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Bacterial Flora in Experimental Dental Caries of the Rat.

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According to Miller's theory[1] dental caries is caused by acids elaborated by microörganisms. Of the acidogenic and aciduric bacteria present in the plaques covering early lesions and in the carious dentine of advanced cavities, lactobacilli and acid-producing streptococci are the forms commonly believed to be implicated in decalcification of human teeth. However, these organisms are regularly found in the mouths of caries-free rats and if they are involved in experimental rat-caries their activities must be dependent upon other not well understood factors. The present investigation was made with animals used by Dr. B. F. Miller[2] in a study of the inhibition of rat-caries and cultures were taken with his kind coöperation.

Material from the surfaces of molar teeth of 60 young rats was cultured aërobically, at the beginning of the diet periods and again after 7 and 13 weeks, on Kulp's agar,[3] Jay's broth,[4] blood-agar, and brom-cresol-green serum-dextrose agar. The methods were not quantitative but all samples were handled in the same way and the results therefore have comparative value. In the first culture-period streptococci comprised from half to three-fourths of the colonies obtained. They were in general about equally divided between α, β and γ types and in most cases about twice as many were acidogenic as non-acidogenic. The remaining organisms were largely lactobacilli, staphylococci, and micrococci. Other forms were encountered less frequently and usually in small numbers. From Jay's and modified Kulp's media lactobacilli were recovered in all cases but since these media tend to suppress organisms that are not aciduric, comparative counts of acidogenic organisms were confined to indicator-plates.

The studies of Miller[2] have shown a high incidence of cavities in animals on a caries-producing diet, fewer lesions in a group receiv-

[1] Miller, W. D., *The Microorganisms of the Human Mouth*, Philadelphia, 1890.

[2] Miller, B. F., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 389.

[3] Kulp, W. L., *Science*, 1927, **66**, 512 (Hadley modification: Hadley, F. P., *J. Dent. Res.*, 1933, **13**, 415).

[4] Jay, P., and Voorhees, R. S., *Dent. Cos.*, 1927, **69**, 977, and personal communication.

TABLE I.

Comparative Percentages of Lactobacilli and Acidogenic Streptococci Cultivated from the Tooth-surfaces of Rats on Caries-Producing and Caries-Inhibiting Diets.

	First culture period (7 wk)			Second culture-period (13 wk)			
	Total organisms	Acidogenic organisms		Total organisms	Acidogenic organisms		Caries† at 16 weeks
Exp. group	% acidogenic	% lactobacilli	% streptococci	% acidogenic	% lactobacilli	% streptococci	
Normal stock* (60 rats)	52	18	23	52	18	23	
Caries diet (20 rats)	35	18	43	36	18	25	+++
Caries diet with:							
NaF (10 rats)	24	<1	19	51	22	19	±
CaF_2 (10 rats)	27	1	45	48	14	42	+
Iodoacetic acid (10 rats)	—	—	—	72	3	90	—

*The cultures from the normal stock rats were made at the outset of the experiment and were not repeated. The data are placed in both sets of columns for comparison.

†+++ = high incidence of dental caries, + and ± = low and very low incidence. — = no carious lesions.

ing this diet together with calcium fluoride and little or no caries in groups with added sodium fluoride or iodoacetic acid. Neither the non-acidogenic flora nor the acidogenic flora as a whole showed changes which could be correlated with the development of cavities or with the diets of the animals. Of the acidogenic organisms only streptococci and lactobacilli were regularly found in sufficient numbers to have probable significance. It should be noted that because variations in the amount of inoculum colony-counts cannot here be compared directly and percentages are therefore used. As shown in Table I streptococci varied erratically from 19% to 45% of the total acidogenic flora and reached 90% in the iodoacetic acid group which had no caries. The data indicate no relationship between the presence of acidogenic streptococci on the tooth surface and the development of caries. Lactobacilli comprised 18% of the acidogenic organisms at the beginning of the experiments and remained at that level in the caries-diet group. However, they were reduced to 3% in the final cultures from the group on iodoacetic acid and to 1% in the cultures at 7 weeks from the groups on the fluoride-diets. These reductions, although temporary in the fluoride-groups, may be of possible significance with respect to the failure of caries to develop in these animals.

After 16 weeks 10 carious and 10 non-carious teeth were removed, carefully cleaned with alcohol and sterile water, ground with mortar and pestle, and cultured. The average bacterial count of 9 non-carious teeth, omitting one specimen with a high staphylococcus-count, was only 28 per mg. The flora in general was the same as in swab-cultures from normal rats' teeth, about 30% of the organisms being acidogenic streptococci.

The total bacterial counts from 10 carious teeth averaged about 500 per mg. About 60% of the organisms were acidogenic streptococci. Lactobacilli were found in small numbers in cultures from only 6. The other organisms had about the same relative distribution as in the swab-cultures of the teeth *in situ*.

The very low incidence of lactobacilli in cultures from carious teeth suggests that they may not be involved in the advanced carious process. On the other hand, the high proportion of acidogenic streptococci might be taken as evidence of some relationship to the lesion. Data obtained from human teeth,[5] indicating that lactobacilli may be involved in early lesions of the enamel, and that cocci may predominate in carious dentine, are in a measure substantiated by the findings in this investigation.

[5] Blayney, J. R., personal communication.