce yellow pigmentation and eventual necrosis are always present. He believed the responsible organism is a streptococcus. Belding and Belding (10) stated that it is the streptococci fermenting cereals that form the acids which dissolve the tooth enamel. They further stated that the increase in susceptibility to caries is caused by relative or absolute increase in the pathogenicity of the oral flora and ensuing accelerated formation of acid. The incidence of caries is determined by the number of food retention areas. Tunnicliff (31), investigating the smooth and rough greening streptococci in the pulps of carious teeth, on subculture found colonies composed of bacilli, crescent and coiled forms and straight and undulating filaments that may correspond to the cocci, bacilli and threads described by Miller (1). She stated that streptococci can invade dentin as easily as the lactobacilli, provided enough acid is produced. She also stated that in natural caries, cocci are the predominant and often the only organisms present in the tubules, indicating that the invading organism in dental caries is a coccus, whatever the predisposing factors. Kligler (17), Frobisher (9) and Anderson and Rettger (32) all found the acidogenic cocci much more prevalent than L. acidophilus in carious mouths.

Notwithstanding the mass of evidence indicating that the acidogenic cocci may be the etiological factor in dental decay, other investigators have claimed that the Lactobacillus species is the chief organism

causing caries. Bunting (33,34,35), Jay (36), and Bradel (37) have shown that there is an apparent increase in the lactobacilli count in carious mouths. Bunting (14) said, "L. acidophilus is not present in all mouths as has been claimed by other investigators. It is wholly absent or only sporadically present in the mouths of caries-free individuals and is continuously present and usually in a high degree of concentration in all cases in which the disease is active." However, Bradel (37) found in examining 4000 cases of dental caries, over 900 cases in which L. acidophilus could not be isolated.

The genus Lactobacillus because of the evidence implicating it in the development of caries has been studied intensively. There is at this time no satisfactory basis of classification available to differentiate between the species. Rahe (38,39) attempted to classify the genus by means of carbohydrate fermentation reactions. Many strains, however, apparently identical, differ in fermentative reactions and are not very stable in these characteristics. Davis and Rogers (40) separated the species by means of their respiratory mechanisms. Curran, Rogers and Whittier (41) investigated the possibility of differentiation by means of the end products of metabolism.

Early investigators studying the lactobacilli believed that the organism isolated from the intestine was different from the strain isolated from the oral cavity. Weiss and Rettger (42) in their study observed that, although there were great variations among different strains of each of the two groups, L. acidophilus and L. bifidus, L. bifidus was considered to be a variant of L. acidophilus. Both are normal inhabitants of the

intestine. Harrison and Opal (43) using carbohydrate precipitation methods showed that the intestinal and oral strains of L. acidophilus from the same individual were apparently identical. Thomas (44) showed that L. acidophilus of the intestine was serologically the same as doderleins vaginal bacillus. Kulp and Rettger (45) found no difference between Lactobacillus Bulgaricus and L. acidophilus and concluded that the former is a variant of the latter organism. Lactobacilli have the unique property of dissociating from the rough to the smooth form, the reverse of the ordinary dissociation reaction. Rogosa, studying this change from the rough colonial form to the smooth colonial form, found that sorbitan monoleate in 0.1% concentration in tomato agar could change the total population of rough colonies to smooth colonies. Since the colonial form can be reversed at will, he concluded that no mutation is involved. Upton and Kopeloff (47), however, showed that the rough forms of L. bulgaricus and L. acidophilus were antigentically the same as were the smooth forms of these two organisms. But, conversely, the rough and smooth forms of either species were antigenically dissimilar.

Hemmens (48) in a review of the oral lactobacilli concluded that L. acidophilus (Moro) and the lactobacilli found in the oral cavity are similar. Howitt (49) as a result of antigenic studies on the oral lactobacilli suggested that they belong to separate groups rather than all belonging to one group. Harrison et al (50) showed that there existed at least four serological types in one group of oral strains while another group consisted of heterogenous types. Later Harrison (51) reported that by their fermentation reactions oral lactobacilli fell into two groups

essentially identical with his two large serological groupings. Orland (52) detected the same major antigen in a number of strains of lactobacilli isolated from different sources, including the oral cavity. Many of these organisms with the same major antigen had different species names. He was unsucessful in attempting to induce a change in antigenicity in these organisms and concluded that the antigenic structure is rather stable. Canby and Bernier (53) on the basis of serological reactions concluded that the antigenic structure of the lactobacilli must be extremely complex.

Coolidge (54), investigating the changes in properties of the lactobacilli, found that certain strains of oral lactobacilli undergo nutritional and metabolic changes accompanied by changes in fermentative properties. These alterations include changes in the requirement for serine and thiamin, changes in the amount of acid produced from glucose, and in the final pH attained by cultures in synthetic media.

Hadley, studying the role of L. acidophilus in caries, tested the properties of the rough and smooth colonies. She found that organisms from the smooth colonies coagulated litmus milk very fast, and were able to ferment sorbitol and mannitol but not maltose. Organisms from rough colonies coagulated litmus milk much slower and were able to ferment maltose but not sorbitol and mannitol. She also observed that the rough colony inoculated into glucose broth in 24-28 hours produced a granular, clumpy precipitate. The clumps, when examined microscopically, were seen to be composed of long filaments and in some places were broken up into chains of cocci or rods. She stated, "These filaments, if seen in direct

smears, would undoubtedly be called Leptothrix. They are seen to form only a phase but possibly the most important phase of this group of oral aciduric rods."

Clapper (56) in his work on caries stated that the presence of caries has been found to be associated with a ralatively high incidence of a special type of Lactobacillus in the saliva and in dental plaques. This type of Lactobacillus produces a pH of less than 5 in glucose broth, has an active dehyrogenase for glucose, ferments salicin, mannitol and rhamnose but not raffinose. Harrison (57) in his work on dental caries in rats found that lactobacilli could be isolated from enamel caries but not dentinal caries. He believes that there is an apparent relationship between lactobacilli and initial caries and between streptococci and advanced lesions of the dentin.

It will be recalled that the presence of Actinomyces in the plaque and also in the mouths of humans in the absence of actinomycosis has been reported (58). Colebrook (59) has shown that the Actinomyces can grow under atmospheric conditions. Others have reported the presence of Leptotrichia in the oral cavity. It was stated that the genus Leptotrichia is no longer considered a valid genus, many of the species now being found in the genus Lactobacillus. Then Lactobacillus bifidus and L. acidophilus were found by Weiss and Rettger (42) to be closely related. Now Putoni (60) claims that there exists biochemical, morphological, and immunological similarities between Actinomyces bovis and L. bifidus. Could these facts indicate that essentially these organisms are closely related and have been separated by an artificial classification scheme?

The latest innovations in the field of dental research concerns the use of fluorine in the water supply and the use of ammoniated dentrifices. Regarding the use of fluorine, Bibby stated (61), "It is our present opinion that the reduction of dental caries produced by fluorine is a result of a direct combination between the fluorine of the water and food with the dental enamel producing a less soluble tooth substance." Complete data have not been accumulated regarding the effects of fluorine in the water supply and the use of ammoniated dentrifices and most research groups believe that a true evaluation of their worth cannot be made at the present time.

Experimental

A. Changes in the Oral Flora During Caries Activity.

Procedure

Rats, thirty days old, were separated by sex and put into seqarate cages. Eighteen rats of the susceptible strain and three rats of the resistant strain, used as controls were employed. Both strains were fed the same diet. Oral examinations were started at this time, earlier examinations were not feasible because of the difficulty encountered in inserting the speculum into the small mouth of the rat. The teeth on the lower right jaw were always examined at weekly intervals and at approximately the same time to determine the presence or absence of caries. The sample was taken from this same area by means of a small, sterile, cotton swab on the end of an applicator stick. The sample was obtained by rubbing the swab over the molar surfaces. It was realized that this procedure had certain weaknesses, but it was the only way to obtain a sample without destroying the rat.

Because the lactobacilli grow slowly on solid media, the plates were incubated at 37° C for four days. The plates were then examined under the dissecting microscope, a count was made of the various colonial forms and representative colonies transferred into Brain Heart Infusion Broth (Difco) containing an additional 0.1% agar.

Gram - stained slides were made of the organism growing in the brain heart infusion medium and, if pure cultures were obtained, it was then used to inoculate the various sugars, litmus milk, and other media necessary to determine the properties of the organism.

Results

A preliminary survey was made of various plating media to determine which one would best support the growth found in the oral cavity. It was found that Tomato Juice Agar (Difco) was an excellent medium, with one exception. The original medium, on cooling, was extremely soft, making it difficult to streak the plates effectively. This was corrected by the addition of 1.0 gm. of agar per 100 ml. of medium. Later it was found that a more vigorous and luxuriant growth could be obtained with the addition of 0.5 gm. of dextrose per 100 ml. of medium. The final pH was 6.1, exactly the same as the original medium. On this modified medium the organisms encountered grew well, could easily be seen and isolated, and there was no excess drying out of the medium on prolonged incubation.

The susceptible strain of rats developed caries six to eight weeks after the examinations began. No caries developed in the resistant rats during the course of the experiment. This was not unexpected for the resistant rats are caries free up to three hundred days and the resistant rats used here were the same age as the susceptible rats.

Examination of the tomato agar plates under the dissecting microscope revealed that there were two types of flora present. One was regular in appearance from week to week, varying only in numerical proportion with each other. This flora consisted mainly of lactobacilli, filamentous organisms resembling leptotrichiae, streptococci, and yeasts. The second type of flora consisted of gram negative rods of the coliform

group, cocci such as Staphylococcus albus, Micrococcus tetragenus, gram positive rods of the diphtheroid type, and several times colonies appeared that were a variant of Bacillus cereus. There was no definite trend in the appearance of these organisms. Sometimes they were present in only one rat at a time. Because of this, they were not investigated as thoroughly as the regular flora.

Because of the way the sample was obtained, this had to be a qualitative rather than a quantitative study. It was found, however, that among the regular flora, the proportions among the organisms varied within definite limits from week to week.

The first examination usually revealed a high proportion of lactobacilli and streptococci. Yeasts were present to a lesser extent, and the filamentous organisms were the least numerous. The lactobacilli and the filamentous organisms were separated on the basis of colonial morhology, staining characteristics and fermentative properties as described in Part B.

Continuing weekly examinations revealed that the lactobacilli decreased in numbers as did the streptococci. The number of filamentous organisms made a slight increase. On numerous occasions it was noticed that around the third or fourth examination there was a decrease in the total number of colonies on the plate, as if some inhibiting force was at work. This was noted always with the susceptible rats, it never occurred during the course of the experiment in the resistant rats.

From the third examination on, the filamentous organisms increased in numbers. The streptococci also showed some slight increase in numbers, the yeast and lactobacilli appearing only sporadically or in very small numbers.

Examination of the plates just prior to the appearance of caries in the susceptible rats always revealed that the flora was predominantly of the filamentous and streptococcal forms. One to two weeks after the development of caries, the plates revealed an increase in the number of lactobacilli colonies with a decrease in the filamentous forms. The proportions of streptococci were usually the same as in the pre-caries period. It was noted that whenever lactobacilli and streptococci were present in the same plate, the streptococci were always numerically superior.

The flora of the resistant rats, used as control, was composed mainly of yeasts, gram negative rods, cocci resembling staphylococci, gram positive diplococci, and only an occasional filamentous organism or Lactobacillus. The number of colonies growing on the plates from the resistant group was always much less than the plates of the susceptible group.

Figure 1.

Lactobacillus sp. isolated from the mouth of the Albino Rat.

Figure 2.

Filamentous organism isolated from the mouth of the Albino Rat showing club-shaped form.

Figure 3.

Filamentous organism isolated from the mouth of the Albino Rat showing the whip-like form.

B. Fermentation Reactions of Organisms Isolated from the Oral Cavity and the Total Titratable Acidity Produced by These Organisms.

Procedure

It has been stated that caries may be developed because of a change in fermentative properties of the organisms found in the oral cavity, accompanied by a relative increase in the amount of acid produced. All the organisms isolated during the weekly examination were tested on 14 carbohydrates, litmus milk and a partly synthetic medium developed by Orland (52). The composition of this synthetic medium was as follows:

Peptone	1.0 gm.
Cystine	0.1 gm.
Yeast extract	2.0 gms.
Glucose	2.0 gms.
K_2HPO_4	100 mg.
KH_2PO_4	100 mg.
$MgSO_4$ .7 H_2O	40 mg.
Nacl	2 mg.
$FeSO_4$ .7 H_2O	2 mg.
$MnSO_4$ .7 H_2O	2 mg.

Distilled Water q.s. 200 ml.
Media tubed in exactly 10 ml. amounts. Final pH, 5.8

This medium favored the lactobacilli, both as to growth and acid production, but all the organisms were tested on it in order to have a basis of comparison. A loopful of organisms from the brain heart infustion broth was used to inoculate the sugars, litmus milk, and the synthetic medium. The sugars were incubated for 14 days with readings taken at 1, 2, 3, 7, and 14 days. The acid produced in the synthetic medium was titrated after 7 days of incubation to obtain the maximum amount of acid, by using N/10 NaOH. Neutrality was determined by colorimetric means, standard buffer solutions were used for comparison.

Results

Preliminary studies of the fermentative properties of the lactobacilli disclosed that the organism gave consistent results on litmus milk, but that the sugar reactions were often erratic. The fermentation of lactose, sucrose, and dextrose was particularly irregular. Many times positive reactions took place only after a five-to-seven-day incubation period. The broth used in these tests was a Tryptose Base Broth (Difco) plus the respective sugars in 0.5 per cent concentration. When Veal Infusion Broth (Difco), which contained an additional 0.1 per cent agar was used, results were more complete and consistent. The same results could be obtained in broth media not containing additional agar if a rubber stopper was inserted into the mouth of the tube, then incubated at 37° C. The indicator used was Brom Cresol Purple.

The lactobacillus colonies growing on the tomato plates were about 1 mm. in diameter, irregular, rhizoidal in shape with a maze of twisted fuzzy projections. They produced acid, coagulation and reduction in litmus milk in one to two days. Every lactobacillus colony isolated from the 21 rats gave the following reactions consistently:

Table 1.

Carbohydrate	Days 1	2	3	7	14
Litmus Milk	-	ACR	ACR	ACR	ACR
Lactose	-	-	↗	↗	↗
Dextrose	-	↗	↗	↗	↗
Sucrose	-	↗	↗	↗	↗
Raffinose	-	-	-	-	↗
Dulcitol	-	-	-	-	-
Rhamnose	-	-	-	-	-
Sorbose	-	-	-	-	-
Sorbitol	-	-	-	-	-
Mannitol	-	-	-	-	-
Maltose	-	↗	-	↗	↗
Xylose	-	-	-	-	↗
Inulin	-	-	-	-	-
Dextrin	-	-	-	-	↗
Arabinose	-	-	-	-	-

↗ = acid
ACR = acid, coagulation and reduction
- = negative

A gram stain of the above organism showed a gram positive rod, 2 to 2.5 microns long and 0.7 to 0.9 microns wide. Occasionally a very long rod was seen. The organism was stained evenly. It was calalase negative, did not liquify gelatin, did not reduce nitrates to nitrites, and did not produce indol. It was non-sporulating.

The filamentous organisms, with Gram's stain, showed long filamentous forms 50 to 100 microns in length and 1 micron in width. The forms were usually decolorized, with gram positive granules discernable within the organism. Very often long tapering forms were seen that were clubbed on one end. They did not change litmus milk, did not reduce nitrates to nitrites, did not produce indol, were catalase negative and grew well aerobically. They were non-sporulating. Every filamentous colony isolated from the 21 rats gave the following reactions:

Table 2.

Carbohydrate	Days 1	2	3	7	14
Litmus Milk	NC	NC	NC	NC	NC
Lactose	-	≠	≠	≠	≠
Dextrose	≠	≠	≠	≠	≠
Sucrose	≠	≠	≠	≠	≠
Raffinose	≠	≠	≠	≠	≠
Dulcitol	-	-	-	-	-
Rhamnose	-	-	-	-	-
Sorbose	-	-	-	-	-
Sorbitol	-	-	-	-	-
Mannitol	-	-	-	-	-
Maltose	≠	≠	≠	≠	≠
Xylose	-	-	-	-	-
Inulin	-	-	-	≠	≠
Dextrin	-	-	-	≠	≠
Arabinose	≠	≠	≠	≠	≠

≠ = acid
NC = no change
- = negative

The differences between the two organisms are apparent. The lactobacilli changed litmus milk, fermented raffinose late and did not ferment arabinose.

The streptococci isolated fermented lactose, sucrose, dextrose, raffinose, maltose, inulin, and dextrin in 24 hours. At the end of 14 days xylose was fermented. Litmus milk showed acid, coagulation, and reduction.

The synthetic medium inoculated with pure cultures of the organisms revealed no significant difference in the total titratable acidity produced. The lactobacilli isolated before, and after, caries development required 3.8 to 4.8 ml of N/10 NaOH. The filamentous organisms required from 3.6 to 5.2 ml of N/10 NaOH. The streptococci required from 2.5 to 3.0 ml of N/10 NaOH. The streptococci reached their maximum production of acid in two days and thereafter the amount of titratable acidity produced did not increase noticeably. The growth of the streptococci in the synthetic medium was not as profuse as in Veal Infusion Broth. The yeasts grown in the synthetic medium needed only 0.5 ml of N/10 NaOH for titration.

After the tomato plates were lightly streaked, the swab was placed into the synthetic medium for 1 minute. The acid produced would represent the production of the complete flora growing together in the synthetic medium. After inoculation the tubes were incubated for 2 days at 37° C. They were then titrated in the same manner as before. Titration of the cultures gave the following readings:

Table 3.

Titratable Acidity in ml of N/10 NaOH

	Week	1	2	3	4	5	6
Susceptible Rat	1	3.2	5.4	4.0	4.6	6.1	5.4
	2	2.8	4.6	4.6	5.3	5.0	5.4
	3	3.5	5.9	5.3	4.9	6.3	5.6
	4	4.0	4.8	3.7	5.4	4.9	3.6
	5	3.2	5.5	6.6	5.3	5.5	5.0
	6	3.2	5.5	4.6	5.4	5.5	5.2
Resistant Rat	1		2.3	2.6	3.8	3.2	
	2		3.6	3.0	3.6	3.0	
	3		2.4	3.4	3.7	3.2	

It can be seen that acid production in the susceptible line prior to caries is a series of peaks followed by a period of decline. In the resistant line the tendency is for acid production to be rather steady. It will be seen that the highest point in acid production on the part of the resistant strain just reached the lower limits for the susceptible strains.

C. An Evaluation of the BCG Dextrose Agar (Difco) Used in the Colorimetric Diagnosis of Caries Activity.

Procedure

The use of BCG Dextrose Agar (Difco) for determining caries activity is based on the acid production in a carbohydrate medium by acidogenic microorganisms found in the buccal cavity and is evidenced by a change in the color of the indicator, brom cresol green, from a blue green to a yellow color. In human subjects, the patient chews paraffin for three minutes, with the saliva being collected in sterile tubes. Then 0.2 ml of the saliva is added to the melted medium, cooled to 45° C by means of a sterile pipette. In testing the rats, the swab containing the oral flora sample was first lightly streaked on the tomato plates and then immersed into the BCG medium for one minute. The tubes were incubated for 48 hours at 37° C, readings being taken at 12, 24, and 48 hours. The tomato plates, after incubation at 37° C for four days, were examined and representative colonies transferred into brain heart infusion broth. After growth of the organism in this medium, a loopful was removed and inoculated into the BCG medium as described above.

Results

Six rats were tested and in general the tests agreed with the status of the rats in regard to the presence or absence of caries. The majority of the rats yielded negative tests during the first few examinations. In the tubes giving positive tests, the isolated colonies from the tomato plates and grown in the B.H.I. broth were inoculated into fresh tubes of BCG Dextrose Agar. In every case except one, and that organism did not produce good growth, the streptococci and the filamentous organism produced a color change in 24 hours. The lactobacilli did not produce a significant color change at 24 hours and only at the end of 48 hours was a change from a blue green to a yellow color discernable.

The explanation of the above results may lie in the fact that the BCG Dextrose Agar is not particularly suited for the cultivation and production of acid by the lactobacilli.

Discussion

The rapid development of dental lesions in approximately 6 weeks in susceptible rats fed a cariogenic diet while resistant rats fed the same diet remained caries free, indicates that some change must occur in the oral cavity. This could be due to a complete change in the oral flora or a percentage change in the number of important organisms comprising the oral flora.

It was shown that there is no change in the basic flora but rather a change in composition and numbers of the organisms comprising the oral flora. The filamentous organisms and the streptococci showed a significant increase in numbers in the period immediately preceeding the development of caries. After caries development, lactobacilli were usually present in all animals examined and in increased numbers.

The classification of the filamentous organism was not attempted. It had characteristics of both the lactobacilli and the Leptotrichia. If this filamentous organism could be shown to be a lactobacillus variant, then it could be said that the lactobacilli increase in numbers significantly during caries development. Further study by serological means would probably be necessary to accomplish this.

These findings pertain only to the action that occurs in the oral cavity of the Albino Rat. No attempt is made to relate this action to the development of dental lesions in humans.

Summary

1. Lactobacilli, as characterized by colonial morphology, staining properties and fermentative reactions were present only sporadically before the development of caries. After caries had developed, they were present regularly and in greater numbers.

2. The oral flora of the susceptible strain of the Albino Rat contained a greater variety of organisms than that of the resistant strain. The number of organisms growing on the plates was always larger in the susceptible group.

3. The titratable acidity produced by the organisms in the oral cavity of the susceptible strain was characterized by a high point usually followed by a slight decline. Titratable acidity produced by the resistant strain of rats was relatively steady. The titratable acidity produced by the resistant rats was significantly below that of the susceptible rats.

4. In those tests in which BCG Dextrose Agar was used to determine caries activity colorimetrically, positive results obtained in 24 hours, were usually produced by organisms other than lactobacilli.

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