STUDIES UPON DENTAL CARIES.

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(Researches conducted under the auspices of the Forsyth Dental Infirmary and the Research Institute of the National Dental Association.)

THE following paper is a report of the work done under my direction in this institution as a part of the investigation of the problem of Tooth Decay. At the Forsyth Infirmary there is a remarkably good opportunity to work upon this problem because of the large numbers of children who visit the infirmary daily, and the system by which the case of each child treated is followed up, and because of the excellent equipment and resources of the laboratory.

The work has been done upon children where caries is active. In the adult, one is never certain but that the active carious process has ceased and but that it has been supplanted by microbic flora of an entirely different type.

The nineteen cases of children that had no decay and yet who had never given their teeth attention are only to be found in a clinic like the Forsyth's.

The results of this investigation support the study and ideas of Dr. Klinger who says that in caries the character of the oral flora is changed from a streptococcal to an acid rod and thread flora.

These results are also supplementary to our other studies upon the bacteriology of dental caries in which we have shown that the prevailing flora consists of the highly aciduric microorganisms of the Moro-Tissier group.

The work here presented has been examined by Prof. Hilliard of the Biological Department of Simmons College and has been made the basis of the thesis that Miss Gillette prepared as a part of the requirements for her B. S. degree. Miss Gillette's paper describes the work fully and we are giving it here.

This work is to be followed by a similar study using anaerobic and semi-anaerobic methods of culture.

THE RELATIONS OF THE NUMBERS OF MICROORGANISMS IN TOOTH DEPOSIT, AND THEIR ACID PRODUCTION, TO THE PRESENCE OF DENTAL CARIES.

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HISTORICAL. The work on organisms from tooth deposit is the oldest we have as the first ocular demonstration of bacteria was of the bacteria in tartar scraped from teeth, by Leuwenhock in 1683. Since the presence of bacteria in the mouth has been known for so long a time it seems surprising that the actual knowledge of the normal inhabitants of the mouth, and of the causative agents in common diseases of the mouth, such as pyorrhea alveolaris, and dental caries, is so scarce. Aside from the fact that it has long been appreciated that the mouth is the common port of entry for many pathogenic organisms, and that cleanliness of the oral cavity is essential to good health, and that many forms of bacteria are found associated with decayed tooth substance, nothing in the bacteriology of the mouth has been definitely established. The theory of tooth decay formulated by Miller in 1892 that "the enamel is decalcified by acids elaborated by bacterial fermentation of carbohydrates in the mouth. The bacteria now enter the softened tooth and destroy it by their ferments," is the most generally accepted one at the present time, even tho it fails to account for many facts.

On the qualitative side of oral bacteriology there has been a large amount of investigation, most of which is study of specific organisms and the frequency of occurrence, but as nearly every common form of bacteria finds its way at some time into the human mouth, a vast amount of data is necessary before we can determine just which forms are normal, which are invaders in case of disease, and which are accidental invaders.

Among the most important attempt at

the classification of bacteria obtained from the mouth, are those of Miller in 1893, Goadby, and Gies and Kligler in 1915. Miller divided the organisms he found into two classes; (1) the cultivable forms, (2) the uncultivable forms. As many of his "uncultivable" forms have since been isolated, his basis of classification is decidedly out of date. Goadby's work was, in the main, based on the work of other investigators. He classified the organisms into those found in superficial and deep layers of decay, and subdivided them into liquifiers and non-liquifiers. Kligler's classification is based on biological relationship under the following heads; (1) Animal parasites, or protozoa; (2) Intermediate forms, or Spirochaetes; (3) Plant organisms, or Bacteria. Kligler's work is an attempt to organize the work of preceding investigators, under his own classification, as well as to gather and classify considerable original data.

Among his most significant and interesting studies of specific microorganisms of the mouth, is the study of the mouth ameba in relation to pyorrhea alveolaris. The work is Bass and Johns (1914), and Barrett and Smith (1915) has attracted wide attention. The ameba has not been established as a causative factor in pyorrhea, as yet. In fact, the most recent work tends to indicate that this type of organism is a normal inhabitant of the mouth, and perhaps a scavenger, rather than a disease producer. Much of the modern treatment of the disease is based on the assumption that the endameba is intimately concerned with the disease.

The discovery of the spirocheta of syphilis by Schaudim in 1906, stimulated interest in the study of mouth spirochetes. Repaco, and Nogouchi, both working in 1912, described these forms and reported their successful cultivation.

Many aerobic cocci, bacilli, and organisms belonging to the Trichomycete group have been isolated and described

by a great many workers. The work of Rodella in 1905 on amebic forms of the mouth, which had hitherto been neglected by students of oral bacteriology, suggested a possible importance of these forms, as many are putrefactive.

Kligler, in his work on Oral Micro-organisms in 1915, attempted to discover whether the numbers of organisms in tooth deposit collected from forty cases, ranging from mouths in which no dental caries was then present, thru various stages of decay to very extreme cases, bore any relation to the condition of the mouth. He plated 1/1000 (or greater) dilutions of weighed deposit on litmus glucose agar, and plain agar plates cultivate aerobically for four or five days, and on glucose agar, and litmus-glucose-serum-agar plates cultivated anaerobically for the same length of time. As sources of material, he used mouths in varying conditions of cleanliness, such as before and after eating, before and after brushing the teeth, etc., and of adults who had received more or less dental care all their lives. The two immune cases also, are persons whose teeth have had excellent care. Microscopic counts were made from 0.1 cc. of the 1/10 dilution and twenty fields counted and averaged, and the number of organisms per milligram computed by this method as well as by the plate count method. He reports that, in general the number of bacteria varied directly with the mass of deposit on the teeth under normal conditions, but that under pathological conditions, the count varied with the stage of decay.

PURPOSE OF THIS WORK. The purpose of the present work is to show whether the conclusion of Kligler in regard to the numbers of bacteria present in deposit found on teeth remained true in cases in which the deposit was obtained from teeth which had never received professional care or daily brushing since eruption. Forty-four cases were examined, but ten were discarded because

they did not fall into the desired groups or because of accident in handling. All cases were between the ages of eight and fourteen. To make the result as striking as possible, it seemed advisable to select mouths which were entirely free from decay and those in which were large numbers of decayed teeth for study. All cases recorded, therefore, belong to one or the other of these classes, and with the exception of two cases were children whose teeth had had no care whatsoever. The two exceptions were brothers of eight and ten years, whose teeth were in perfect condition, but who were in the habit of brushing their teeth. They were instructed to neglect their teeth for eight weeks. At the end of that time, when the cultures were made, there was much deposit.

No attempt was made in this work to make anaerobic cultures, or to isolate forms for study, but to gain a knowledge of comparative numbers of organisms found in the two types of cases. With this quantitative study, of experiments were undertaken to show roughly the character of the organisms with respect to their power of producing acid.

COLLECTING SAMPLES. The method of collecting material was essentially that of Kligler. A small vial about 4 cm. long, was fitted with a glass spatula having a thin connection with the handle. The spatula was held in place by the cotton stopper. The vials were stoppered with the spatulas in their places, and then sterilized and placed in a desiccator kept for the purpose. Just before using each vial was weighed to tenths of milligrams. The deposit was collected on a sterile excavator and transferred to the spatula with greatest possible care. The vial was then weighed immediately, and the difference between this and the former weight recorded as the weight of deposit used. This process was carried on as rapidly as possible to make the conditions as to the evaporation of water from the material as uniform as they

could be. The material collected was made representative by removing some of the deposit from the necks of as many teeth as could be easily reached.

MAKING CULTURES. After the second weighing of the vials, the spatula was removed, and the material placed in a dilution bottle containing 10 cc. of sterile water, and glass beads. This was done by pressing the spatula against the side of the tube until the end broke off and fell into the water, carrying the material with it. When thoroly mixed 1 cc. of this was transferred by a sterile pipette into a dilution bottle of 99 cc. of sterile distilled water. Using 1 cc. of this dilution for each plate, three glucose agar plates were made. Another 1 cc. was pipetted into a tube containing 10 cc. of maltose bouillon (1% maltose) of known acidity, which was as near neutral as possible.

For some of the cases plates of lactose agar, and litmus-glucose, and litmus-lactose-agar were made. The litmus plates were made with the intention of obtaining a differential count of acid formers and forms not producing acid, in order to estimate what proportion of organisms were acid formers for each type of case. In every case the entire surface of the plate was so reddened that such a count was impossible. In several cases litmus plates were made from dilutions as high as 1/100,000, but in such cases all the organisms were surrounded by a reddened area. In some cases the litmus plates were reddened when first examined (after 48 hours) and, at the end of four days were almost entirely blue again, due to the presence of slow growing spreaders and yeasts. On lactose plates the count was always very much greater than on glucose.

On some of the first cases examined I attempted to make microscopic counts by Kligler's method, described above, but found it impracticable, on account of a large amount of debris composed of

food particles, crystals of lime salts found in tartar etc., which made it very difficult to find and count the bacteria on the slide.

OBSERVATION OF CULTURES. The glucose agar plates were counted after four days incubation at 37.5° C. The counts of the time plates were averaged (unless one was so different from the others that its count must be thrown out) and the average number of organisms per milligram of material used computed and recorded.

After twenty-four hours incubation at 37.5° C. the Maltose broth cultures were titrated against N/20 NaOH with phenol-phthalein, and again after 48 hours incubation, and the per cent. of normal acidity computed. The second day's titration did not differ from the first in most cases, but was done as a check on the first work.

The observation of yeast growth was accidental, and no significance attached to it until too late to gather adequate data concerning the presence of yeasts in decay.

RESULTS. The results of these experiments are given in the accompanying tables. Table I shows the results of the cases of decay. Fifteen cases are recorded under this heading. In Table II nineteen cases of no decay are recorded. In comparing these two tables it will be noticed that the average number of organisms counted is only slightly greater for the decay cases than for the cases in which no decay is present. When the wide variation in the figures for the different cases in each type is taken into consideration, this difference will be seen to be insignificant.

As the figures for the bacterial count are computed on the basis of the number of organisms per milligram of deposit, it was necessary to put the figures expressing the degree of acidity on a similar basis. Under Acidity in Maltose broth, in the tables, the first column

gives the per cent normal acidity of the cultures as titrated, after correction for the acidity of Maltose broth before and after incubation, has been made. It will be remembered that 1 cc. of the 1/1000 dilution was inoculated into the Maltose broth. In this way the number of organisms varied, according to the amount of deposit used in making the dilution. In order to obtain a convenient number for comparison I have divided the number of organisms per cent. in 1 cc. of dilu-

tion (as shown by plate count) into the number representing the acidity for the culture. The second column under acidity, headed "Acidity per organism" is made up of figures obtained in this way. The difference between the average "Acidity per organism" for the cases of the decay type will be readily seen to be much greater than that for the cases of the no decay type.

Under "Remarks" I have noted the general appearance of the plate, as re-

TABLE 1—CASES OF DECAY.

Results of Bacterial Count of Plates				Acidity in Maltose Broth—24 hours		Remarks
Case	Wt. of Deposit	Total No. of Organisms	No. of Organisms Per Milligram	Total Acidity per Organism		
IV	0.0059	1058000	179000	...	.....	Some yeast present. Colonies larger than Table II.
VII	0.0160	9000000	562000	...	.....	
X	0.0163	7500000	470000	...	.....	
XV	0.0021	646000	308000	4.3	0.00665	
XXI	0.0051	288000	576000	3.8	0.00322	
XXII	0.0062	1920000	320000	4.5	0.00234	
XXIV	0.0077	584000	760000	5.7	0.00958	
XXVI	0.0151	1632000	108800	3.2	0.00134	
XXIX	0.0058	1536000	265000	3.6	0.00234	
XXX	0.0011	297600	270000	4.2	0.01310	
XXXII	0.0036	1124500	350000	3.9	0.00347	Small colonies
XXXV	0.0102	62000	62000	4.3	0.06933	
XXXVII	0.0018	900000	546000	...	.....	
XXXVIII	0.0032	24000	7800	...	.....	
XXXIX	0.0068	595000	87400	...	.....	
Averages			324800	4.15	0.01282	

TABLE 2—CASES OF NO DECAY.

Results of Bacterial Count of Plates.				Acidity		Remarks
Case	Weight Deposit Used. Grams.	Total Number of Organisms.	No. of Organisms per mgn. of Deposit.	Total	Acidity Per Organism.	
III	0.0029	372000	128000	...	.....	Pinpt Col.
IX	0.0119	6000000	500000	...	.....	
XI	0.00295	1800000	610000	...	.....	
XIII	0.0169	1587000	94000	4.6	0.00280	
XIV	0.0057	544000	95000	3.4	0.00625	Perfect teeth Pinpt Colonies Pinpt Colonies Some yeast pres. Pinpt Col. Pinpt Col. Bros. teeth Neglected 8 Wks.
XVI	0.0027	672000	50000	3.5	0.00506	
XVIII	0.0109	5728000	520000	3.7	0.00064	
XIX	0.0127	2624000	220000	3.5	0.00132	
XX	0.0138	6400000	450000	3.8	0.00059	
XXIII	0.0077	11000	1500	0.0	0.0	
XXV	0.0208	1789000	86000	2.8	0.00156	
XXVII	0.0069	6400000	910000	3.8	0.00059	
XXVIII	0.0100	3200000	320000	3.8	0.00119	
XXXI	0.0051	3517500	162000	2.8	0.00019	
XXXIII	0.0053	1126500	240000	3.6	0.00325	
XXXVI	0.0040	714000	166000	4.6	0.00644	
XL	0.0081	4100000	506000	3.9	0.00095	
XLI	0.0085	7566000	606000	3.7	0.00048	
XLIV	0.0064	860000	132000	...	.....	
Averages			310340	3.5	0.00228	

gards the type of colonies, whether very small or not, and also the appearance of yeast growth. It is interesting to note that in almost every case of no decay the colonies were very small, almost microscopic in size, with the exception of one case, no spreaders or yeast forms were found. In the cases of decay, there was but one case noticed in which colonies resembling yeasts were not present. In a large number of cases, smears showed that suspected colonies were really yeasts. In this type of plate all the colonies were somewhat larger than those on the plates of cases where decay was absent.

I should like to call attention to cases numbered XXIII, XL, and XLI in the second table. Case XXIII was a girl of about ten years, whose teeth, the examining dentist said, were the most perfect he had ever seen. There was as much deposit on the teeth as in most other cases, but, as the table shows, there were not many organisms in it. The maltose broth culture showed some growth, but there was no greater change in acidity than there was in uninoculated tube on incubation, even after 48 hours. Cases XL and XLI are those of the brothers mentioned above.

SUMMARY. 1. The number of organisms found in a known weight of material scraped from tooth surfaces in uncared for mouths in which caries is present, as determined by counting plates incubated aerobically, does not vary significantly from the number of organisms found in uncared for immune mouths.

2. Plates made from deposit obtained from carious mouths showed, in general, larger colonies, among them yeast forms, than those made from the deposit obtained from non-carious mouths.

3. Titrations of Maltose broth cultures made from 1 cc. of a 1/1000 dilution of the oral material showed a no-

ticeable difference in the power of acid production in the two types of cases, the factor per organism for the carious type being about five times as great as for the non-carious type.

CONCLUSIONS. Altho the bacterial count made in this way cannot be taken as absolute values for the number of microorganisms in the material used, these counts can be considered relative, and inasmuch as they were taken from such widely differing types of cases, show rather strikingly that as far as aerobic microorganisms are concerned, the number of bacteria in the mouth are directly proportional to the amount of deposit present, regardless of the presence of caries.

The difference between the flora of immune and carious mouths, as indicated by the acid production and difference in appearance of the colonies, is chiefly a qualitative difference. There is a predominance of organisms capable of producing larger quantities of acid when caries is present than when there is no caries.

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