A STUDY OF THE RELATIONSHIP OF CERTAIN FOOD QUALITIES, AND THE ANTIBIOTIC REACTIONS OF ACIDURIC, PROTEOLYTIC, AND OTHER MICROORGANISMS OF THE ORAL FLORA TO THE PRODUCTION OF DENTAL CARIES.

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A THESIS

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by

Don Chalmers Lyons.

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A RESUME OF THE PRESENT STATUS OF RESEARCH INTO THE CAUSES OF DENTAL CARIES.

Dental caries, or caries of the teeth, is the most prevalent disease known today. It has no limitations as to age, sex, station in life, or race. It is a universal health problem, although certain groups of people and a few individuals through-out the world as has recently been shown by Price (82) are relatively immune to the disease. Our present knowledge of the causes of dental decay is the accumulation of centuries of thought and investigation. Guernini (34) shows by frequent illustration in his text on dental history that dental decay is as old as the ages, that it has been known and written about since the earliest time. Egyptian skulls, remains of the early Empire days show extensive decay. Mummery (73) reported that of 364 skulls belonging to the Stone Age which he examined, 64 showed dental decay. However, ancient as dental decay is, we find that the sum total of our information concerning its etiology is exceedingly meager in many respects.

Great stimulation was given to research into the causes of dental decay by the discovery that caries is a major health problem in that

defective teeth frequently act as foci of infection. This discovery some years ago stimulated increased interest in the teeth. With the development of the roentgen ray or x-ray which revealed hidden infection at the apices of defective teeth, further interest was added to the study of the etiology of decay. (23)

Detailed discussion of the history of dental decay has been amply covered in various other papers and texts. It will suffice to say that with the discovery of the relationship between disease and bacterial growth by Pasteur and with the promulgation of the bacterial theory of dental decay by Miller (64) in 1884, the present era of research in this field was started.

The fifty years that have passed since Miller's work have seen the establishment of a number of well worked out theories concerning the destruction of teeth by caries. Today these sum up to four major premises and a number of minor ones concerning the problem. The first major premise is that of the bacterial growth in the mouth, or what has been termed the local environmental factor. The second concerns the nutritional relationships, namely deficiencies in amounts or quality of vitamins and mineral salts. The third deals with the

salivary secretion relationship to the problem. The fourth premise is that of the influence of heredity to dental caries. The minor ones will be mentioned later.

THE BACTERIAL THEORY.

In 1884 Miller (64) demonstrated that bacteria found in the mouth of the normal average human produced end-products of high lactic acid content by the fermentation of carbo-hydrate food debris adhering to the teeth. He further discovered that lactic acid decalcified the tooth enamel and that decalcification of the enamel took place in the mouth in areas of the teeth where food debris were retained, such as in pits and fissures and in inter-proximal spaces. He therefore concluded at this early date that dental decay was a bacterial disease.

There were however a number of points to his theory which did not agree with the actual conditions found to exist in the human mouth. The repeated failure of early investigators into the cause of dental decay to explain the following facts in the light of Miller's theory led to its abandonment for many years. (a) The attempt to produce typical carious areas in extracted human teeth or parts of teeth by lactic acid was a failure, for

the enamel decalcification thus produced did not resemble natural decay in various respects, particularily in the mode of invasion of the enamel. (b) No explanation was offered or could be found for the fact that certain individuals having filthy mouths containing teeth well coated with residual food debris were apparently immune to caries, and that the decalcifying action which was to be expected of the acids produced in the fermentation of the carbohydrate debris upon the teeth did not take place. (c) There was no explanation in Miller's thoery as to why carious processes might stop for long periods of time without further destruction of the tooth or teeth. (d) There was no explanation of the fact that caries is prone to develop more rapidly during pregnancy, malnutrition, and long debilitating sickness. (e) It did not seem logical that, if bacterially produced acids were the entire cause of dental decay, certain limited areas of the tooth would be affected and not the entire tooth.

Lactobacillus Acidophilus.

Most of the recent work concerning the etiology of dental caries from bacterial standpoint has been done in the belief that the L. acidophilus is the acidogenic bacterium which causes dental decay. In 1900 Moro (70) isolated an acid producing bacillus

from the mouths, stomachs, and intestines of breast fed infants, which has since been termed the Lactobacillus acidophilus. His observations were soon substantiated by Goadby (31), Kendall (54) and others. However, the presence of this bacillus in the mouths of infants did not create any particular interest from a dental standpoint until 1917 when Howe and Hatch (41) reported that they persistently found large numbers of L. acidophilus of the type reported by Moro (70) in decayed teeth which they were examining. Further renewed interest in bacteria as a cause of dental decay developed with the finding of better culture media for this bacillus, such as those suggested by Hunter (46), Kulp (59), and Grigoroff (35). In 1922 Mc Intosh, James, and Barlow (65) reported finding L. acidophilus in dental caries, but they failed to reproduce caries in rabbits which they used for experimental purposes. Rodriguez (85, 86) the following year came to the definite conclusion that this organism was the etiologic factor in dental decay, but failure of other investigators to get consistent results made the etiology questionable. Sierakowski and Zajdel (91) found the organism in eighty percent of the carious teeth which they examined. Cannon (18) Kopeloff (57) and other investigators about this time

also found the organism in many decayed teeth. In 1925 Bunting et al (10, 11, 12, 13, 14, 15 and 16) started a series of researches into the relationship between L. acidophilus and dental decay and found the organism in 90% of cases showing advanced caries as against 16% in mouths where there was an apparent immunity to caries. There was however still difficulty in obtaining consistent results; for seven years later,(1932) Enricht, Friesell, and Trescher (25) were only able to find the organism in from 52 to 61% of the carious areas which they examined.

This great variation in incidence has been a stumbling block for many investigators as has been that of the fact that until recently it was apparently impossible to produce caries in teeth of experimental animals. Howe (42) tried feeding animals a high carbohydrate diet for months without success. Until the past few years inoculation of food with L. acidophilus as a means of stimulating dental decay has also been a failure. Bunting (10, to 16), Hoppert (40), Devereux and Etchells (19) have overcome this objection by improvements in technic and have actually produced carious teeth in humans (Bunting), and in rats (Hoppert and Etchells).

There has been considerable objection to the theory that L. acidophilus is an etiologic factor in the disease because it has been thought that there

was a difference between the organism isolated from intestines or milk and that found in the mouth. Morishita (71, 27) stated that he found marked differences between the L. acidophilus of the mouth and that of the intestines, but Rosenbury (87) who was able to isolate 21 strains from the mouth and nine from other sources, declared that he could find no morphologic or biochemical difference. Rahe (83, 84) who classified 6 sub-strains agreed with him as have Etchells (27), Enricht (26), Ross (88), Kulp and Rillzer (59), Bunting (10 to 16) and many others. Arkwright (2) found that L. acidophilus cultures had an ambiguous character. Howitt (45), Kulp (59) and others have found that there are two types commonly found in the mouth, a rough colony form, and a smooth one. Upton and Kopeloff (95) showed that pure line colonies of R and S strains were antigenically distinct. Ross et al (88) compared the milk strains of L. acidophilus with dental strains and found them serologically related.

NUTRITIONAL CAUSES.

Another group of investigators have developed a very conclusive program showing the relationship between dietary deficiencies and dental decay. This group believes that dental decay is the result of nutritional disturbances which may

be either deficiencies in mineral salts, vitamins, or both. Drain and Boyd in 1930 (20) reported a series of experiments in which the dental and general physical condition of children under their care improved greatly under a dietary regime, which increased the daily intake of fresh vegetables and milk. Hoppert et al (40) have recently brought a new factor into the problem of dental decay, namely a quality of the food. They found that it was very easy to produce dental caries in the rat by feeding a diet which was adequate in all the known food elements, if the mixed food contained coarsely ground corn meal. Others have repeated this experiment with rice and have had similar results.

Vitamins.

Since the discovery of those food factors which are still called vitamins because no other terminology seems better at this time, considerable attention has been focused upon their relationship with caries. The failure of Miller's theory to offer an explanation for the fact that decay occured more rapidly during pregnancy, malnutrition, and sickness, probably was more instrumental in starting research along this line than any other one thing except possibly the fact that Waugh (97 to 99), Leigh (60),

Price (77 to 82) and many others have noticed and reported the fact that dental decay is very infrequently found in various groups of peoples living in isolated or semi-isolated locations and on distinctive diets. For example, Waugh (97 to 99), and Stefansson (94) found that Eskimos living on a carnivorous diet consisting mostly of sea-bird eggs, fish, and the flesh of mammals, were practically immune from decay. Mitchell (69) on the other hand reported in a very comprehensive survey of Labrador that the people there, making their living by fishing, had a diet very deficient in certain minerals etc., and as an apparent result suffered from many dental disorders.

Vitamin C.

The earliest knowledge which we have of the disease now known as scurvy concerns its relationship with dental disorders. Starting with John Colbatch in 1699, those who have written of adventure, and exploration, have frequently made reference to the sufferings from this disease by the members of these parties and in most instances such recordings are excellent accounts of the ravages of the associated dental troubles. With this long history of the relationship between scurvy and dental disorders before us, it is not surprising that among the first investigations which were made after it was found that a deficiency of

vitamin C is the cause of scurvy, was a study of the importance of this vitamin to dental health. Jackson and Moore (49) in 1916 seem to be the first to report scorbutic changes in the teeth from a histologic standpoint. Since that time other investigators have substantiated their findings. The report of Hojer (47) gives a splendid summary of the changes which one finds in the teeth of the laboratory animals (guinea pig) under a scorbutic diet. He found that;

1. The odontoblasts become shorter, more rounded, and change in arrangement, and may change to osteoblasts.
2. There is an amorphous calcification of the predentine.
3. There is a widening of the Tomes canals.
4. There is a change to bony structure by the forming dentine of a spongy porous character.
5. There is a dilation of the blood vessels and hemorrhages in the dental pulp.
6. There is an atrophy of the pulp.
7. There are abnormalities in the dental canals.

While Hojer found many changes in the structure of the teeth due to vitamin C deficiency he did not find or report dental decay.

Howe (41, 42, 43, 44) by feeding diets deficient in vitamin C was able to produce in the mouths of monkeys all the dental changes which may

be found in the human mouth. He reported such changes as these: The jaws and facial bones of the monkeys remained infantile in character. The alveolar ridge which surrounds the teeth became thin and porous. The teeth became loose, pockets formed around the same with pus formation, the lower teeth moved inwards and the upper ones outwards, and occlusion was so disturbed that the animals could not chew their food with any degree of efficiency. Dental decay was rampant.

Boyd, Drain, and Nelson (9) demonstrated to their apparent satisfaction that dental caries can be controlled by certain diets containing an adequate supply of vitamins. Eddy (21,22), Holton (48) and Kesel (55) have added similar findings. Webber (100) found that the addition of cod-liver oil and orange juice as a means of furnishing vitamins D and C to the diets of the rats which he was testing, as well as the addition of calcium and phosphorus did not seem to have any influence upon decay. He stated that 127 out of 140 rats in the stock colony developed dental decay.

In a recent investigation completed by Hanke (37) and his associates at Mooseheart, covering a period of three years, data are

presented which are pertinent to the present status of research in dental decay. They found that after a year on the standard institution diet, 75% of the 440 children under their care had gingivitis or inflammation of the gums and an incidence of dental decay of 78%. This diet according to the report was;

Food	Girls	Boys	Amount
Milk	.88	.92	Quarts
Bread	.25	.49	Pounds
Butter	.08	.087	Pounds
Sugar	.103	.112	Pounds
Eggs	.566	.6	Eggs
Fruit	.46	.47	Pounds
Vegetables	.846	1.05	Pounds
Meat	.212	.246	Pounds
Cereal	.032	.676	Pounds
Sundries	.206	.227	Pounds
Calories	2,545	3,065	

Hanke (37) stated that these food intake values were figured to represent the average amounts ingested per child per day throughout the entire one year period. The following year these same children had 16 ounces of citrus fruit juices added to their daily standard diet and at the end of a twelve month period, the percentage of gingivitis had dropped to 12.4% and the percentage of dental decay to 33.0%. The standard diet was again instituted the third year and the percentage of cases of gingivitis jumped back to 60.3%, while the percentage of dental decay increased to 83.4%.

This report apparently indicated that large quanities of citrus fruit juices are needed in the American diet for the same report states that the standard diet contained 3 oz., daily of citrus fruit juice, but this amount apparently was not sufficient.

Vitamin D.

Along with these researchs on the importance of vitamin C in the diet, work has been carried on which apparently brings out the fact that vitamin D is also an important factor in the diet. Results point towards the belief that the structure of the teeth is of value in the prevention of dental caries. The investigators correlate the need of vitamin D with its importance in calcium and phosphorous metabolism. Mellanby (66,67,and 68) was probably the first to become interested in vitamin D and mineral salt metabolism in dental decay. She found that by feeding young dogs diets deficient in vitamin D while their teeth were in the developmental stage, that the teeth which erupted were defective in structure and improperly placed in poorly calcified jaws. Macroscopically and microscopically, her evidence appeared to point to the fact that decay is associated with defective enamel. Fiske and Sudbarrow (28), Bonskoe and Rath (8),

Lowry (61), Kramer and Howland (58), Witt (106), Forbes (30), Marshall (62), Bethke (3), Steenbock and Black (93) and Noyes (75) have added to the literature on the relation between phosphorus-calcium metabolism, vitamin D, and dental decay. Jundell and Magnusson (51) found that in children free from caries, the serum-phosphorus varied from 4.2 to 6.2 mg. and serum-calcium between 9.6 and 12.0 mg. per 100 c.c. of blood. For those with advanced caries the serum-phosphorus was 4.1 to 5.3 mg. and serum-calcium 8.9 to 11 mg. per 100 c.c. of blood. They concluded that this difference was not sufficient to be significant. On the other hand, Agnew, Agnew, and Tisdall (1) stated that an adequate supply of phosphorus is an important factor in the prevention of dental caries. Seventy out of 71 rats which they fed a diet low in phosphorus and vitamin D for periods of from two to seven months developed dental caries. Their basal diet was as follows;

Finely ground corn meal 77.4%.
Wheat glutin 20.6%.
Calcium carbonate 1%.
Sodium chloride 1%.

The addition of vitamins A, B, B-1, C, E, to the basal diet did not have any effect upon the development of caries. The addition of vitamin D to the diet prevented caries in six of 19 rats. Steenbock

and Black (93) reported that rachitogenic diets do not contain optimum amount of minerals or vitamins and as a result were decided factors in dental conditions.

Hawkins, Hendricks, and Hills (38) found that those immune to dental caries consume liberal amounts of foods which are high in alkaline ash, in calcium, and vitamin D, and low in cereals. Howe (41 to 44) reported that 500 children patients of the Forsythe Institute showed marked improvement in dental conditions when added amounts of vitamin D were placed in their diets. Price (77, 78, 79, 80, 81, 82) in a long series of researches has presented a volume of evidence showing the importance of foods containing what he calls activators or the vitamins and calcium, in the prevention of carious processes. Webber (100) on the other hand showed that the addition of calcium carbonate or tri-calcium phosphate failed to have any effect upon the prevention of dental caries in Albino and Piebald rats.

Vitamins A and B.

Eddy (22) states that a deficiency in both A and C causes atrophy in the odontoblasts and that it also occured when A alone was left out of the diet. Walback and Howe (101) noted changes in the

incisor teeth in vitamin A deficiency. On the other hand Hess (39) in a recent article comes to the conclusion that the average American diet contains sufficient vitamin A.

Grunig (32) found that an absence of vitamin B from the diet of guinea pigs resulted in discoloring and softening of the tooth enamel, but believes that it was due to chemical changes in the saliva incident to this avitaminosis.

Forbes (30) stated that the determining factor in the production of dental caries is the acid-base equilibrium of the blood and the calcium-phosphorus concentration in the saliva in contact with the teeth in any locality, these concentrations depending upon the calcium content of the saliva and its neutralizing power against acids. Hawkins (38) emphasized the importance of diets yielding a high alkaline ash and increase in calcium to enable the saliva to neutralize the acids of fermentation.

INFLUENCE OF SALIVA.

Klein (56) found that the phosphorus level of the saliva was important and he stated, " A steady flow of saliva will rather effectively prevent the accumulation of acids any place in the mouth." Jones et al (50) in Hawaii also believed that the

influence of acid-base balance of the diet is important. They found that active caries was always associated with diets that contained an excess of acid over basic elements, but also that many persons on such a diet, particularily Filipinos had no decay. Their results are interesting from the fact that they found among the people of Hawaii and New Zealand that sunshine and diets containing cod-liver oil, eggs, orange juice, tomatoes, and cows milk in large quanities did not prevent disintegration of the teeth. They state, "We regard the exciting cause of dental decay as the balance between the saliva and lymph on the one hand, and the acid formed by bacteria in the oral cavity on the other hand."

Karshaw, Krasnow, and Krejci (53) found that there was a greater phosphorus content in the saliva of people immune to caries. Other investigators have been turning their attention to the saliva, since this is the only body fluid in direct contact with the enamel of the teeth, therefore immunity which body conditions exert upon the teeth should depend upon the composition of the saliva. They found that their problem is a difficult one to solve because under ordinary conditions one can grow microorganisms in the

saliva, and no controlled condition has been developed to furnish means of accurate comparison.

Agglutinins.

Bunting (10 to 16) and others have sought for antigens in the blood stream and saliva, and although they have found agglutinins, the work has not progressed to the point where any definite conclusions can be made. Considerable investigation should be done upon the saliva before any conclusions can be reached.

OTHER THEORIES OF DENTAL DECAY.

Bodecker (6, 7), Bibby and Van Huysen (5) and others present another new thought in the cause of dental decay. Bodecker (6, 7) believes that the enamel of the tooth is porous and may be permeated by fluids from the interior of the tooth and that when the alkaline elements of the blood plasma are low, the dental lymph does not possess neutralizing power to combat the acids of fermentation and dental caries result. Bibby and Van Huysen (5) find that there is a separable membrane covering the enamel of the tooth which is resistant to decay. This material is considered as being the organic matrix of the enamel and is continuous with the organic sheath of the enamel rods as observed by Bodecker (6), Nishimura (74),

Fish (29), Cape and Kitchen (17), and Williams (104). This film is not to be confused with Nasymth's membrane which covers the crown of the tooth when it erupts. Bodecker (6) summarized his observations as follows; "We must regard the enamel as a tissue closely dependent for its welfare to its physiologic connection with the body." Such being the case he was of the opinion that dental caries may be caused mainly by systemic disturbances.

Some interest has been focused upon the endocrine glands from time to time as a possible etiologic factor in dental decay. It is well known that the parathyroids influence calcium and phosphorus metabolism, while the thyroid secretion controls metabolism in general. These facts have been amply demonstrated by Plummer (76), Wilson and Kendall (103), Rowe (89) and Williamson (105). Grieves (33) however, was probably the first to suggest that dental decay might be the result of an endocrine disturbance.

Heredity also seems to play some part in dental decay because it is very evident that some families are almost entirely immune to caries, while others are not. It is a clinical observation that many dental characteristics may be inherited.

INTRODUCTION TO PROBLEM

In presenting the results of this investigation into the causes of dental decay, the author wishes to state at the beginning that he is fully aware of the fact that the rat, in the words of Holt, "is not a human being and probably never will be." It is felt however, that the Albino rats which were used throughout this study furnish admirable laboratory animals to use in an endeavor to ascertain if possible the answers to certain questions about the bacterial flora existing in the oral cavity. It is therefore believed that the Albino rat should be looked upon in this connection in the manner similar to that recently mentioned by Sherman (92), who states, "I think it scientifically accurate to state as the conclusion indicated by the great mass of evidence now existing, that what we find for the rat is within the probabilities as applied to the human."

There is very little investigation into the diseases affecting the human being which can be applied directly to humans other than by the mathematical formulas derived for the laws of probabilities and of standard deviation due to the length of time needed and lack of controls necessary to produce well substantiated findings.

The results presented here, however, are specifically applied to the Albino rat. If an application can be made of these findings to the human being, such should be done. The author deliberately fails to enter into any discussion as to the relative anatomical values of the rat molars and the human teeth.

A study of the voluminous literature now existing in published form reveals that among the theories as to the cause of dental decay two are outstanding; that which considers the local environmental factors as the cause, and that which considers defective nourishment as a causative factor. Attempts to correlate the two have consistantly met with difficulties. (96)

In general it is admitted that the bacterio-chemical theory of dental decay is fundamentally sound. Investigationsin the past few years have pointed to the fact that the L. acidophilus is a predominating organism. However, a critical survey of the literature reveals that this organism does not seem to be present in all cases of dental decay. Fish (29), recently came to the conclusion that a microorganism which he called Streptococcus mutans found in many decayed areas was the causative factor. Enright (25, 26) and his group of co-workers found fifteen varieties of bacteria in decayed teeth which they term; Micrococcus conglomeratus, Micrococcus ochraeus,

Corynebacterium pseudodiptheriçum, Neisseria sicca, Streptococcus mitior, Lactobacillus acidophilus (Y type), Gaffkya crassus, Pseudomonas, Leptothrix buccalis, Bacillus mesentericus, N. flavus, B. fusiformis, Staphylococcus albus, N. perflavus, and N. catarrhalis, etc.. They found also that there was a little difference in the incidence of the L. acidophilus between individuals with active decay and those free from decay. They recovered L. acidophilus from the mouths of 61% of their group who had active decay and from 52% of those who were free at the time from decay. Others including Sanborn (90), have studied the oral flora in relation to diets.

Rosebury et al (87) stated, "Thus far we have not been able to satisfy ourselves that lacto bacillus alone among bacteria are capable of decalcifying enamel in vivo or that any critical pH level of enamel solubility exists."

Enright (25, 26), and his co-workers suggested that perhaps the natural association of the lactobacillus with Leptothrichia buccalis, Neisseria flavus, and N. perflavus might be conducive to caries.

PROBLEM.

The object of this study was to ascertain what relationship, if any, exists between acidogenic

bacteria, certain food qualities, and dental decay. Hoppert, Webber, Canniff (40) and Devereux and Etchells (19), demonstrated that if rats were fed a diet which was adequate in every respect, dental decay would result if the corn or rice used in the diet was coarsely ground. On the other hand, if the rice or corn was finely ground, decay did not develop. This injected a new question into the problem of dental decay, that of the importance of food impaction. At first it was thought the reason there was no decay when the food was finely ground was that there was no impaction. This supposition was later shown to be false. There was a food impaction but it was so firmly packed that it is now believed that it formed an almost impervious coating in the dental grooves. However, one must remember that this coating was still a fermentable food. Why then, granting the presence of acidogenic bacteria, did not decay develop? Further, if it is necessary for the saliva to reach every surface of the tooth in order to prevent decay, as is suggested by several investigators, why was there no decay when this fermentable food was packed into a cavity so tightly that the saliva could not permeate it? These questions remain unanswered.

In this investigation a number of common acid producing carbohydrate fermenting organisms were used.

Albino rats of known health and parentage were used in the experiments, their teeth were caries-free and all were gaining weight at a normal rate. Nomenclature as applied to the bacteria used in the experiments is that accepted by the Society of American Bacteriologists and used in Bergey's manual (4), thus avoiding the confusion of terms so commonly found in dental literature.

METHODS USED.

The experiments herewith reported consist of four types of study; (1) a study of cultures obtained from decayed areas in human teeth, (2) a study of the results obtained from different cultures placed in the food of the Albino rat, and the bacterial flora obtained from their mouths, (3) laboratory studies of the cultures, and (4) a study of the relationship between the certain vitamin or mineral deficiencies produced in the Albino rat and dental decay.

EXPERIMENT ONE.

In the first study, cultures were obtained from the teeth of approximately 500 individuals. These cultures were obtained from three locations; (1) from the mucous membranes and gum tissues surfaces, (2) the outer portion of the decayed tooth, (3) and that decayed portion of the teeth last removed before placing the restoration. The specimans were obtained

by the use of either a chrome steel or platinum needle which was flamed and then applied to the area in question. The pabulum or debris picked up was then washed into a small test tube (Wasserman size) which contained 1 c.c. of sterilized 0.85% saline solution.

After the debris had been dispersed through-out the solution by vigorous shakings it was streaked on either or both plates of yeast extract agar or blood agar of a pH of 6.8. A loop (1 cm diameter) of the same material was also placed in a test tube containing 20 c.c. of sterilized yeast extract. The media used were made according to the following formulas;

Yeast Extract Broth.

Yeast extract (dehydrated)	5 gms.
Peptonized milk	10 gms.
Salt	5 gms.
Dextrose	10 gms.
Distilled water qsad	1000 c.c.

Autoclaved for 15 minutes at 15 pounds and adjusted to a pH of 6.8.

Yeast Extract Agar.

Same as above with addition of 15 gms. of agar.

Blood Agar.

Plain agar media + 5% fresh blood.

Examinations were made at frequent intervals to determine the character of the growth and to

identify the cultures obtained. Fermentation tubes of the various sugars were run in all cases to determine acid or gas production and the results were checked further by the use of litmus milk.

The patients studied were divided into six groups according to age, because a survey of the literature failed to show that much attention had been paid to the bacterial flora of the mouth according to age groups. Group A included children from the age of 1 to 10 years, B from 10 to 20, C young adults from 20 to 30, D from 30 to 40, E from 40 to 50, and F all who were 51 or older. Only those individuals having active dental decay were studied. An effort was made especially to determine the L. acidophilus distribution with the following results;

A	84%
B	66%
C	49%
D	20%
E	14%
F	2%

It was further noted that as the individual age increased there was a definite change in the D, E, F groups;flagellated bacilli and streptococci predominated. Both these organisms produced acid in fermentation tubes, and acid and coagulation in litmus milk. The streptococcus recovered was usually 0.6 to

0.8 micron in size , occured in pairs, tetrads, or short chains, gram positive, produced hemolysis on blood agar, made broth turbid, coagulated or made litmus milk acid, and was a facultative aerobe.

Cultures obtained from the gum tissue surface and surface pabulum of the carious teeth were generally a mixture of yeasts, fungi, flagellated bacilli, spirochaetes, and cocci. L. acidophilus was seldom recovered in such cultures but this may be due to the fact that there was an overwhelming growth of the other bacteria in the cultures. No attempt was made to classify the bacteria recovered because only the L. acidophilus was being studied at this point.

EXPERIMENT TWO.

In the second study a number of Albino rats were used. These rats were obtained from two sources; the Biological Chemistry Department stock colony and the Dairy Department stock colony of the Michigan State College. Both groups were fed on the same stock rations before the beginning of the experiments, namely;

Cornmeal	60%
Whole milk powder	30%
Oil meal	6%
Alfalfa leaf meal	3%
Sodium chloride	1%

This ration was adopted as the stock colony ration after considerable experimentation and has proved adequate in every respect for growth, reproduction, lactation, and general health. The rats were placed

in individual sterilized wire cages and individual compartments which did not communicate with each other. The cage bottoms were made of large mesh screening which did much to keep the cages clean. The sterility of the food after autoclaving was checked repeatedly, the food remaining sterile for at least a month; this being the longest test period.

The test periods in groups one and two of experiment two were eight weeks, with three exceptions as will be discussed later. The test periods in groups three and four were generally eight weeks, but in several instances the period was lengthened.

The basal ration used in this experiment was as follows;

Rice	63 parts
Oilmeal	15 parts
Alfalfa	10 parts
Yeast (dried)	5 parts
Casein	5 parts
Sodium chloride	1 part
Calcium carbonate	1 part
Sterile distilled water	ad lib.

The rice in the diet with the exception of the F group was ground so that 52% was retained by a #20 screen, 47% by a #40, 0.05% by a #60. 0.05% was dust.

The rats were divided into the following groups;

Group 1.

Rat numbers

SBR-A	(1,2)	2 rats	Lactobacillus acidophilus from human teeth. Isolated from the A group of individuals.
SBR-B	(1,2)	2 rats	L. acidophilus from human teeth. Isolated from B group of individuals.
SBR-L	(1,2)	2 rats	L. acidophilus; laboratory culture.
SBR-R	(1,2)	2 rats	Bacillus ramosus; laboratory culture.
SBR-RA	(1,2)	2 rats	B. ramosus and L. acidophilus. Isolated from the A group of individuals.
SBR-S	(1,2)	2 rats	No culture added to the sterile diet.
SBR-U	(1,2)	2 rats	The food was not sterilized.
SBR-F	(1,2)	2 rats	Very finely ground rice was substituted for the coarsely ground. The food was not sterilized.

Group 2.

Rat numbers

SBR-C	(1,2)	2 rats	L. acidophilus from human teeth. Isolated from C group of individuals.
SBR-D	(1,2)	2 rats	L. acidophilus from human teeth. Isolated from D group of individuals.
SBR-BA	(1,2)	2 rats	Acetobacter aceti; laboratory cultures.
SBR-BS	(1,2)	2 rats	Bacillus subtilis; laboratory cultures.
SBR-BSC	(1,2)	2 rats	B. subtilis and L. acidophilus. Isolated from C group of individuals.

SBR-S (3,4) 2 rats No culture added to the sterile diet.

SBR-U (3,4) 2 rats The food was not sterilized.

SBR indicates standard basal ration. Letters A, B, etc., represent abbreviations of cultures used.

All food was placed in individual metal food cups, stoppered with cotton, and then sterilized in an autoclave for 30 minutes at 15 pounds pressure. Each food cup containing approximately 50 grams of food was inoculated with 10 c.c. of 48 hour broth culture of the organisms indicated above. This was repeated as the individual rat consumed the food; at least twice a week.

At the end of the test period the rats were etherized and the outer surface of the mouth was sterilized by flaming. The mouth was then opened and a swab culture made of the mucous membrane. The jaws were removed, held in sterile cotton, and cultures made from the impacted food debris and carious areas. Three rats of the first group were etherized at the end of six weeks due to an intestinal disorder which caused abdominal distention. These were rats SBR-A2, SBR-B2, and SBR-LA2.

It was possible to recover the organisms inoculated from impacted food and the carious areas in every case. In rats SBR-S and U an organism was recovered from the carious areas which after various tests proved to be the Cellulomonas deciduosa. This organism and a streptococcus which was not further identified were invariably

recovered from swabbings of the mucous membranes. A test made of the oral flora of 10 stock rats showed that these organisms were the predominating organisms in their mouths. It was therefore concluded that this was a naturally occuring organism in the mouths of this particular colony of rats. In no instance was the L. acidophilus recovered from the oral swabbings of the stock rats.

Group 3.

The basal ration used in this experiment was the same as before. A similar precedure as to sterilization and inoculation of food was followed. The Albino rats used were obtained from the Dairy Department stock colony. Cultures were added to the sterilized food as indicated;

SBR-A	(3,4)	2 rats	L. acidophilus recovered from the mouths of the "A" rats of group 1.
SBR-Ce	(1,2,3,4)	4 rats	Cellulomonas deciduosa recovered from the mouths of the "S" and "U" rats of group 1.
SBR-M	(1,2,3,4)	4 rats	A mixture of; equal parts of Proteus vulgaris, Streptococcus lactus, Escherichia coli, and Bacillus subtilis were added.
SBR-S	(5,6,7,8)	4 rats	Sterile basal ration.
SBR-U	(5,6)	2 rats	Unsterile ration.

In as much as decay developed in the teeth of three rats of the first group, namely SBR-A2, SBR-B2,

and SBR-La2 which were etherized at six weeks on account of illness, three rats of this experiment SBR-A4, SBR-Ce2, SBR-M were etherized after same length of time.

Group 4.

The basal ration used in this experiment was prepared the same as before. The rats which were used were obtained from the Biological Chemistry Department stock colony. After sterilization of the food it was inoculated with the organisms used in group three. This experiment was a duplication of group three in every respect with the exception that several of the rats were kept on the experiment for a longer period of time, nine and ten weeks respectively, to check the possibility that the failure to produce decay in several instances in group three was due to time factor involved in the test period.

EXPERIMENT THREE.

In an effort to determine whether or not there is any relationship between certain vitamin deficient diets and the production of dental caries in the Albino rat, the teeth and jaws of 55 white rats were examined. These rats were on various diets, some deficient in vitamins and others containing a sufficient quanity to maintain growth and health. No attempt was made

to ascertain the presence or absence of aciduric bacteria, for this experiment was concerned mainly with the determination of the incidence of dental decay in the teeth of rats which had been depleted of their vitamin supply.

The vitamin A group contained 17 white rats averaging in weight about 60 grams when the experiment was started. These rats were fed the following basal diet:

Irradiated corn starch	67%
"A" free casein	18%
Dried yeast	10%
McCollum salt mixture No. 185	4%
Sodium chloride	1%

At the end of a depletion period, usually 4 to 5 weeks, or until increase in weight ceased, these rats were given various supplements. One rat was continued on the basal diet without a supplement until definite signs of vitamin A deficiency appeared. Three rats were given 1/2, 1, 1 1/2, grams of a margarine daily. Four were given 2, 3, 4, 5 grams of cabbage respectively. Three received 1/2 unit of a commercial carotene preparation daily, two received 1 unit, and two 1 1/2 units. One rat received 1 drop of cod-liver oil daily, and one was given 2 drops. The seven rats on the carotene preparation developed the various symptoms of vitamin A deficiency and did not live over four weeks after the beginning of the experiment.

Fifteen rats were placed on vitamin B deficient diets for a depletion period of two weeks. The following basal diet was used:

Starch	53%
"B" free casein	18%
Autoclaved yeast	15%
Butterfat	8%
McCollum salt mixture No. 185	4%
Cod-liver oil	2%

At the end of the depletion period the animals were placed on various diets some deficient in vitamin B, while others contained enough to prevent the typical polyneuritis. Two rats were given Rowena feed in 0.5 and 1.0 gram doses, two were given similar amounts of Ralston cereal, two were given 1.5 grams of corn silage, plus the following amounts of whole yellow corn, four with 1.5 grams and three with 2.5 grams, three with 0.5 grams. One was kept as a negative control. The experiment was continued for eight weeks.

Six rats were placed on a diet deficient in "D" for a depletion period of 18 days, the following diet being used:

Yellow corn	76%
Wheat gluten	20%
Calcium carbonate	3%
Sodium chloride	1%

After the depletion period they were given various levels of cocomalt, salmon, etc.. After a period of five days they were etherized and the line test made for healing.

Four rats were placed on the following vitamin G deficient diet for a depletion period of three weeks, or until growth ceased:

Starch	51%
Whole wheat	20%
"B" and "G" free casein	15%
Butterfat	8%
Mc Collum salt mixture No. 185	4%
Cod-liver oil	2%

One was kept on this diet without supplement, one received 1.8 gram dried liver daily, one received 0.4 gram autoclaved yeast, and one 0.5 gram daily. The experiment was carried out for a period of seven weeks.

Thirteen rats were placed on miscellaneous diets consisting of:

Fox-chow
Cake and water
Whole grain cereal
Coffee and whole wheat bread
White bread 55%, meat 5%, milk powder 20%, potatoes 20%, and lettuce.

Each of the above diets were given ad lib for a period of eight weeks.

At the end of the various experimental periods mentioned, or when the rats died before the end of a particular experiment as happened in several instances, the jaws of the rats were removed, cleaned, and bleached. The following results were observed.

In none of the rat jaws examined was there any extensive decay. In the teeth of one of the vitamin D rats (the negative control) there were two pin hole carious spots. The teeth of the rats receiving fox-chow were slightly abraded. The rats on the vitamin A deficient diet showed definite deficiency in growth of the jaw bones, but not of the teeth.

EXPERIMENT FOUR.

It was thought advisable to check the experimental results obtained in the white rats where it was believed that antibiosis existed as a factor in the development or prevention of dental caries. To this end a series of investigations were carried out in the laboratory to ascertain the growth relationships between a colony of L. acidophilus isolated from human teeth of the "A" group of patients mentioned in the first experiment and stock colonies of such micro-organisms as E. coli, B. subtilis, and P. vulgaris. The following results were obtained:

1 loop L.a. plus 1 loop E.c. gave semi-firm curd, no gas.
2 " " " " " " " firm curd, no gas.
3 " " " " " " " " " " "
1 " " " 2 " " " acid curd, " " reduction.
1 " " " 3 " " " " " , gas, "
1 " " " 4 " " " " " " "
1 " " " 5 " " " " " " "

1 loop L.a plus 6 loop E.c gave acid curd, gas, reduction.

1 old culture L.a plus 1 loop E.c gave no change

1 " " " " 2 1" " " " "

1 " " " " 3 " " " " "

1 loop L.a plus 1 loop B.s gave firm curd, no gas, s reduction.

1 " " " 2 " " " s acid, no gas, s reduction.

1 " " " 3 " " " " " " " " " "

1 " " " 4 " " " acid curd, no gas, reduction, putrifaction.

1 " " " 5 " " " " " " " " "

1 loop L.a plus 1 loop P.v gave acid curd, no gas, r and p.

1 " " " 2 " " " " " " " " " "

1 " " " 3 " " " " " " " " " "

1 " " " 4 " " " " " " " " " "

Explanation. The medium used in all tests was litmus milk. Each test was made as follows, first a loopful of one culture was placed in the litmus milk and then a loopful of the second culture placed in it. The test tube was then placed in an incubator and incubated for 48 hours at 37°C. after which readings were made. The loop used was a standard platinum loop 5 mm. in diameter. L.a indicated L. acidophilus. E.c indicated E. coli, P.v indicated P. vulgaris, and B. s indicated B. subtilis. s reduction stands for slight reduction. r and p stands for reduction and putrefaction.

Examination of thesedata shows that a slight increase in amount of E. coli microorganisms or B. subtilis

over L. acidophilus in culture medium is sufficient to prevent the normal development of the L. acidophilus and that if equal amounts of P. vulgaris and L. acidophilus are placed in the medium the P. vulgaris will outgrow and prevent the development of the latter. These findings offer a logical explanation for the antibiosis found in the mouths of the white rats used in the various experiments.

DISCUSSION.

The findings obtained in the series of experiments reported in this thesis add confirmatory data to some of the theories regarding dental decay, and also present new information. It is felt that the data obtained in these investigations lead to a new theory of dental decay, perhaps open a new pathway to the ultimate solution of this public health problem, and warrant continued and further investigation.

Experiment one was undertaken in the first place as a means of obtaining fresh active cultures of aciduric bacteria, particularily L. acidophilus from active human dental caries. A criticism which has been made in the past to experiments of this type is that old cultures,or stock cultures of various origins, grown on artificial media for long periods of time, have been used. It was our wish to avoid this criticism in this investigation. Throughout the study which was made of the literature on the subject of dental decay it was noted that there was great variation of reported incidence of L. acidophilus in dental decay. No previous effort seems to have been made to classify patients

according to age. In several instances, however, it was apparently implied that children were the only clinical sources of cultures.

The incidence of L. acidophilus reported in the A and B groups of this experiment closely correspond with that report by Bunting (10 to 16), Enright, (25), and others, but there is considerable difference in the C, D, E, F figures which represent results obtained in older patients. These are apparently a confirmation of theories previously expressed by others, namely that the problem of dental decay after eruption and positioning of the teeth has taken place, is not a problem of a single species of microorganism, but one of various types of aciduric bacteria, with perhaps L. acidophilus predominating during childhood or early adult years. It is believed that herein lies the solution of the variance of incidence of L. acidophilus in carious teeth as reported by various investigators.

An attempt has been made to correlate as far as possible in this limited group of experiments, the so-called "local" theory of dental decay with the nutritional. The work of Hoppert et al (40),

Rosebury (87a) in producing dental caries in the teeth of the Albino rats fed a balanced adequate diet was used as a basis for the experimental work reported herein. Their experiments and these, which are confirmatory, show that dental decay, at least after the eruption of the teeth, is not dependent upon nutritional factors other than such local influences as the food presents as a source of aciduric bacteria and as a nidus for bacterial multiplication.

The basal diet of experiment two which was first used by Hoppert (40) has been shown by repeated analysis and experimental results to be complete in every respect. Cod-liver oil is not used in this diet as a source of vitamins A and D because they are found in other items of the diet.

No attempt has been made to classify bacteria found in the mouth or in the food from the standpoint of aciduric qualities. This has been amply covered by others; Etchells (27) for example. It was felt that better control could be obtained over the experiment by sterilizing the food and then inoculating it with bacteria commonly found in nature and known to be aciduric,

purtifactive, or proteolytic. The bacteria used were L. acidophilus, B. ramosus, Esch. coli, B. subtilis, Acetobacter aceti, St. lactis, C. deciduosa and Prot. vulgaris.

It was realized that one objection which might be presented to these findings is that of the inability to control the bacterial flora of the mouth of the rat. This is a logical objection, but the author wishes to point to the fact that under stock conditions on the usual ration, few if any white rats develop dental caries. Therefore it is believed that this objection may be considered negligible. Separate partitions were placed between each cage, the cages washed in sterilizing solutions, the water and food sterilized, and wide mesh wire placed in the bottom of the cage to prevent the rat access to feces. These precautions eliminate many contaminating factors.

The caries produced were similar in character to those found in human teeth as is seen in the figures 1, 2, 5, 6. It is interesting to note in the x-rays, figures 5, 6, that the dental decay developed both on the occlusal surfaces and between the teeth. In some instances the decay progressed so far that the pulps of the teeth were exposed and abscesses or

granulomas developed similar to what might be expected in the human mouth under similar conditions.

In the instances where caries developed in the teeth of the rats fed an inoculated food, bacteria similar to those inoculated were recovered from the carious rat teeth as indicated in Tables (1,2,4). While this can not be construed as complete evidence these particular bacteria were involved in the carious process, it is at least presumptive that they were associated with the process. In view of the findings in the "M" group of rats, one may assume that if the aciduric bacteria heretofore mentioned did not actually produce the decay, they were instrumental in producing conditions favorable to naturally occuring aciduric bacteria. This assumption is confirmatory of the work of Rodriguez. In his discussion of Enright et al (25), he states, "my quantitative examinations have confirmed my original theoretical view that massiveness of oral invasion, that is, maintained over growth, is the determining factor in enamel deterioration."

The recovery of a microorganism in many of the mouths of the test animals and in their carious

teeth which upon isolation proved to be C. deciduosa leads to the assumption that this organism is a predominating one in this particular group of rats studied, and may be a factor in the production of dental decay.

Unsterilized adequate diets such as mentioned by Hoppert et al (40) produced dental decay, but diets which were deficient in vitamins as reported in Experiment three did not cause the production of dental decay, demonstrating the fact that vitamin deficiency alone is not a factor in the production of dental caries in the teeth of the white rat. We desire to limit this statement to rats which have received an adequate diet during intra-uterine development and the first thirty three days after birth, or during the period when the teeth are developing or calcifying.

Experiment four confirms the conclusion developed upon the basis of the results obtained with the "M" group animals and some others, namely that where there is a greater number of proteolytic and putrefactive bacteria in the mouth than aciduric at any given time, dental decay is prevented or inhibited because of the antibiosis between the

types of bacteria mentioned. The results obtained in the laboratory experiment point to the value of further and extensive research on the antibiotic factors present in the oral flora with regard to their influence upon dental decay.

CONCLUSIONS.

It is believed that the observations on the incidence of Lactobacillus acidophilus in the mouths of individuals of various age groups may offer an explanation as to some of the variations in results reported by other investigators. While there may be some objection to these findings on the basis that the medium used has a pH of 6.8 or was approximately neutral, it is believed that such a medium offers a more natural representation of the normal oral cavity in general and therefore gives a truer picture of actual mouth conditions than one of the pH of 5.0 which has been used by some. The use of a yeast extract broth medium should counterbalance any disadvantage to the growth factors of the lactobacilli as compared to other bacteria because it is a very satisfactory medium for growth of aciduric bacteria.

The experiments with the Albino rat lead to the conclusion that many types of aciduric bacteria can produce or are associated with the process of the destruction of the tooth structure, among which the Cellulomonas deciduosa is the most common naturally occuring aciduric microorganism in the oral cavity of the colony of rats studied.

The attempt by feeding vitamin deficient diets to produce dental caries in the molars of the rat after the teeth had fully erupted was a failure, while on the other hand carious teeth were produced at will by feeding adequate balanced diets containing coarsely ground food in which a number of aciduric bacteria were to be found or which were added after sterilization. These results substantiate the observations of others that vitamins have little or no effect upon the teeth after eruption or calcification and that the carious process is purely one of local environmental factors.

Evidence is presented which seems to point to the relationships between the oral bacteria as factors in dental decay. When the diet was sterilized and inoculated with an attentuated culture of L. acidophilus, no decay developed. The results obtained in the "M" group of animals and others demonstrated that it is possible for putrefactive or proteolytic organisms to overgrow or produce products antibiotic to the aciduric organisms. This was also demonstrated by laboratory experiments under controlled conditions. It is thought that in each instance wherein the inoculated organism was recovered instead of some naturally occuring one, the excess of culture in

the food acted in an antibiotic manner to the naturally occuring ones.

The foregoing observations lead to the assumption that the antibiotic relationships of the oral flora are important factors in causing or preventing carious teeth. The theory of antibiosis answers many of the questions which have been raised regarding the bacterial theory of dental decay, particularily immunity in the presence of gross fermentation, accumulated food debris, and high bacterial count.

These results present added information regarding the ultimate solution of that great public health problem, the decay and destruction of human teeth.

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SUMMARIZED EXPLANATION OF CHARTS

The appended tables show briefly data which have been stated as results through-out the discussion and reports of experiments. Tables 1, 2, 5, 6 give the weight of each animal at the beginning and end of the experiments. These tables also show the cultures used for each rat and cultures recovered, as well as the number of carious teeth developed during the experimental period.

Table 3 gives the results obtained with the stock rats examined and demonstrates that aciduric bacteria may be found in their mouths under normal conditions, indicating that if decay is to be produced some factor must be introduced to upset normal bacterial relationships.

Table 4 indicates briefly the identification of the cultures recovered in experiment 2.

Table 7 gives the results obtained by the sugar media tests for acid production. The pH mentioned in two instances was obtained by chemical indicators and therefore is only relative.

TABLE 1.

EXPERIMENT 2. GROUP 1.

Animal.	Wght. beg.	Wght. end.	Cult. used.	Cult. rec.	Results.
A1 w ♀	57 gm.	130.5	L.a A	L.a	4 large cavities
A2 st ♀	52 gm.	64.0	L.a A	L.a	4 cavities. Etherized -6 weeks
B1 w ♂	57 gm.	170.0	L.a B	L.a	3 cavities
B2 st ♀	52 gm.	62.2	L.a B	L.a	4 cavities Etherized-6 weeks
L1 w ♀	52 gm.	117.0	L.a L	L.a	4 cavities 1 small
L2 st ♀	51 gm.	66.6	L.a L	L.a	5 cavities Etherized-6 weeks
R1 w ♀	52 gm.	118.0	B.r	B.r	3 cavities
R2 st ♀	56 gm.	137.0	B.r	B.r	3 small cavities
RA1w ♀	52 gm.	134.0	B.r L.a	B.r L.a	4 cavities destruction 2
RA2st ♂	58 gm.	136.0	B.r L.a	B.r L.a	5 cavities
S1 w ♀	52 gm.	121.0	---	--- C.d	6 cavities
S2 st ♂	59.9gm.	124.9	---	--- C.d	6 cavities
U1 w ♂	54 gm.	176.6		St. C.d	4 cavities
U2 w ♀	68 gm.	148.5		St. C.d	3 cavities
F1 w ♀	71 gm.	210.0		St.	No decay
F2 w ♂	62 gm.	165.0		St.	No decay

Legend L.a - Lactobacillus acidophilus
B.r - Bacillus ramoseus
C.d - Cellulomonas deciduosa
St. - Streptococcus
A. - Lactobacillus acidophilus from "A" group individuals
B. - From "B" group individuals
L. - Laboratory stock culture of L.a

TABLE 2.

EXPERIMENT 2 GROUP 2.

Animal		Wght. beg.	Wght. end	Cult. used.	Cult. rec.	Results.
C1	w ♂	51 gm.	175.2	L.a C	L.a	5 decayed teeth
C2	w ♂	48 gm.	121.5	L.a C	L.a	3 decayed teeth
D1	w ♂	49 gm.	112.5	L.a D	L.a	4 decayed teeth
D2	w ♂	41 gm.	99.0	L.a D	L.a	4 decayed teeth
Ab1	w ♀	49 gm.	122.0	A.a	A.a b	2 decayed teeth
Ab2	w ♀	38 gm.	119.2	A.a	A.a b	1 decayed tooth 1 pin hole
Bs1	w ♂	54 gm.	156.0	B.s	B.s b	3 decayed teeth
Bs2	w ♀	39 gm.	139.0	B.s	B.s mc	4 decayed teeth
BsC1	w ♀	50 gm.	99.0	B.s C1	B.s L.a	5 decayed teeth
BsC2	w ♂	44 gm.	119.0	B.s C1	B.s L.a	6 decayed teeth
S3	w ♂	54 gm.	110.0	--	mc	3 decayed teeth
S4	w ♀	38 gm.	84.0	--	--	no decay
U3	w ♀	48 gm.	124.0	--	f C.d	3 decayed teeth
U4	w ♀	50 gm.	152.0	--	C.d	4 decayed teeth

Legend

L.a - Lactobacillus acidophilus
A.a - Acetobacter aceti
b - Bacilli
B.s - Bacillus subtilis
mc - Mixed culture
f - Flagellated bacilli
C.d - Cellulomonas deciduosa

TABLE 3

SWABBINGS TAKEN FROM THE ORAL MUCOUS MEMBRANE AND DENTAL GROOVES OF STOCK RATS.

Rat	Broth	Plate	L. Milk	Culture
1	sl. T sed.	R.s. Tr nC	Coagulated	St. C.d Y. f
2	" " "	" " " "	Coagulated Acid	" " " "
3	" " "	" " " C	Acid only	" " " "
4	" " "	" " " nC	" "	" " " "
5	" " "	" " " "	Coagulated Acid	" " " "
6	" " "	" " " "	" "	" " " "
7	" " no sed.	" " " "	" "	" " " "
8	" " "	" " " "	Acid only	" " " "
9	" " floc.	" " " "	Coagulated Acid	" " " "
10	" " "	" " " "	" "	" " " "

Legend

- sl. - Slight
- T - Turbid
- sed. - Sediment
- floc. - Flocculent
- R - Rough edges
- s. - Small colonies
- Tr. - Transparent
- nC - No confluency
- C - Confluency
- St. - Streptococcus
- C.d - Cellulomonas
- f - Fungi
- Y - Yeast forms
- L. milk - Litmus milk

TABLE 4.

TYPICAL LABORATORY REACTIONS OF CULTURES RECOVERED FROM ALBINO RATS USED IN EXPERIMENT TWO.

Culture from		Broth	Litmus Milk	Strain of Culture as Identified
A1	w ♀	T sed	S curd	L. acidophilus
B1	w ♂	" "	"	" "
C1	w ♂	" "	"	" "
D1	w ♂	" "	"	" "
La1	w ♀	" "	"	" "
R1	w ♀	Film	acid, some gas	B. ramosus, Streptococcus fungi
RA1	w ♀	"	curd, some gas	B. ramosus, L. acidophilus?
BA1	w ♀	H T sed	curd	?
Bs1	w ♂	Ppt. floc. sed	gas, whey	B. subtilis
Ce1	w ♂	T	curd	Cellulomonas deciduosa
M1	w ♂	H T sed	curd, some gas	Numerous, E. coli P. bulgaris
S1	w ♀	floc	curd	St. ? short rods, motile
U1	w ♂	floc Ppt.	"	Mixed short rods motile
F1	w ♀	"	"	" " " "
St1	w ♀	"	acid, gas	" " " "

Legend

T - Turbid
sed - Sediment
S curd - Smooth curd
Film - Top film
H - Heavy sediment
Ppt. - Precipitate
floc - Flocculent
St. - Streptococcus

TABLE 5

EXPERIMENT 2 GROUP 3.

Animal		Wght. Beg.	Wght. End.	Cult. Used	Cult. Rec.	Results
A3	w ♀	72.0	168.0	L.a	L.a	no decay
A4	w ♀	79.0	216.0	L.a	L.a	no decay
Ce1	w ♂	85.0	229.0	C.d	C.d	3 decayed teeth
Ce2	w ♀	75.0	188.0	C.d	C.d	4 decayed teeth
Ce3	w ♂	60.0	180.0	C.d	C.d	4 decayed teeth
Ce4	w ♀	67.0	171.0	C.d	C.d	2 decayed teeth
M1	w ♂	66.0	154.0	*	Mc	no decay
M2	w ♂	61.0	156.9	*	Mc	no decay
M3	w ♀	68.0	155.0	*	Mc	no decay
M4	w ♀	65.0	156.0	*	Mc	no decay
S5	w ♂	70.0	186.1	-	St. Mc C.d	2 pin hole
S6	w ♂	66.0	186.0	-	" " "	2 decayed teeth
S7	w ♂	72.0	173.5	-	" " "	4 small decay
S8	w ♀	70.0	173.0	-	" " "	4 decayed teeth
U5	w ♀	60.0	180.0	-	" " "	3 decayed teeth
U6	w ♀	65.0	167.0	-	" " "	5 small decay

Legend

* - Esch. coli, P. vulgaris, B. subtilis, S. Lactis.
Mc - Mixed cultures including the above.
C.d - Cellulomonas deciduosa
L.a - Lactobacillus acidophilus
St. - Streptococcus

TABLE 6.

EXPERIMENT 2 GROUP 4.

Animal		Wght. Beg.	Wght. End	Dur. Exp.	Organ. Used	Organ. Rec.	Results
A5	st ♂	66.5	137.0	8 wk.	L.a (1)	L.a	no decay
A6	w ♂	62.5	146.5	9 wk.	" " (1)	" "	" "
A7	st ♂	65.0	167.0	10 wk.	" " (1)	" "	2 pin hole cavities
Ce5	w ♂	69.0	140.0	8 wk.	C.d (1)	C.d Mc	3 cavities
Ce6	st ♀	69.9	134.6	8 wk.	" " (1)	" " Mc	3 cavities
Ce7	st ♂	57.0	170.9	9 wk.	" " (1)	" " Mc	5 cavities
Ce8	st ♂	63.5	161.0	10 wk.	" " (1)	" " Mc	4 cavities 1 very large
M5	w ♀	64.0	137.8	9 wk.	Mc	Mc	no decay
M6	w ♂	59.0	136.0	" "	"	"	" "
M7	st ♂	57.0	130.0	" "	"	"	" "
M8	st ♂	70.0	died 3 wk.	---	---	---	inflammation in lungs
S9	st ♂	56.5	177.8	9 wk.	---	St. Mc.	2 small cavities
S10	st ♂	60.0	170.0	" "	---	St. Mc.	4 cavities
S11	st ♂	59.5	172.0	10 wk.	---	St. Mc.	no decay

Legend

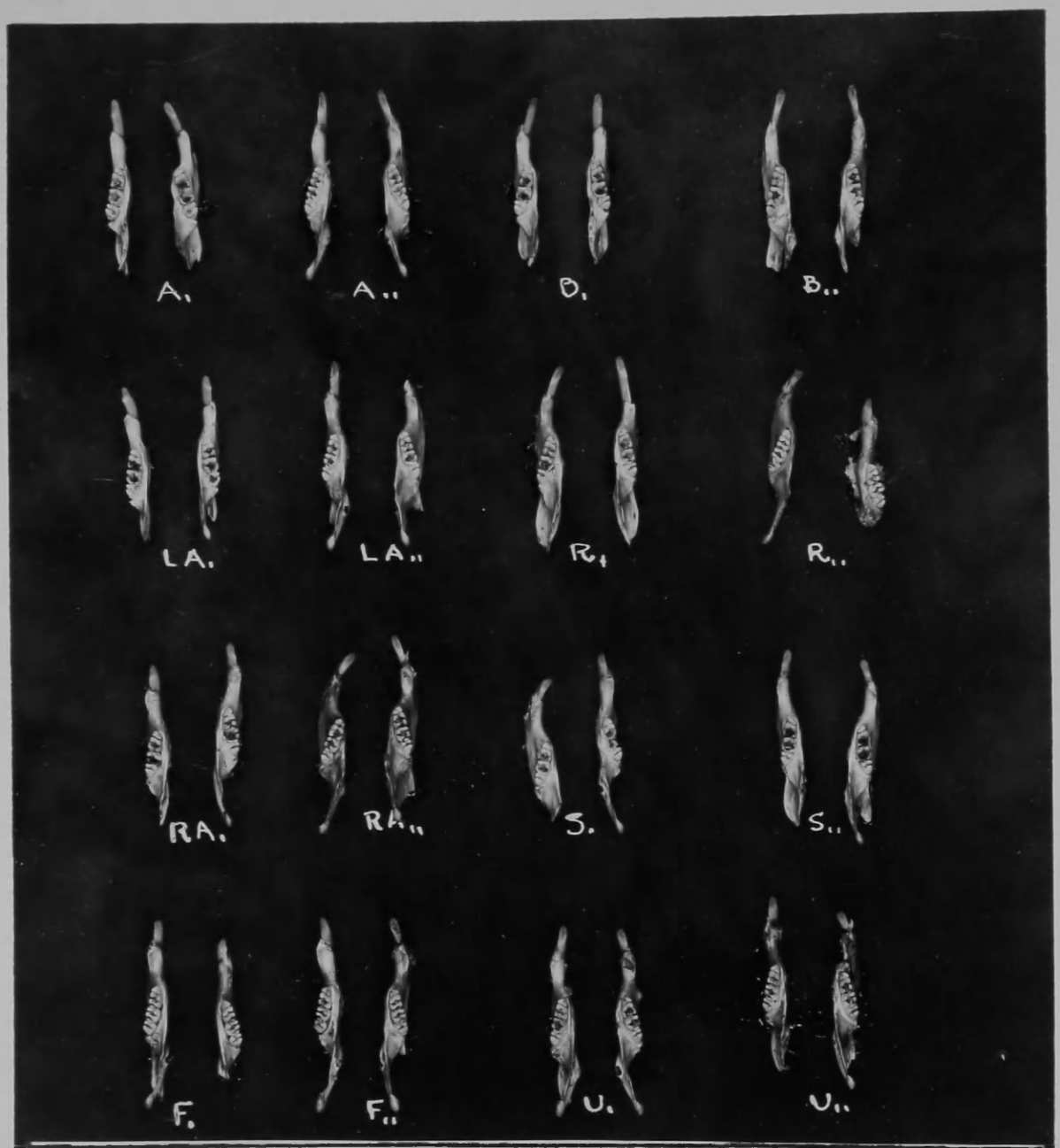
L.a - Lactobacillus acidophilus
Mc - Mixed culture
C.d - Cellulomonas deciduosa
(1) - As recovered in Experiment 2 Group 1.
St. - Streptococcus

TABLE 7.

TYPICAL LABORATORY REACTIONS OF CULTURES RECOVERED FROM ALBINO RATS USED IN EXPERIMENT TWO. SUGAR TESTS FOR ACIDITY.

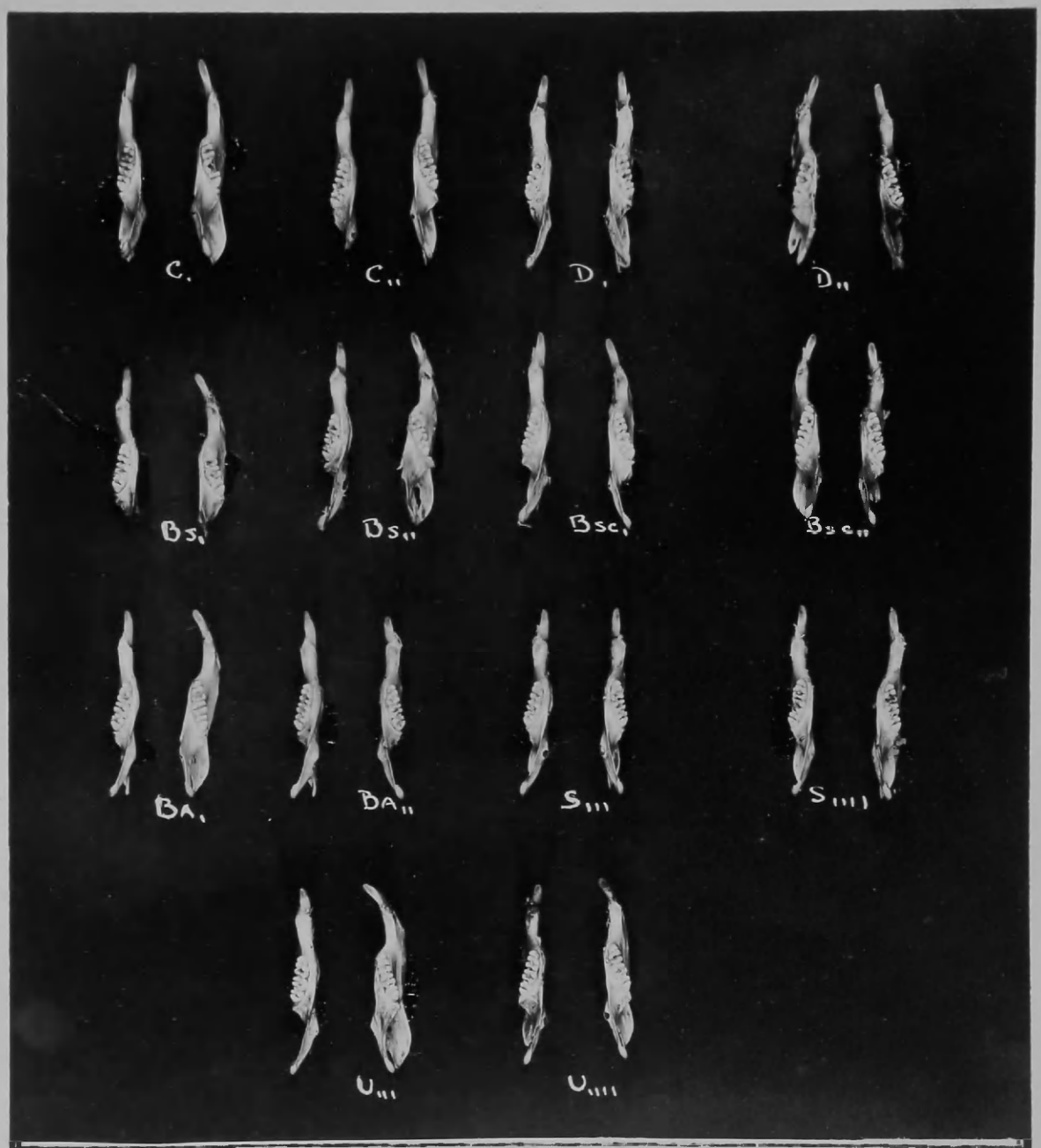
Culture From	Sucrose	Lactose	Maltose	Dextrose	Raffinose
A1 W ♀	*	*	*	B*	*
B1 W ♂	*	*	*	*	*
C1 W ♂	*	*	*	*	*
D1 W ♂	*	B*	*	*	*
La1 W ♀	*	*	*	*	*
R1 W ♀	*	*O	*	*	–
RA1 W ♀	*O	B*	B*	*	pH 6.6
BA1 W ♀	*	*	B*	*O	*
Bs1 W ♂	*	*	*	B*	*
Ce1 W ♂	* pH 4.8	*	*	*	B*
M1 W ♂	B*	B*	*O	B*	*
S1 W ♀	B*	*O	B*	*O	*–
U1 W ♂	*	*O	*O	B*	B*
F1 W ♀	B*	*O	*O	*O	*–
St1 W ♀	*–	*–	*	*	*

Legend * – Positive
– – Negative
*O – Positive with gas
B* – Positive with bubble
*– – Positive and negative tubes.



MANDIBLES OF WHITE RATS NUMBERS AS INDICATED.
EXPERIMENT 2 GROUP 1 D.C.LYONS

FIGURE 1.



MANDIBLES OF WHITE RATS NUMBERS AS INDICATED.
EXPERIMENT 1. GROUP 2 D.C.LYONS

FIGURE 2.

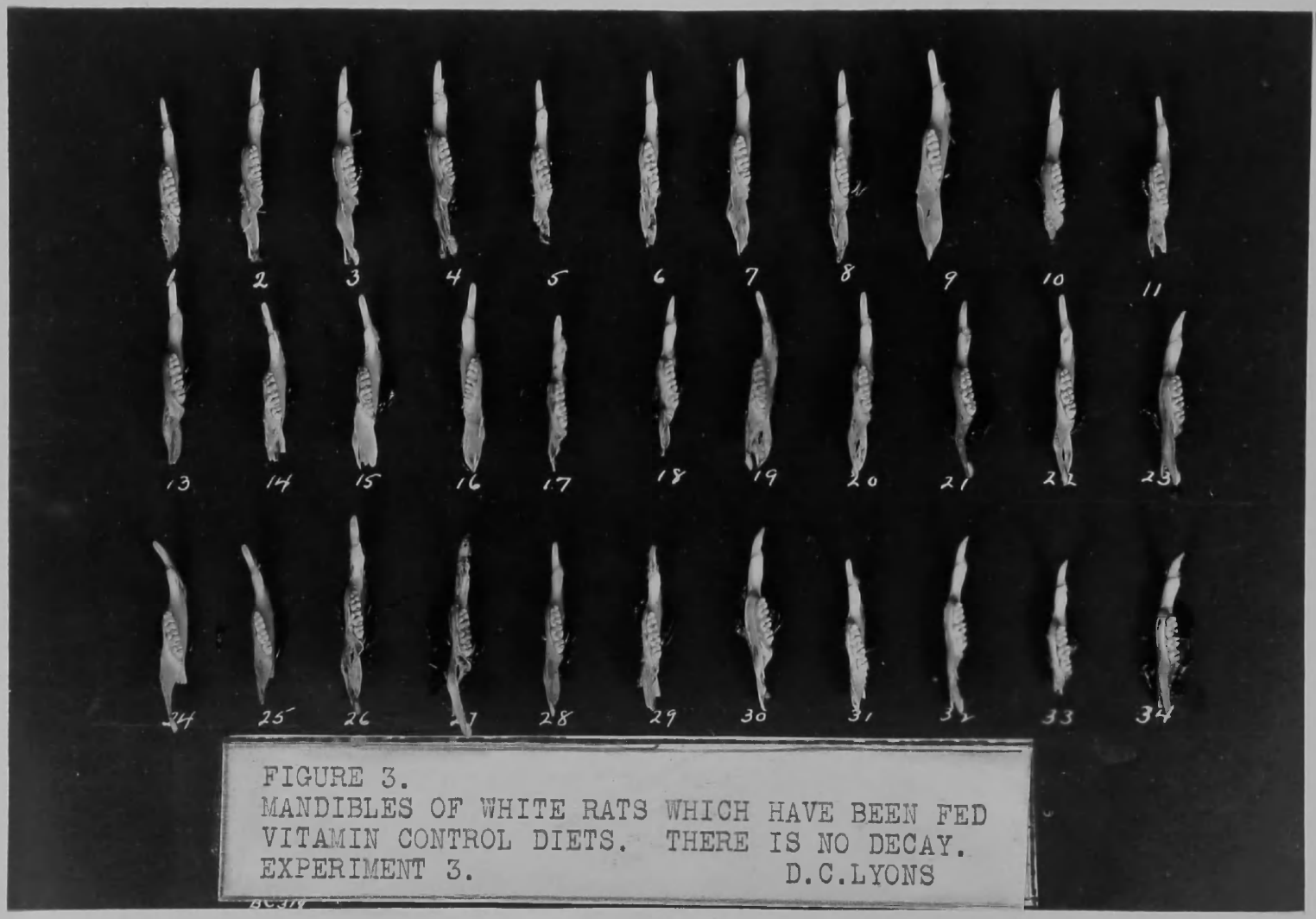


FIGURE 3.
MANDIBLES OF WHITE RATS WHICH HAVE BEEN FED VITAMIN CONTROL DIETS. THERE IS NO DECAY.
EXPERIMENT 3. D.C.LYONS

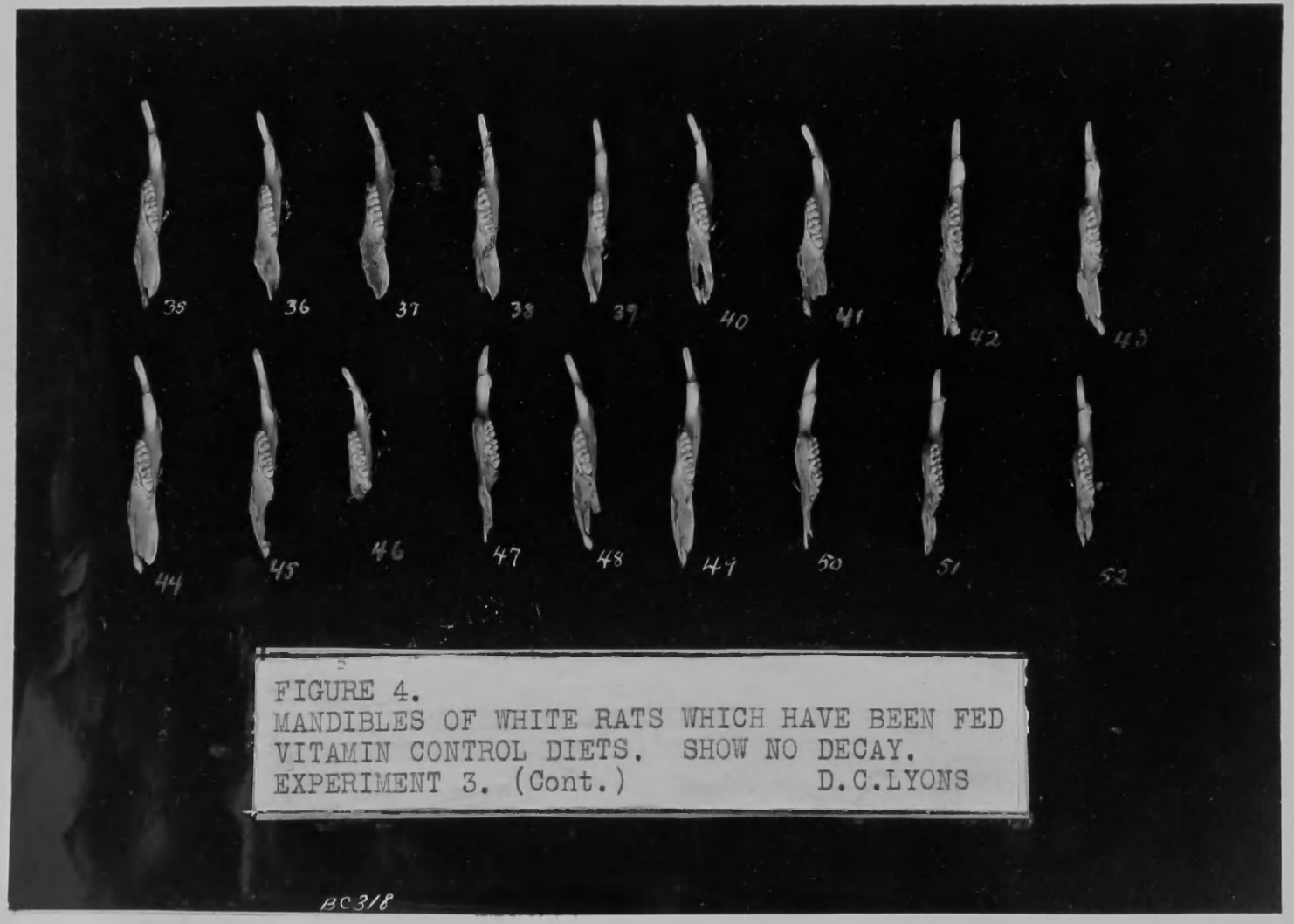


FIGURE 4.
MANDIBLES OF WHITE RATS WHICH HAVE BEEN FED VITAMIN CONTROL DIETS. SHOW NO DECAY.
EXPERIMENT 3. (Cont.) D.C.LYONS

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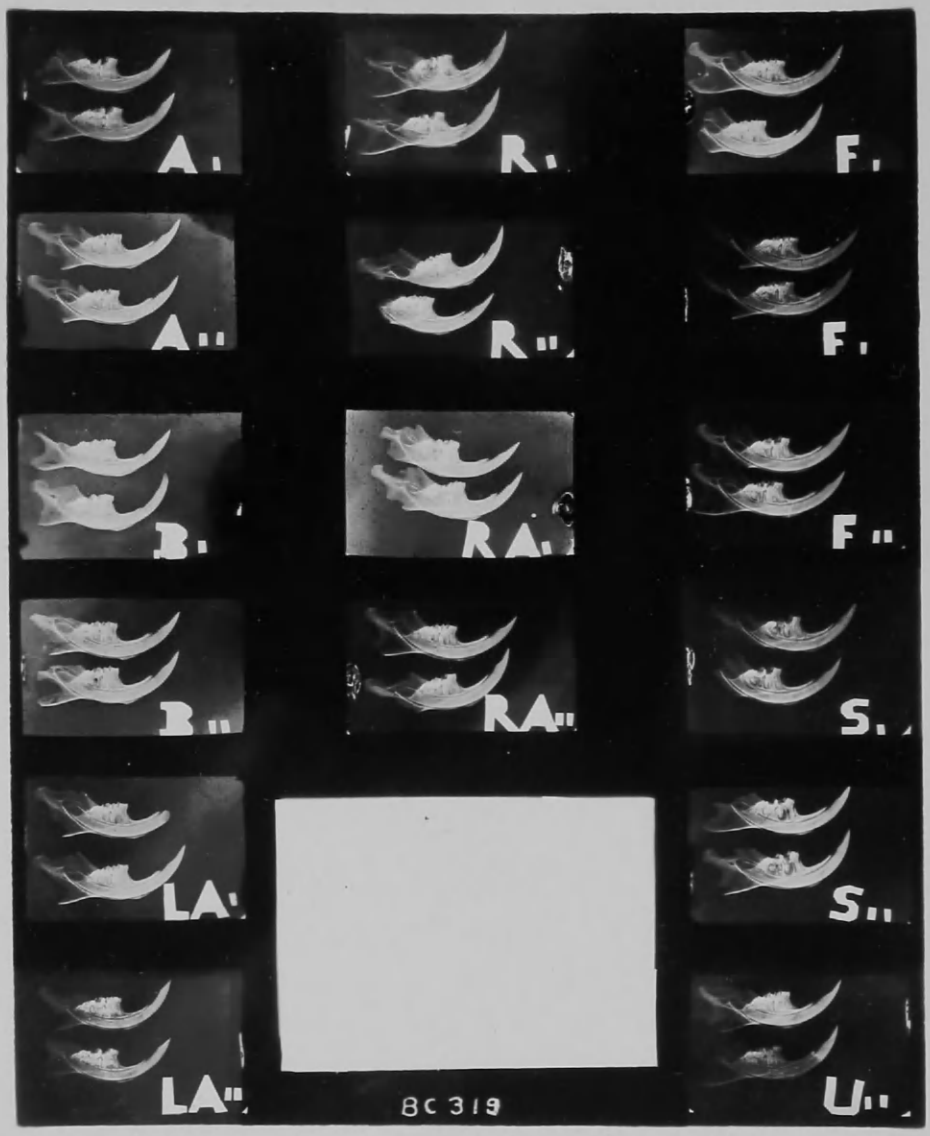

FIGURE 5.
X-RAYS OF MANDIBLES OF WHITE RATS, EXPERIMENT 2, GROUP 1, SHOWING EXTENSIVE DECAY AND APICAL ABSCESSES. D.C.LYONS

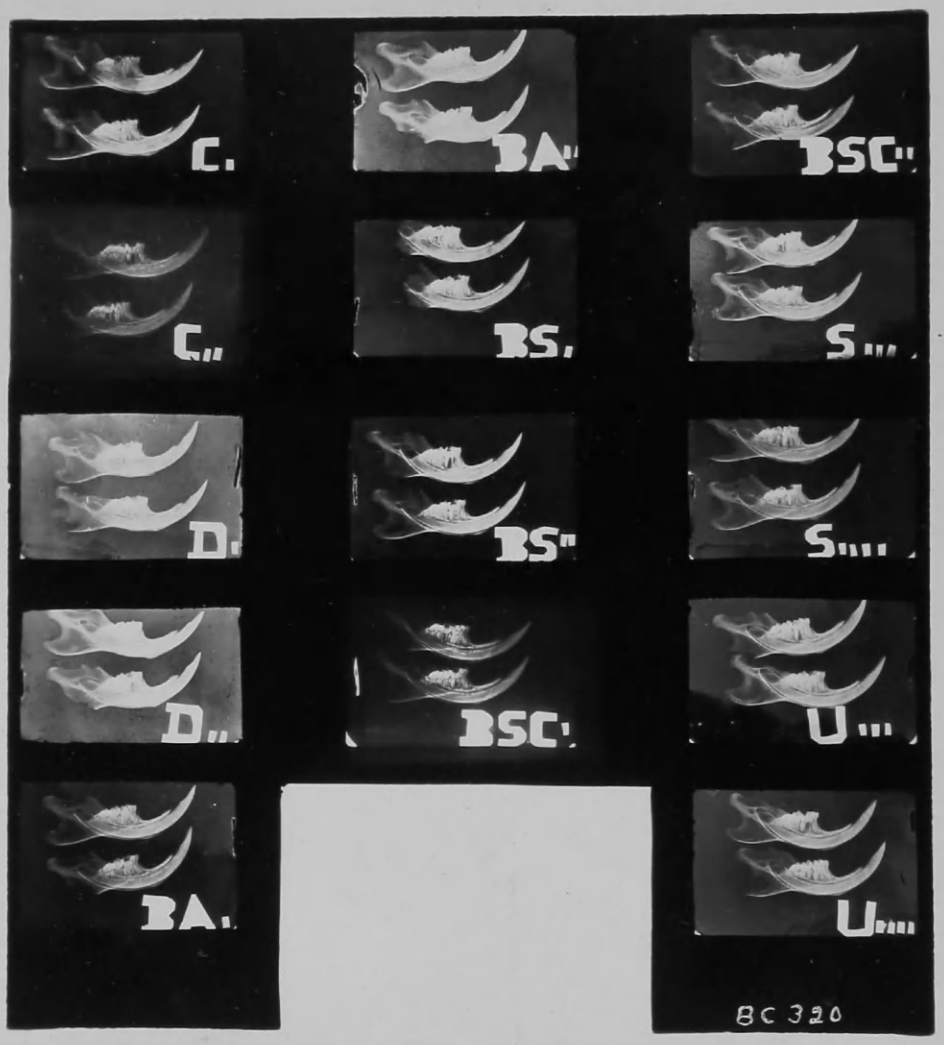

FIGURE 6.
X-RAYS OF MANDIBLES OF WHITE RATS, EXPERIMENT 2, GROUP 2 , SHOWING EXTENSIVE DECAY AND APICAL ABSCESSES.

D.C.LYONS