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The Effect of Sodium Fluoride on the Metabolism of Oral Lactobacilli.*

WILLIAM E. CLAPPER.† (With the technical assistance of Anne Adams.)
(Introduced by L. P. Gebhardt.)

From the Department of Bacteriology, University of Utah School of Medicine, Salt Lake City, Utah.

Dental caries has been shown to be associated with high lactobacillus counts in saliva by the investigations of Jay,[1] Dean and his associates,[2] Snyder,[3] Collins *et al.*,[4] and others.[5] Almost all investigators now agree that the number of lactobacilli in the saliva is a rough index of caries activity.

Children of school age in the areas in which the drinking water contains 1 p.p.m. or more of fluoride have a low caries incidence. Dean[6] found that the lactobacillus counts in such areas were lower than in areas in which the water contained no fluoride. More recently,[7,8] the use of a solution of NaF as a mouth wash decreased the number of lactobacilli. Fosdick and Starke,[9] have shown that *Lactobacillus acidophilus* will degrade glucose according to the Meyerhof scheme to lactic, pyruvic, and phosphoglyceric acids. Any of these acids are capable of dissolving enamel. Sodium fluoride has been shown to inhibit the formation of acid from glucose by lactobacilli in concentrations as low as 2 p.p.m.[10] Concentrations from 2 to 100 p.p.m. did not inhibit growth.

There has as yet been no complete explanation of how fluoride water inhibits caries or of how the fluoride inhibits the activities of oral lactobacilli.[11] Some information should be secured by studies of its effect on specific enzymes and of the effect of continued growth in the presence of fluoride. With this in mind an investigation was made of (1) the effect of NaF on the acidogenic properties of several strains of lactobacilli from saliva, (2) the acidogenic properties of a lactobacillus grown in the presence of NaF for 6 months, and (3) the effect of NaF on the activity of 9 dehydrogenases of a lactobacillus.

Effect of Added NaF on Acid Production in Glucose Broth. Eleven strains of lactobacilli were isolated from saliva and maintained in peptonized milk agar. Five-tenths ml of a 48-hour tryptose phosphate broth culture of these organisms was planted in flasks containing 100 ml of tryptose phosphate broth with .5% glucose. Sodium fluoride was added to some of the flasks to make a concentration of 1 p.p.m. and to others to make a concentration of 100 p.p.m. The acid was titrated at intervals with N/10 NaOH. The titratable acidity of duplicate flasks never varied more than 2 ml of N/10 acid per 100 ml medium.

Table I shows that NaF in concentrations of 1 p.p.m. and 100 p.p.m. inhibit acid formation from glucose at all stages investigated in the growth of the lactobacilli. The acid production was inhibited to some extent for

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† Present address: University of Colorado Medical School, Denver, Colorado.

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TABLE I.
Average Reduction in Acid by NaF. Eleven Strains of Lactobacilli.

	Reduction per 100 ml medium	Hours incubated			
		24	48	72	96
1 p.p.m. NaF	ml N/10 acid	3.3	6.2	6.8	9.2
	% reduction	8.8	10.9	10.5	12.6
100 p.p.m. NaF	ml N/10 acid	4.4	12.5	16.5	20.2
	% reduction	11.4	21.5	25.0	27.3

each organism. The smallest reduction was shown in the first 24-hour period, and the greatest reduction in the 96-hour period. The higher concentration of the fluoride was roughly twice as effective as the lower in preventing acid formation. Growth of the organisms was not decreased by either concentration of fluoride as far as could be determined by visual observation of turbidity.

Effect on Acid Production of Continued Growth in the Presence of NaF. A single strain of lactobacillus, Strain 1, isolated from the saliva of an individual with rampant caries was grown in peptonized milk agar containing 100 p.p.m., 1 p.p.m., and no NaF and was maintained by weekly transfers over a period of 6 months on these media. This organism, Strain 1, was a strong acid producer. At the end of 6 months, organisms from the plain peptonized milk agar, organisms from the agar containing 1 p.p.m. NaF, and those from agar containing 100 p.p.m. NaF were inoculated into tryptose phosphate broth containing .5% glucose. The acid was titrated as before.

Continued growth in the presence of 1 p.p.m. NaF had no effect on the acidogenic properties of the strain used, but in the presence of 100 p.p.m. NaF, the organism lost 53% of its ability to produce acid. Repeated titrations showed little or no variation. To check this result the original Strain 1 was again grown for 6 months, in a medium containing 100 p.p.m. NaF. It again showed a marked loss of ability to produce acid when planted in broth with no NaF present. Maintenance of this trained strain on plain peptonized milk agar for 6 months did not cause it to regain its acidogenic properties. Gram stains showed no morphological changes.

Effect of NaF on Dehydrogenase Activity. The Thunberg technic was used. Two control tubes containing the substrate but no NaF, 2 with substrate and 1 p.p.m. NaF, and 2 with substrate and 100 p.p.m. NaF were set up simultaneously. The lactobacilli were obtained by growing in 100 ml of tryptose phosphate broth for 48 hours, centrifuging, washing once with distilled water, and suspending in distilled water. The suspension was adjusted to a standard turbidity (500 on a Kletts-Summerson photoelectric colorimeter) and was used immediately since storage in the refrigerator seemed to decrease the dehydrogenase activity. Light suspensions were relatively inactive. Slight variations from the above standard density produced only small changes in activity. The organisms were placed in the side arm of the tube and tipped in after the tubes had reached a temperature of 37°C. The time was noted for the decolorization of the control tubes and the increase or decrease in time for the tubes containing NaF. The time of decolorization of the control tubes was given a value of 100, while values for those with NaF were calculated as percentages of activity of the controls. A tube requiring twice as much time for decolorization as that of the control was given a value of 50 as its dehydrogenase activity. The results are shown in Table II. One hundred p.p.m. NaF inhibited all but 1 of 9 dehydrogenases but 1 p.p.m. imparted no inhibitory, slight inhibitory, or even stimulating action.

Discussion. As little as 1 p.p.m. NaF decreased acid production from glucose. This inhibition may explain the low caries incidence in those areas in which the water supply contains fluoride. There was considerable variation among the strains in their susceptibility to fluoride, 4 showing no signi-

TABLE II.
Effect of 1 p.p.m. and 100 p.p.m. NaF on the Dehydrogenase Activity of Strain 1.

Substrate	1 p.p.m.	100 p.p.m.
Glucose	87*	60
Levulose	100	80
Maltose	100	71
Sucrose	100	0
Lactose	140	100
Glycerol	100	0
Pyruvic acid	95	43
Lactic acid	94	89
Formic acid	180	25

* Values are expressed as percentages of activity of the control which had no added NaF.

ficant decrease with 1 p.p.m., although 100 p.p.m. did inhibit acid production. Less inhibition was found in the first 24 hours than in the later periods of growth. Bibby and Van Kestern[10] working with 12 strains of lactobacilli from healthy mouths obtained values similar to those found in this work.

Continued growth of a strain of lactobacillus in the presence of 100 p.p.m. NaF decreased its ability to produce acid from glucose. This suggests that an alternate enzyme system is used. Wiggert and Werkman[11] obtained fluoride resistant strains of *Propionibacterium pentosaceum* by growing the organism in a medium containing fluoride. These strains were incapable of fermenting phosphoglyceric acid. They suggested that this organism was capable of fermenting glucose in the presence of fluoride by a route other than that involving phosphoglyceric acid. Nord and Mull[12] point out that in the fermentation by *Fusaria,* phosphoglyceric acid was neither isolated in the presence of fluoride nor utilized as a carbon source. The strain of lactobacillus reported in this paper, Strain 1, which was grown in media containing fluoride, may have a similar metabolism. Such a change in metabolism may occur in lactobacilli in plaques on the teeth. The plaque being repeatedly exposed to small concentrations of fluoride in drinking water may adsorb sufficient quantities to bring about such changes in the organisms with which they are in contact. NaF, then, may reduce decay by the prevention of acid formation from fermentation by lactobacilli in the mouth. This acid production may be reduced in one or both of 2 ways. First, by the direct inhibition of the enzymes necessary to the formation of acid and second, by altering the enzymatic pattern of the organism by being constantly present in the environment.

That NaF is capable of affecting the oxidative mechanisms of the lactobacillus is shown in the results of the dehydrogenase determinations. The substrates were chosen because they were carbohydrates likely to be encountered in the mouth by lactobacilli, or were possible intermediates in the breakdown of sugars. Previous experimental work in this laboratory had shown that the activities of the dehydrogenases for these substrates were remarkably constant for 5 strains that were tried. No dehydrogenase activity was shown for either succinic acid or acetic acid. The inhibition of oxidation of formic, lactic and pyruvic acids would appear to have no relation to decreased acid production from glucose since oxidation of these acids would lead to less accumulation of acid. Preventing the oxidation of glycerol if it is formed, would prevent acid formation. The point of inhibition of acid formation of lactobacilli from glucose would seem to lie in the inactivation of an enzyme involved in changes before phosphoglyceric acid is formed. The dehydrogenase for glucose is inactivated by both concentrations of fluoride.

Just what part is played in the prevention of caries by the action of NaF on the dehydrogenases for sucrose, maltose, and lactose is impossible to determine from this experiment. It may be of no significance, since all of the above carbohydrates are hydrolyzed to glucose in the normal mouth. One hundred parts per million of NaF prevents acid formation from lactose but does not inactivate the dehydrogenase for lactose. The inability to inactivate a carbohydrate dehydrogenase does not always indicate inability to prevent acid formation.

Summary. 1. Of 11 strains of lactobacilli tested, all were reduced in their ability to

[12] Nord, F. F., and Mull, R. P., *Advances in Enzymology, Vol. V., Interscience,* New York, 1945, p. 165.

produce acid by the addition of 1 and 100 p.p.m. of NaF. Resistance to the action of fluoride on acid production varied among strains.

2. Continued exposure to 100 p.p.m. NaF lowered the ability of an actively acidogenic strain of lactobacilli to produce acid from glucose. This reduced acidogenic activity continued even in the absence of NaF. One p.p.m. of NaF had no demonstrable effect.

3. One hundred p.p.m. NaF inhibited the dehydrogenases of lactobacilli for 8 out of 9 substrates. The inhibition by 1 p.p.m. was much less.

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Effect of the Fat Content of Diets on Blood Sugar.

MICHAEL SOMOGYI AND ROBERT J. COOK.
From The Jewish Hospital of St. Louis, Mo.

The adverse effect of fat feeding on the carbohydrate tolerance of normal persons has been amply demonstrated. Staub[1] and Kageura[2] 25 years ago discovered the fact that healthy men, after being kept on carbohydrate-free meals for 2-3 days, responded to glucose feeding with "diabetic-like" blood sugar-time curves and glycosuria. Himsworth[3] obtained identical results with high fat diets even when including carbohydrates not in excess of 50 g per day. In these studies no attention was paid to the postabsorptive blood sugar level, but the data in the reports of these authors disclosed no rise above the normal level.

Changes in the postabsorptive glycemic level after fat feeding were first reported by F. M. Allen and his associates.[4] Their observations were made on epileptic persons who were fed 260 to 580 g of fat daily, with only 12 to 22 g of protein and 2.5 to 3.7 g carbohydrate. Periodically performed determinations of the plasma sugar showed marked and gradual increases, as for instance (in the most responsive subject) from the initial 135 mg % to 357 mg % in 7 weeks.

Allen's observations, and a number of other studies related to the subject, were carried out under rather extreme experimental conditions. Our interest lay in the effect of high fat diets which, at the time of our studies here reported, were (and very widely still are) prescribed to diabetic patients. Accordingly, the carbohydrate ration was 100-150 g, daily, with unrestricted protein consumption. We selected for the experiment healthy persons, whose carbohydrate tolerance was normal and whose eating habits fitted into a pattern which can be considered as representing the normal, average type. They customarily consumed 80-120 g protein, 80-120 g fat, and 200-300 g carbohydrate. For the experiment the protein was kept at the usual level, the fat was increased to 200-250 g, and the carbohydrate was limited to approximately 100 g. Just prior to changing to the high fat diet the sugar content of the venous blood was determined in the postabsorptive state. In most instances several determinations were made on consecutive days while the subjects were still on the normal diet in order to ascertain the extent of normal fluctuations of the fasting glycemic level. These were very slight, ranging from 0 to 3 mg %, provided that the subjects adhered to a fairly uniform though unmeasured diet.

Since rather slight changes in the blood sugar were to be detected, accuracy of the analytical procedure required special attention. At the time of these studies—1936 and 1938—we used the copper-iodometric

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[3] Himsworth, H. P., *Clin. Sci.*, 1933, **1**, 1.

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