DENTAL CARIES OF RATS PRODUCED BY ACIDOGENIC ORGANISMS

THESIS FOR THE DEGREE OF M. S.

John Lincoln Etchells

1932

Teeth - Diseases

Title

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Submitted to the Faculty of the Michigan State College
in partial fulfillment of the requirements for the
Degree of Master of Science

by

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ACKNOWLEDGMENT

I wish to acknowledge my indebtedness to Doctors E. D. Devereux and C. A. Hoppert for their interest shown throughout this investigation, and for the helpful advice freely given.

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I. Introduction

The subject of dental caries has been studied in great detail since 1867 when Leber and Rottunstein (1) wrote a most interesting book on the subject. They presented a theory of dental caries which does not vary in great detail from the conceptions of the investigators of the present day, who recognize the role of bacteria in relation to dental decay. However, Miller (2) not only confirmed the earlier investigations as to the action of organic acids upon the inorganic constituents of the teeth, but showed by experiment the action of these acids upon their structure. For the following half century, work on cause of dental decay was carried on under the stimulus of Miller's (2) theory.

Recent research on dental caries has, in the greater part, been confined to the part played by diet and nutrition. The present day investigators, while recognizing Miller's theory, find it inadequate in some respects and would place the most emphasis upon the dietary deficiencies, rather than acknowledge the role of acidogenic bacteria.

Hoppert et. al. (3) showed that dental caries could be produced in rats fed on an adequate diet by retention of coarse food. Using this work as a foundation, this study demonstrates the bacteriological

role of acidogenic organisms, by using such bacteria to produce dental caries in rats, a procedure which heretofore has met with little or no success.

II. Review of Literature.

Although a review of the etiological factors of dental caries has been presented by Hanke (4), Bunting (5), and Marshall (6), it appears necessary that investigation pertaining to the bacteriology of this disease be reviewed again as it is of primary importance to the work presented by the writer.

In 1883 Miller (7) first recognized the relationship between bacteria and dental caries. He was of the opinion that although bacteria were not the sole cause of dental caries, there was no case of dental caries in which they did not play an important part. Due to a lack of suitable culture media he was unable to give complete discriptions of the bacteria he studied.

Hartzell and Henrici (8) in 1917 reported that mouth streptococci are the only true parasitic organisms of the normal, characteristic mouth flora. They state definitely that these bacteria were the cause of dental caries as well as a number of other dental diseases.

In 1920 Kahnert (9) made a study of dental caries in the horse. He concluded that as in human beings, dental caries is not caused by any one specific organism. He stressed the importance of acid-forming and proteolytic organisms.

Rodriguez (10) in 1922 classified a high acid-producing type of bacteria as types I and II, on the

basis of morphology, and found them constantly in the deep portions of the foci of active dental caries.

McIntosh, Lazarus-Barlow and James (11) in 1923 produced artificial caries by placing teeth in broth inoculated with acidogenic organisms. After a prolonged period the teeth showed erosion of the enamel, penetration of the dentinal tubules, almost identical to natural caries. They were, however, unable to produce dental caries in vivo by feeding acidogenic bacteria. In their study of fifty carious teeth, two types of organisms similar to Lactobacillus acidophilus were found. One organism occurred in 88 per cent of the teeth examined, and the other in 42 per cent. They proposed the name Bacillus acidophilus odontolyticus, I and II.

Howe (12) in 1924 was able to produce dental diseases in monkeys by feeding diets solely deficient in vitamin "C", but he reports that earlier work on production of dental caries in animals by acid-forming organisms was unsuccessful.

Sierakowski and Zajdel (13) in 1925 confirmed the work of McIntosh et. al. regarding the role of L. acidophilus in dental caries. They isolated this organism from 80 per cent of the dental caries examined. Two types were found, long slender rods, and short thick rods. Both grew feebly in liquid media. They noted that the best growth occurred in glucose

broth. Pure cultures of these organisms caused typical caries in a tooth in a test tube in three months.

Bunting and Palmerlee (14) further confirmed these findings when in 1925 they found an acidogenic and aciduric type organism which appeared to be L. acidophilus in practically every lesion of active dental caries.

Schlirf (15) who made a bacteriological investigation of dental caries in Germany in 1926, stated that the decalcification of the teeth was due to the production of acid produced by bacterial metabolism. He noted large numbers of acid-forming rods in carious dentine. These conclusions as to decalcification were discussed most clearly by Miller (2) in 1883.

Rosheny, Linton and Buchbinder (16) in 1929 studied the aciduric organisms and L. acidophilus in dental decay. They could see no differentiation between these two types of organisms. Out of 21 strains of aciduric organisms isolated from teeth no differences could be detected between the two groups in the morphology and bio-chemical study. There was no clear distinction in the serological reactions, as there was marked cross agglutination.

In contradiction to the investigators that report finding L. acidophilus in practically every lesion, Morishita (17) reported that from 105 strains of

aciduric organisms isolated from dental caries only 7 bore a close resemblance to L. acidophilus (Moro).

In 1929 Bodecker (18) proposed a theory as to the cause of dental caries. He maintained that fermentation of carbohydrates formed lactic acid, which attacked mineral constituents, while the organic substance is destroyed by another type of bacteria. Thus a cavity is formed. This theory was propounded almost identically by Miller (2) in 1883 and by others later.

Morishita (19) in 1929 found that high acid tolerating organisms were found in constant occurrence in tooth enamel in the process of decay. Relatively few were noted in non-carious teeth. He could produce decalcification in vitro but not in vivo.

Marshall (6) in review of the etiology of dental caries states "An historical resumé of the etiology of dental caries presents at least one interesting fact; namely, more has been written and less known of this disease than any other dental lesion".

III. Experimental.

A. Bacterial study of rat foods

Procedure

One gram each of rice, powdered milk, and corn was taken from stock and after making proper dilutions was plated on yeast extract agar*. The typical colonies from each food were noted as to morphology, and then picked into litmus milk to find the acid reaction.

Results

The types of organisms naturally occurring on the grains studied, that were used for food for rats, had a close relationship to the types isolated from the teeth. The production of acid by the organisms from the food would probably be sufficient if once established in the food-impacted teeth to give rise to cavities.

It must be noted that while a total of 15 acid-forming organisms were isolated from the foods studied, there were also 16 proteolytic organisms noted. This fact may have a direct bearing on the more advanced stages of dental caries, where the decomposition has reached the bone tissue. Miller recognized the

*Formula for yeast agar:

Peptone----------	5.0 grams
Peptonized milk--------	10.0 "
Dextrose----------	5.0 "
Yeast extract----------	5.0 "
Agar----------	15.0 "
Water----------	1000.0 c.c.

proteolytic enzymes, as his theory of decay was divided into two parts, first, fermentation of carbohydrates to form organic acids and secondly, action of proteolytic enzymes on the protein constituent of the teeth.

Of the 12 organisms isolated from the rice, six were acid producers, which formed a typical smooth curd in 48 hours while one produced acid indicated by litmus change in 48 hours, and formed curd after several days.

In the case of the powdered milk, being a laboratory product, the acid formers were not as numerous. Of the 11 organisms isolated, two formed smooth curds, and one formed acid in 48 hours incubation at 37°C. in litmus milk.

From the 11 organisms isolated from ground corn, five were found to produce smooth curd in litmus milk.

B. Bacterial study of carious teeth.

Procedure

Organisms associated with dental lesions were isolated from rats (old animals with pronounced caries) by etherizing them, removing the jaw bone, and with a sterile, straight needle digging into the foci of infection and smearing direct on yeast extract agar plates. These plates were incubated for 48 hours at 37°C. The typical organisms usually appeared in pure culture and were then studied as to morphology and acidogenic properties.

Results

From the total of 12 rats, (old females) with caries well developed, 48 organisms were isolated. Of this number 30 were producers of acid curd in litmus milk.

Organisms from rats, Nos. 5 to 12, were obtained by smearing direct from the carious area with straight platinum needle. The preceeding organisms, those from rats Nos. 1 to 5, were isolated by dilution method made from material taken from the tooth. The former method proved superior, as the needle reached the focus of the carious lesion, and hence limited the organisms obtained to those having direct association with decay.

From the plates that were streaked directly on yeast extract agar, the typical colonies found were

repeatedly noted. They were (1) streaks of small white, glistening colonies, gram positive cocci, and (2) streaks of veily, grey filamentous colonies of large, plump gram positive rods, resembling L. acidophilus. These two organisms always predominated on the smeared plates and many times occurred in pure culture. They were vigorous acid formers and it was interesting to note that the same organisms were observed on ten of the direct smears made from human caries.

The coccus forms always appeared as small, round, white, glistening colonies which would be lifted off with a needle. They readily fermented the seven diagnostic sugars for L. acidophilus and in 48 hours incubation produced a pH of 4.6 in broth.

The L. acidophilus colonies occurred in three, more or less typical forms, (1) regular, rough, fuzzy, filamentous type (2) more compact, rough, irregular edge type (3) round, irregular colony with projecting, fuzzy filaments.

Chart I. Data on acidogenic organisms from caries of rats.

Organism	Motility	pH in broth adjusted to pH 7.0 24 hr.	2 days	3 days	Gr. stain	Ident.*
1-R2	+	6.3	6.5	4.9	+ rod	S.L.acidophilus
1-R3	-	4.8	4.6	4.6	+ coccus	-
1-R5	-	4.7	4.7	4.7	+ rod	S.L.acidophilus
1-R6	-	4.8	4.7	4.6	+ coccus	-
1-R7	-	4.7	4.6	4.7	+ coccus	-
1-R8	-	4.8	4.5	4.6	+ coccus	-
1-R16 (control)	+	6.0	7.3	7.5	- rod	-
F9	-	5.7	5.7	5.1	+ rod	R.S.L.acidophilus
R20	-	4.8	4.8	4.75	+ rod	R.L.acidophilus
1-LA	-	4.3	4.0	4.0	+ rod	R.L.acidophilus
R12	-	4.5	4.4	4.2	+ rod	R.L.acidophilus
1-R21	-	4.6	4.8	4.8	+ rod	R.L.acidophilus
BR-11	-	-	-	4.5	+ rod	R.L.acidophilus
AR-11	-	-	-	4.5	+ rod	R.L.acidophilus

*S = smooth type L. acidophilus

R = rough type L. acidophilus

RS = rough-smooth variant L. acidophilus

Chart II. Data on acidogenic organisms from human caries.

Organism	Motility	pH (7 days incubation)	Gram stain	Identity of organism
K-RS	-	4.25	+ rod	R.S.acidophilus
K-1	-	3.8	+ cocco-bacillus	S. acidophilus
B-1	-	5.0	+ coccus	-
K-12	-	4.8	+ coccus	-
H-1	-	4.8	+ rod	R.S. acidophilus
H-2	-	4.5	+ rod	R. acidophilus
H-3	-	5.0	+ rod	R.S. acidophilus
H-4	-	4.0	+ rod	R.S. acidophilus

The last four human strains (Chart II) obtained from extracted carious teeth, remained in the R.S. state when transferred daily in broth, however, when cultured on two per cent dextrose chicken infusion agar and allowed to grow at room temperature for three weeks, typical smooth L. acidophilus colonies appeared, and upon staining revealed short bi-polar rods. If the smooth colony was transferred into broth, it would revert to the original R.S. stage. Time did not permit a thorough study of this behavior, though it was repeated three successive times.

C. Production of dental caries with acidogenic organisms

Procedure

Source of cultures.

1. R20 - R. L. acidophilus, oral strain, isolated from old rat (female) with extensive caries.*

2. K-RS - R.S. or rough variant L. acidophilus, isolated from a student having typical dental caries.

3. K-1 - Cocco-bacillus, isolated from a student, having typical dental caries.

4. F-1 - Coccus form of fowl origin. Isolated from a fowl having an impacted beak with extensive fermentation so that the bill had been partially disintegrated. This organism appeared in pure culture, upon streaking on yeast extract agar from the infected area.

These organisms were chosen because of their widely separated sources, and inasmuch as they were known to be directly related to dental decay.

The experiment was based on the fact that rats although fed upon an adequate diet, when food impactions can be made by use of coarse rice or corn, will contract dental caries.

*These organisms were first smeared directly upon yeast extract agar with a straight needle. They were then picked into yeast extract broth, and after 24 hours incubation at 37°C. were streaked with a loop needle on yeast extract agar slate and incubated at 37°C. for 24 hours. After picking into yeast extract broth for the second time and incubating they were considered ready to be used.

Hence by use of acidogenic cultures isolated from dental caries, when placed in such an environment will reproduce a typical case of dental caries as observed so often in nature.

Experimental set-up

Cage No. 1 control - sterile diet - no organism

Cage No. 2 culture R20 + sterile coarse ration

Cage No. 3 culture K-RS + sterile coarse ration

Cage No. 4 culture K-1 + sterile coarse ration

Cage No. 5 culture F-1 + sterile coarse ration

Cage No. 6 impaction control - unsterilized coarse (corn or rice)

Cage No. 7 impaction control - unsterilized fine (corn or rice)

The selection of a proper diet that was suitable for such an experiment involved much time and difficulty inasmuch as each experimental procedure took a period of eight weeks. The diet finally chosen was one that was adequate for growth, retained its characteristics upon sterilization and was palatable to the rat. The formula for the ration in percentages by weight was as follows: Alfalfa 10, corn 63, oilmeal 15, yeast 5, casein 5, sodium chloride 1, and calcium carbonate 1.

The ration, after being thoroughly mixed, was placed in suitable containers, plugged with cotton and autoclaved for 30 minutes at 15 pounds pressure. A control can was plated for bacterial growth to insure sterility. After being sterilized the food was broken up with a sterile glass rod.

The organisms were added to the rations daily, one c.c. of a 24 hour broth culture added by means of a sterile pipette and mixed. Each day a complete set of rations were made. This was done to insure an excess of the culture, and to control the microflora of the mouth.

The animals on the experiment were isolated and daily the animals and cages were exposed to ultra-violet light treatment to avoid outside contamination.

After a period of eight to ten weeks the animals were etherized, the jaws removed and streak plates made from carious areas of the teeth upon yeast extract agar (pH 7.0)*. After incubation the plates were ready for identification of organisms.

The stock cultures were identified according to the following:

1. characteristics of colonies
2. staining morphology
3. sugar reactions
4. litmus milk reactions

*The teeth specimens, after culturing were washed and dried and reserved for further study.

Results.

This experiment was performed to produce dental caries by controlling the micro-flora of the mouth, by use of acidogenic bacteria. These organisms were inoculated into sterile rations containing coarse corn or rice to cause retention of food. This impaction of food resulted in carbohydrate fermentation by the acidogenic organisms, which in turn disintegrated the dental structure.

The experimental work was retarded considerably at the beginning until a ration containing coarsely ground grain could be selected that would upon autoclaving remain palatable to the rats and also retain its physical characteristics and be adequate for growth.

After two preliminary runs of eight weeks each, coarse corn was found to be the ideal grain for impaction. This also withstood autoclaving without change of hardness. These investigations on corn revealed that L. acidophilus, which is a delicate organism to grow and requires an enriched medium, would grow most readily on finely ground corn emulsion.

Chart III. Continued. Characteristics of isolated organisms from cavities

Organism	Morphology	L. milk	pH	Sugars							Plate descirption
				dex.	lev.	gal.	mano.	malt.	lact.	raff.	
Cage No.2	large rod, 1 x 3 u singly and chain sacs	+++ acid	4.55	+	+	-	-	-	+	-	identical with R20
Cage No.3	short rod 0.5 x 0.1 u singly	smooth curd	4.30	+	+	+	-	-	-	+	small, round, granular, irreg. resembling R.S. L. acidophilus
Cage No.4	bi-polar rods singly and groups, 0.5 x 1 u	smooth curd	4.00	+	+	+	+	+	+	-	identical with K-1
Cage No.5	coccus, large 1 to 1.5 u clumps, short chains	smooth curd	4.35	+	+	+	+	+	+	+	identical with F-1 (smaller)

Chart III. The characteristics of stock cultures.

Organism	Morphology	L. milk	pH	Sugars							Plate description
				dex.	lev.	gal.	mano.	malt.	lact.	raff.	
RRO (cage No.2)	plump rod, 1 x 3 u in short chains and singly	smooth curd	4.75	+	+	+	-	+	+	-	rough, fuzzy, filamentous colony
K-RS (cage No. 3)	short rods 5 x 1 u singly and groups	+++ acid	4.25	+	+	-	-	+	-	-	round, granular, irregular edge
K-1 (cage No. 4)	cocco-bacillus 0.5 x 1 u singly and chains	smooth curd	3.8	+	+	+	+	+	+	-	round, white, glistening, granular, regular edge
F-1 (cage No. 5)	coccus, 1 to 1.5 u singly and clusters	smooth curd	4.1	+	+	+	+	+	+	-	round, regular edge, slightly yellowish

Chart IV. Controls of the experiment.

Control	Result expected	Result obtained	Animals
Cage No. 1, sterile food only	no caries	small cavity	1 animal*
Cage No. 6, coarse corn not sterile	caries	extensive caries	both animals
Cage No. 7, fine corn not sterile	no caries	no caries	both animals

*Note - In cage No. 1 examination of the carious lesion revealed no L. acidophilus, which are normally found in caries of rats.

The sugar reactions obtained in the identification of cultures isolated from the caries produced in the rats, were relatively inconsistant, and it must be pointed out that sugar reactions can not be used as diagnostic agents for delicate organisms such as L. acidophilus. Duplicate sugar determinations made on this and similar strains gave varied results.

The isolation and identification of stock cultures was based on morphology, cultural characteristics, pH changes and litmus milk reactions. As to the use of serological reactions authors differ in their opinions. Hadley, Bunting and Delves (20) report cross-agglutination of "S" antigen by "R" serum through a dilution of 1 to 1,280 and the "R" antigen was completely agglutinated by the "S" serum through a dilution of 1 to 5,120. In contradiction to these results Upton and Kopeloff (21) state that pure line "R" and "S" strains appear to be antigenically distinct. They demonstrated also that "R" forms of L. acidophilus and L. bulgaricus could not be distinguished.

Thus, with the serological question unsettled it can not be taken too strongly as an index of identification. Reliance should be placed on cultural and morphological characteristics, which were adhered to in the identification of the cultures used in this experiment.

From the chart it appears that R20, K-1 and F-1 were identified as the cause of the carious lesions in their respective animals, leaving K-RS as the only questionable organism. Although the cultural and morphological characteristics of the organism isolated from the carious teeth, and assumed to be K-RS coincided to a fair degree of accuracy to the characteristics of the stock culture the organism did not appear as numerous on the plate of the direct smear from the cavity as R20, K-1 and F-1.

L. acidophilus was not observed on the direct smears from the carious areas of the teeth of the animals receiving the cultures. There was an exception to this in the case of R20 which was a rough L. acidophilus used on animals in cage No. 2. The absence of rough L. acidophilus on the other plates demonstrated that the microflora, of the mouths of the experimental animals, were adequately controlled. In cage No. 6 receiving coarse corn, (not sterile) the organism isolated from the caries of both experimental rats was identified as L. acidophilus. In cage No. 7 L. acidophilus was also observed, but due to the fine corn no impaction resulted, consequently no fermentation or caries developed.

D. Fermentation studies on organisms occurring naturally in rat food, and those isolated from carious teeth.

Procedure

One gram samples were taken from stock supply of corn, rice and powdered milk. Ten c.c. amounts of sterile water were added to each one of the tubes, and after incubation for 48 hours at 37°C. 5 c.c. of the fermented material was titrated with N/20 NaOH.

For fermentation of corn by organisms isolated from dental caries, one gram samples of each were taken and autoclaved for 30 minutes at 15 pounds pressure. Ten c.c. amounts of sterile water were added and after 48 hours incubation at 37°C. 5 c.c. portions of the fermented material were titrated with N/20 NaOH.

For fermentation of sugars by organisms isolated from dental caries the following procedure was observed. The diagnostic sugars were inoculated with a loop from 24 hour broth cultures then incubated for 48 hours at 37°C. and recorded.

Chart V. Unautoclaved stock foods with naturally occurring organisms.

Corn samples	c.c. of N/20 NaOH used	c.c. of material titrated	Per cent normal acid present
No. 1	1.2	1	6.0
No. 2	1.15	1	5.75
No. 3	3.35	3	5.58
No. 4	1.5	5	1.5
No. 5	0.60	1	3.0
No. 6	0.90	1	4.5
Rice samples			
No. 1	0.8	3	1.33
No. 2	2.5	5	2.5
No. 3	2.2	5	2.2
No. 4	1.9	5	1.9
Powdered milk samples			
No. 1	2.75	5	2.75
No. 2	3.50	5	3.50
No. 3	2.2	5	2.20
No. 4	2.8	5	2.80

Chart VI. Autoclaved corn meal + sterile salt solution + organisms isolated from dental caries.

Organism used	c.c. of N/20 NaOH used	c.c. of material titrated	Per cent normal acid present
R20 - R.L. acidophilus	1.25	1	6.25
R12 - R.L. acidophilus	0.95	1	4.75
K-1 - human cocco-bacillus	0.55	1	2.50
K-RS - human R.S.L.acid-ophilus	0.60	1	3.00
F-1 - coccus from fowl	0.65	1	3.25

Chart VII. Sugar reactions of organisms isolated from carious teeth.

Organism	Source	Sugars*						
		1	2	3	4	5	6	7
1-R12	rat	+	+	+	+	+	+	-
1-R2	rat	+	+	-	-	-	-	-
1-R21	rat	+	+	-	-	+	-	-
H-B	human	+	+	-	-	-	-	-
H-A	human	+	+	-	-	-	-	-
H-D	human	+	+	-	-	-	-	-
H-C	human	+	+	+	-	+	+	-
L.A. S	stock culture	+	+	-	-	-	-	-
L.A. R	stock culture	±	±	-	-	-	-	-
R20	rat	+	+	-	-	+	-	-
R19	rat	+	+	-	-	±	-	-
F-1	fowl	+	+	+	+	+	+	±

*1. dextrose
2. levulose
3. d-galactose
4. mannose
5. maltose
6. lactose
7. raffinose

The acid production of those organisms, naturally occurring in corn and those isolated from caries was very high. Artificial caries in vitro have been produced by investigators by use of one per cent lactic acid. Hence, with acidogenic organisms producing acid from 2 to 5 per cent in corn emulsion, it would not be unreasonable to believe that carious lesions could be readily produced with the retention of the corn particles. Thus the two important factors in dental decay are: (1) The presence of the acidogenic bacteria as demonstrated by this experiment, and (2) the retention of the corn particles as demonstrated by control cage No. 6 in which the natural occurring organisms in the unautoclaved diet were used.

E. An attempt to demonstrate agglutinins in rats on experiment.

Procedure

It was assumed that in the case of an extensively decayed tooth, in which the cavity proceeded to the bone tissue (which is often the case) that the organisms might be introduced into the blood stream, and give rise to agglutinins. This being the supposition the following technique was observed.

The rats, at the time they were examined for dental caries, were etherized and three c.c. of blood was drawn from the heart with a sterile syringe. The blood was allowed to clot, and after standing over night in the refrigerator it was centrifuged and the serum drawn off.

Antigens were prepared by growing the test organisms in yeast extract broth for 18 hours at 37°C. They were then centrifuged and washed with phenolized salt solution, resuspended and adjusted to the proper turbidity standard. The agglutination tests were conducted in the usual macroscopic manner.

Results*

Chart VII. Agglutination reactions of stock culture antigens and serum from rats on experiment. (Second run).

Antigen	serum of cage	1-1	1-2	1-4	1-8	1-16	1-32	1-64	1-128	1-256	control
R20	No.1	-	-	-	-	-	-	-	-	-	-
R20	No.2	+	+	+	+	+	+	+	+	+	-
K-RS	No.3	+	+	P	T	-	-	-	-	-	-
K-1	No.4	+	P	T	-	-	-	-	-	-	-
F-1	No.5	P	P	T	-	-	-	-	-	-	-
R20	No.6	+	+	+	+	+	+	+	+	+	-
R20	No.7	-	-	-	-	-	-	-	-	-	-

Note: + = agglutination
P = partial agglutination
T = trace of agglutination
- = no agglutination

*Results on the first run of rats (on production of dental caries) showed positive agglutination through the first three dilutions of K-1 antigen, by the serum of the animal being fed sterile diet + K-1 organism. This animal had well developed caries.

IV. Discussion

It is regretted that the bacteriological studies on dental caries were limited since time did not permit a more thorough investigation. While one successful run on production of dental caries by the use of acidogenic organisms, did not constitute definite proof, however, it did give a satisfactory technic which was imperative in such a study.

According to these studies, it was evident that acidogenic bacteria were present in great numbers on the normal food of the rats, and that with these organisms and food retention, decay is inevitable. If this is proven to take place (in animals fed adequate diet) as shown by control animals in cage No. 6, then it is highly improbable that dental caries can be attributed solely to dietary deficiencies.

Thus these data presented uphold and strengthen the bacteriological theory (Miller) of decay. The use of acidogenic bacteria to control the micro-flora of the oral cavity and to isolate those cultures from the resulting cavities (after an eight week period) certainly favors the bacteriological theory and leaves little or no ground for those investigators who are prone to disregard the role of bacteria in dental decay. It is not the writer's wish to engage in controversy over the theories existing as to the cause of dental caries, but it is hoped that these

data will show the role of bacteria in dental decay. The bacteriology of dental caries has been worked on for the last fifty years, and is not to be wholly supplanted by new studies, those of dietary deficiencies which attempt to explain more conclusively the mechanism of dental caries.

V. Summary

1. Acidogenic organisms were found in rat food.
2. L. acidophilus was isolated from approximately 60 per cent of the dental caries of rats examined.
3. Proteolytic organisms were isolated from caries of rats.
4. Dental caries in rats was produced by use of acidogenic organisms.
5. Fermentation of rat food by naturally occurring organisms, and those isolated from carious teeth of rats, resulted in high acid production.
6. Production of agglutinins in rats fed acidogenic organisms was inconsistent.

VI. Literature Cited.

1. Leber and Rottenstein: Untersuchungen uber die Karies der Zahn, Berlin: Hirschfeld.

2. Miller, W. D. Tr. IV. Internat. Cong. 1:237.

3. Hoppert, C. A., Webber, P. A. and Canniff, T. L. The production of dental caries in rats fed an adequate diet. Jour. Dental Research, 12:161-170, 1932.

4. Hanke, M. T. The role of diet in the cause, prevention and cure of dental diseases. Jour. Nutr., 3:433-451, 1931.

5. Bunting, R. W. A review of recent researches on dental caries. Jour. Amer. Dental Assoc., 18:785-806, 1931.

6. Marshall, J. R.. The etiology of dental caries. Phys. Review, 4:564, 1924.

7. Miller, W. D. The agency of microorganisms in the decay of human teeth. Dental Cosmos, 25:1, 1883.

8. Hartzell, T. B. and Henrici, A. T. Mouth streptococci and dental caries. Abst. of Bact., 1:272, 1917.

9. Kahnert, B. Etiology of dental caries in the normal horse. Abst. of Bact., 4:242, 1920.

10. Rodriguez, G. E. Studies on the specific bacteriology of dental caries. Abst. of Bact., 7:28, 1922.

11. McIntosh, J., Lazarus-Barlow, P., and James, W. W. Investigations into etiology of dental caries. Abst. of Bact., 7:442, 1923.

12. Howe, P. R. Studies of dental disorders following experimental feeding with monkeys. Jour. Am. Dental Assoc., 11:1924.

13. Sierakowski, S. and Zajdel, R. Bacteria causing dental caries. Abst. of Bact., 9:164, 1925.

14. Bunting, R. W. and Parmerlee, Faith. Role of B. acidophilus in dental caries. Jour. Am. Dental Assoc., 12:381, 1925.

15. Schlirf, K. Bacteriological investigation of dental caries. Biol. Abst., 1:898, 1927.

16. Rosheny, T., Linton, R. W., Buckbinder, L. Study of dental aciduric organisms and L. acidophilus. Jour. of Bact., 18:395, 1929.

17. Morishita, T. Further studies on certain aciduric organisms of dental and salivary origin. Jour. Bact., 17:7, 1929.

18. Bodecker, C. F. A new theory of the cause of dental caries. A. J. P. H. A., 19:1104, 1929.

19. Morishita, T. Studies on dental caries with specific reference to aciduric organisms associated with this process. Jour. Bact., 18:180, 1929.

20. Hadley, F. P., Bunting, R. W. and Delres, E. A. Recognition of B. acidophilus associated with dental caries. Jour. Amer. Dental Assoc., 17:2041-2058, 1930.

21. Upton, M. F. and Kopeloff, N. Agglutination and dissociation studies with lactobacilli. Jour. Bact., 23:455, 1932.