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Relationship between Caries Activity and the Number of Lactobacilli in the Oral Cavity

By

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Since the report, by KLIGLER (1915), of the occurrence of lactobacilli in deposits on carious enamel, the lactobacilli have been the centre of interest in bacteriologic investigations of dental caries, and with but few exceptions (ANDERSSON & RETTGER 1937, BOYD *et al.* 1949, MARSHALL DAY *et al.* 1949 and BOYD 1950) most workers in this field have found a close correlation between caries activity and the number of lactobacilli in the oral cavity. Readers interested in a survey of these studies are referred to APPLETON (1944) or a *Survey of the Literature of Dental Caries* (1952).

There is, however, still divergence of opinion concerning the nature of this correlation. Some authors expressed the view that lactobacilli represent the main etiological factor in dental caries (MCINTOSH *et al.* 1922, BUNTING *et al.* 1928), while others suggested that the occurrence of these bacilli in the oral cavity is only a secondary phenomenon, *i.e.* secondary to the development of caries (BIBBY, 1935).

Various investigations in which the amount of carbohydrates in the food has been increased or decreased have shown that the variation in the dietary carbohydrate level is accompanied by a corresponding variation in the number of lactobacilli (APPLETON 1950, p. 458). Thus KOEHNE, BUNTING and MORELL

(1934) found that an increase in the dietary cabohydrate resulted in an increase in the number of lactobacilli as well as in an increase in caries activity, and HADLEY (1933), JAY (1940), BECKS *et al.* (1944) and several others observed that drastic restriction of the dietary carbohydrates is accompanied or followed by a decrease in the number of lactobacilli and, as a rule, also by a decrease in caries activity.

In this connection it might be useful to mention that BUNTING *et al.* (1929) and JAY *et al.* (1933) failed to "implant lactobacilli in mouths" which were naturally free from them.

The number of lactobacilli found in the saliva has been used as a measure of caries activity and, judging by the literature, lactobacilli have been demonstrated in the saliva of some 80 per cent of all patients with rampant caries, as against 10 to 20 per cent of caries-free or caries-inactive individuals (COLLINS *et al.* 1942, BECKS *et al.* 1944, STRÅLFORS 1947, and others).

As a clinical investigation of the influence of dietary factors on caries activity was in progress at Vipeholm Hospital, where groups of patients had been living under well-controlled dietary conditions for a long time and been regularly dentally examined, the writer utilized the opportunity of studying this material bacteriologically in an attempt to find answers to the following questions:

1. Did the dietary groups differ with respect to the distribution of the patients among lactobacillus counts?
2. Was any correlation demonstrable between lactobacillus count and caries activity in individual patients in each dietary group?

Material and Methods

The material consisted of adult mental defectives in whom caries activity had been studied under various well controlled conditions (GUSTAFSSON *et al.*, 1954). The patients had been divided into different groups, all of which received one and the same basic diet (2700 Cal. per day). For the composition of this

diet, see GUSTAFSSON *et al.*, (1954). This basic diet was supplemented by the addition of a ration (carbohydrates or fat) varying from one group to another, but equivalent in calorie value. Each patient thus received the basic diet plus an addition bringing up the total daily calorie supply to 3000.

The patients were dentally examined at least once a year, and the groups were afterwards compared with regard to caries activity.

The bacteriologic studies described below were carried out in connection with the Carbohydrate Study II (1949–1951) of the Vipeholm investigation. The material consisted of male groups receiving the following diets:

Basic diet plus 40 g. fat (Control Group)
Basic diet plus 75 g. sugar in solution served at meals (Sucrose Group)
Basic diet plus bread rich in sugar (345 g. containing 50 g. sugar) served at all meals (Bread Group)
Basic diet plus chocolate (max 65 g. per day) served as 4 portions between meals (Chocolate Group)
Basic diet plus toffees (max. 8 per day) served between meals and 25 g. sugar in solution served at meals (8-toffee Group)
Basic diet plus caramels (max. 22 per day) served between meals (Caramel Group)

As judged by clinical examination, the first two of these dietary groups, the Control Group and the Sucrose Group, may be regarded as caries-inactive, the remainder, Bread, Chocolate, 8-toffee and Caramel Groups as caries-active (GUSTAFSSON *et al.* 1954).

The present investigation was based on bacteriologic examination of all of the patients belonging to the Caramel Group and of those patients of the other groups that were included in the 1946–1951 part of the Vipeholm Investigation, *i.e.* those patients providing the basis for the calculation of caries activity accounted for in the main publication by GUSTAFSSON *et al.* (1954). The present material included patients in whom the number of new cavities recorded was high, and some in whom only few or even no new cavities appeared, *i.e.* the material was

unselected regarding the occurrence and degree of caries activity.

Sampling and Culture

The method usually employed for the collection of material in bacteriologic studies, *i.e.* requesting the patient to chew a piece of paraffin wax to stimulate the flow of saliva, which is then collected for culture (HADLEY 1933), was not possible in the present investigation, because many of the patients (in some groups more than 60 per cent) were unable to co-operate sufficiently. It was therefore necessary to resort to passive methods. Material for culture was collected from the patients' teeth by means of a Ward's carver. Of the material thus collected a standard amount, about 40 mg., as measured in stainless steel spoons, made specially for this purpose, was used for culture.

The results obtained did not differ appreciably from those recorded in a parallel study using the HADLEY method.[1]

Three salivary samples collected from every patient at intervals of 10–14 days were cultured on tomato agar plates.[1]

In some preparatory studies the samples were collected partly by means of a sterile swab and partly with the aid of a tooth brush.[1] However, these methods proved less suitable and were abandoned. Therefore, unless otherwise stated, use was made throughout of the so-called "spoon" method. The examinations were made at a time when the patient had been on one of the various diets for at least 1 year.

Classification of the Results of Culture

In the study of the results of culture of samples collected by the "spoon" method or by means of the tooth brush, 3 classes were distinguished, according to the number of colonies per plate. In experiments using material collected by the sterile swab the results were classed simply as positive or negative, according to the presence or absence of growth of lactobacilli.

[1] For further particulars of the methods used and the composition of the medium see GRUBB and KRASSE (1954).

In view of the extensiveness of the investigation, no attempt was made systematically to study various dilutions of the salivary samples from one and the same patient. In some of the plates in which the number of colonies exceeded 400, growth was so dense that differentiation beyond this limit was no longer possible.

In the classification of the results use was made of a logarithmic scale with a class interval of 1. The results were thus classed as follows:

Class I: 0– 10 colonies per plate
II: 11–100 colonies per plate
III: more than 100 colonies per plate.

Control studies using the method of HADLEY showed that Class I corresponds to about 0–1,000 lactobacilli per ml. saliva, Class II to 1,100–10,000, and Class III to more than 10,000.

For assignment of a patient to any of these three classes, the results of at least two of the samples must have fallen within the class in question (GRUBB & KRASSE, 1954).

Results

1. *Comparison between the Dietary Groups*

Table 1 summarises the types of diet used, the caries activity in the various groups and the results of culture.

The Control and Sucrose Groups were clinically inactive, while the Bread, Chocolate and 8-toffee Groups were active. It is apparent that the inactive groups did not differ appreciably from the active ones concerning the results of culture. This is still more apparent from Table 2, where the inactive groups are compared with the most active ones, i.e. the Bread and the 8-toffee Groups.

The table shows that of the inactive groups as well as of the active groups, 17 per cent of the patients belonged to the class with the highest number of lactobacilli and that the groups did not differ appreciably regarding the percentages assigned to the other two classes either.

Table 1. *Distribution of the patients among lactobacillus counts* (Classes I, II and III)

Group	Diet	Number of new carious surfaces per patient per year	Number of patients	Number of patients in lactobacillus class		
				I	II	III
Control Group	G5 + 40 g. fat served at meals	0.47	36	17	10	9
Sucrose Group	G5 + 75 g. sugar in solution served at meals	0.82	54	23	25	6
Bread Group	G5 + 345 g. bread rich in sugar served at all meals	2.32[1]	38	20	14	4
Chocolate Group	G5 + 65 g. chocolate served between meals	1.40[1]	45	29	8	8
8-toffee Group	G5 + 25 g. sugar in solution served at meals + 8 toffee between meals	4.05[1]	35	16	11	8

[1] This increase was statistically significant in comparison with the period before the introduction of the experimental conditions.

Table 2. *Comparison between caries-active and caries-inactive groups*

Group	Caries activity	Number of patients	Distribution of patients among lactobacillus counts		
			I	II	III
Control Group Sucrose Group	Inactive	90	44 %	39 %	17 %
Bread Group 8-toffee Group	Active	73	49 %	34 %	17 %

Thus, as far as the distribution of the patients among lactobacillus counts (Classes I, II, III) is concerned, the active groups did not differ from the inactive ones. (It might be worth mentioning that preliminary analysis of the results of culture of material collected one year earlier from the same patients by means of the tooth brush technique did not show any statistical difference between these groups either.)

Although it might appear that relatively many of the patients belonging to the Chocolate Group were assigned to the class with the lowest number of lactobacilli (Table 1), the χ^2 test (KEMP 1942) failed to reveal a statistically significant difference between this group and any of the others.

2. *Comparison between Patients in One and the Same Group*

Table 3 shows the relationship between the number of lactobacilli and caries activity in the individual patients in each group. However, as the inactive groups (Control and Sucrose) closely resembled one another regarding caries activity, they were taken together as a single group. In this table those persons in whom no new cavities were found during the study period (1 year) are compared with those in whom caries activity exceeded the mean value found for the entire group.

It is apparent from the table that in the inactive Control and Sucrose Groups, of the percentages assigned to the various lactobacillus counts the caries-inactive patients did not differ from those in whom caries activity exceeded the mean value for the group (1 new cavity). It might be mentioned that the distribution according to lactobacillus count of the 9 cases in which

Table 3. *Relation between caries activity and distribution of patients among lactobacillus counts*

Group	Caries activity	Number of patients	Number of patients in lactobacillus classes			Percentage of patients in lactobacillus classes	
			I	II	III	I	II + III
Control & Sucrose Groups	0	73	33	29	11	45	55
	> average	17	7	6	4	41	59
Bread Group	0	17	10	7	0	59	41
	> average	10	3	4	3	30	70
Chocolate Group	0	17	12	4	1	71	29
	> average	14	7	3	4	50	50
8-toffee Group	0	6	6	0	0	100	0
	> average	13	2	6	5	15	85

2 or more new cavities were noted did not differ either from that of those in whom no new cavities were seen during the period under consideration.

In the 8-toffee Group, in which caries activity was highest, all of the 6 patients in whom no new cavities were detected were found to belong to Class I (0–10 colonies per plate), while only few of those with pronounced caries activity were assignable to this class. For the group, statistical analysis (χ^2 with Yates' correction) showed that, regarding lactobacillus count, the distribution of the inactive patients differed significantly from that of those with the highest caries activity ($\chi^2 = 8.8$ and $0.01 > P > 0.001$). A corresponding correlation, though not so close, between caries activity and lactobacillus count was also found for the Bread Group.

Caries activity and the number of lactobacilli are compared in Fig. 1, which also shows the average clinical caries activity for each class according to lactobacillus count in all the dietary groups.

It is clear that a distinct correlation was found between caries activity and the number of lactobacilli in the Bread and 8-toffee Groups, the caries activity being highest in patients with the greatest number of lactobacilli (Class III) and lowest in those with the lowest number of lactobacilli (Class I). No such correlation was found for the remainder of the groups. In these

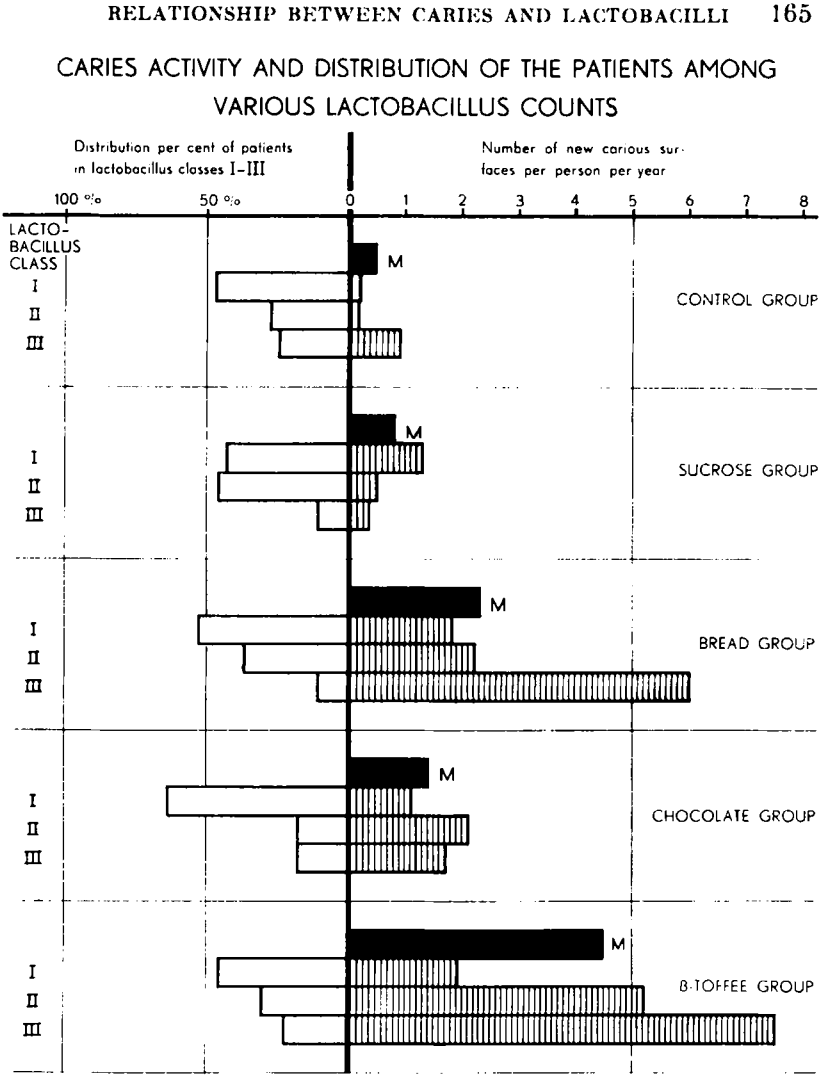

Fig. 1. *Left of midline*: — Distribution per cent of the patients among different lactobacillus counts (Class I<1.000 bacteria per ml saliva, II 1100–10.000 and III>10.000 bacteria per ml saliva). It is clear that, generally speaking, the dietary groups did not differ essentially with regard to the percentage of patients in the respective classes.

Right of midline: — Mean caries activity for the various classes. The black field (M) indicates the mean activity for the group. It is apparent from the diagram that caries activity was correlated with the lactobacillus count in the two groups with the highest mean caries activity (Bread and 8-toffee Groups), in both of which the patients with a high lactobacillus count had the highest mean caries activity and those with a low count, the lowest mean activity. No such correlation was found for those groups with a low mean activity (Control, Surcose and Chocolate Groups).

the average caries activity was approximately one and the same for all three classes of lactobacillus counts and thus showed no tendency to be higher for Class III.

On the basis of samples collected by the "swab" method, the Caramel Group was studied for any correlation between the occurrence of lactobacilli and caries activity. The results are summarised in Table 4.

Table 4. *Relation between caries-activity and lactobacillus count in Caramel Group*

Caries activity	Number of patients	Occurrence of lactobacilli	
		Negative	Positive
0	13	6 (46 %)	7 (54 %)
> average	32	1 (3 %)	31 (97 %)

It is clear from the table that a correlation was found between caries activity and the occurrence of lactobacilli, such micro-organisms having been demonstrated in 97 per cent of all those with a caries activity exceeding that of the mean value found for the entire group, as against only 54 per cent of those who were caries-inactive throughout the experimental period. Statistical analysis (χ^2 test with YATES' correction) showed that the difference was statistically significant ($\chi^2 = 9.96$ and $0.01 > P > 0.001$).

Thus, in the three most active groups — Bread, 8-toffee and Caramel Groups — caries activity was related to the number of lactobacilli in the individual patients, high activity being found in association with a high number of lactobacilli, low activity being found in the patients with a small number of lactobacilli. On the other hand, no such correlation was demonstrable for patients in the Chocolate Group or in the inactive groups.

In Tables 3 and 4 the material is classed according to caries activity, in Table 5, however, according to the number of lactobacilli. This table compares the caries inactive Control and Sucrose Groups as well as the caries active Bread and 8-toffee

Groups regarding the caries activity in patients with the lowest (Class I) and highest (Class III) number of lactobacilli.

Table 5. *Caries-activity in lactobacillus classes I and III*

Group	Lactobacillus class	Number of patients	Caries activity	
			0	> average caries activity of the group
Control and Sucrose	I	40	33 (81 %)	4 (10 %)
	III	15	11 (73 %)	2 (13 %)
Bread and 8-toffee	I	36	16 (44 %)	4 (11 %)
	III	12	0 (0 %)	8 (67 %)

It is apparent from the table that:

in the inactive Control and Sucrose Groups, many of the patients with a high number of lactobacilli showed no caries activity;

in the active Bread and 8-toffee Groups, a fair percentage of the patients with the lowest number of lactobacilli showed caries activity, sometimes fairly high, while 44 per cent were caries inactive;

in the active Bread and 8-toffee Groups, all of the patients with the highest number of lactobacilli (Class III) showed caries activity, the caries activity in 67 per cent of them exceeding the mean value for the group, the corresponding figure for Class I patients being only 11 per cent.

Of the patients belonging to the caries active Bread and 8-toffee Groups, those with few lactobacilli (Class I), differed statistically from those with many (Class III) regarding caries activity (χ^2 with YATES' correction $= 11.8$ and $P < 0.001$).

Discussion

Most investigations of the correlation between the number of lactobacilli in the oral cavity and caries activity are based on comparisons between patients with rampant caries and caries-free or caries-inactive individuals (BUNTING *et al.* 1925, COLLINS *et al.* 1942, BECKS *et al.* 1944, STRÅLFORS 1947, and others). In the present investigation no such primary selection

was made, the material having been studied independently of the presence, absence or degree of caries activity.

BOYD *et al.* (1949) stressed that, if the relationship between the number of lactobacilli and caries activity is to be judged, the study must be carried out on unselected material. In a study of a small series (64 teen-age girls) unselected with regard to caries, they found no relationship between the number of lactobacilli and the degree of caries activity.

It has been shown that the number of lactobacilli in the oral cavity can be enhanced or depressed by an extreme increase or decrease in dietary carbohydrates (KOEHNE *et al.* 1934, BECKS *et al.* 1944). In the present investigation the dietary groups were not found to differ regarding the percentages of patients assigned to different classes according to the number of lactobacilli (Tables 1 and 2). If the results reported by KOEHNE *et al.* (1934), JAY (1940) and BECKS *et al.* (1944) be accepted as valid, this lack of difference between the dietary groups in the present investigation might suggest that the differences in carbohydrate level were not large enough to produce any demonstrable difference in the number of lactobacilli. *The differences in carbohydrate supply between the diets were, however, sufficient to produce a demonstrable difference in caries activity* (GUSTAFSSON *et al.* 1954). In this conjunction it might be useful to mention an observation made by BOYD, ZENTMIRE and DRAIN (1933). They cultured salivary samples from 45 children, 34 of whom had for long periods been on diets capable of arresting caries. The authors state: "Summarizing the observations pertaining to the children receiving diets capable of arresting caries: There is nothing to indicate that the prolonged use of such diets led to any characteristic change in the oral flora, to disappearance of B. acidophilus, or to a lessened acid-producing power of the flora".

As the caries-active groups did not differ from the inactive ones concerning the distribution of the patients among different lactobacillus counts, the investigation failed to provide support for the view that lactobacilli represent a primary factor in the etiology of dental caries (MCINTOSH *et al.* 1922, BUNTING *et al.* 1928) or that they are a consequence of caries (BIBBY 1935).

It should, however, be stressed that the results of the present investigation showed a correlation between the number of lactobacilli and the degree of caries activity. As shown in Tables 3 and 4, a clear correlation was found between the number of lactobacilli and the degree of caries activity in the individuals of each of the three dietary groups in which caries activity was high (8-toffee, Bread and Caramel Groups).

However, as pointed out earlier, this correlation can hardly be taken as evidence of lactobacilli being a cause or a consequence of dental caries, because the caries-active groups were found not to differ from the inactive groups regarding the distribution of the patients among lactobacillus counts. Neither can the correlation be explained by the assumption that the same factor or factors favour both the development of dental caries and growth of lactobacilli, *because patients with a large number of lactobacilli were also found in the inactive dietary groups* (Table 2). As no correlation was found in the clinically inactive Sucrose Group, in which carbohydrate consumption was highest, the correlation must apparently be associated with the carbohydrate consumption *and* the caries activity at the same time. This infers that the correlation might be explained by the assumption that lactobacilli are involved in the development of caries following the consumption of carbohydrates *in a caries-promoting manner*. It appears that the observations made in the present investigation might be summarised as follows. On introduction of a certain type of caries-promoting diet, the diet will exert a greater cariogenic effect on the teeth of individuals with an originally large number of lactobacilli than on others (cf. Table 5 and Fig. 1), *i.e.* in general, caries susceptibility varies to no small extent with the number of lactobacilli in the mouth of the individual.

The validity of this line of thought calls for further investigation. The question cannot be decided on the basis of reports of studies using an increased carbohydrate supply under well controlled conditions, because in one of the investigations on record (BOYD 1950), no increase in caries activity was noted, and in the other (KOEHNE, BUNTING & MORELL 1934) it is difficult to judge the clinical caries activity from the data given.

Judging by the analysis of the data obtained in the present investigation, the presence or absence of a correlation between caries activity and the number of lactobacilli is to no small extent dependent on the degree of caries activity (cf. Table 3 and Fig. 1). This would readily explain why no such correlation could be demonstrated in series with low activity (ANDERSSON & RETTGER 1937, BOYD *et al.* 1949).

In the Chocolate Group, in which a statistically significant increase in caries activity was noted, no correlation between caries activity and the number of lactobacilli was observed. This group thus differed from the other clinical caries-active groups. This difference is receiving attention in an investigation lying beyond the scope of the present paper.

In the evaluation of the number of lactobacilli as a measure of caries activity in a given individual, it should be mentioned that a high lactobacillus count is by no means always associated with high caries activity, just as a low count is not regularly seen in persons with low caries activity. Thus, in most investigations, the lactobacillus count was found to be low in some 20 per cent of patients with rampant caries and equally often high in caries-inactive patients. Such cases were also seen in the present material, and as some of these patients had been observed for several years, they appear worthy of mention. Thus, of the caries-inactive Control and Sucrose Groups, a large number of lactobacilli (Class III) were seen in 11 patients in whom no new cavity was noted during the last year of the study. With one exception they all had at least 33 intact dental surfaces at the beginning of the investigation. In 6 of these patients the number of lactobacilli in the mouth was regularly found to be high, and in 3 of them the lactobacillus counts made one year earlier had also been high. Examples of high caries activity in association with low lactobacillus count were also seen. In 1 patient in the caries-inactive Sucrose Group and 1 in the caries-active Bread Group, 18 new cavities appeared during the last year of the investigation, during which time the numbers of lactobacilli found on 4 different occasions were: 0, 10, 0, 0, and 0, 0, 0, 0. As such combinations are by no means uncommon, it appears that the number of lactobacilli

as a measure of caries activity in a given case is of but limited value. This does not, however, imply that lactobacillus counts are of no value in research work, because it appears that, under certain circumstances, lactobacilli play an important role in the etiology of dental caries.

Summary

The following observations were made in a study of the relationship between caries activity and the incidence of lactobacilli in patients living under well controlled dietary conditions.

1. The distribution of the patients among lactobacillus counts in the caries-inactive dietary groups did not differ from that of the active groups.
2. A distinct individual relationship was found between caries activity and the occurrence of lactobacilli in the three most active dietary groups, the activity being highest in patients with a large number of lactobacilli and lowest in those with a small number of lactobacilli.
3. The presence or absence of a correlation between caries activity and the number of lactobacilli is to no small extent dependent on the degree of caries activity.

The correlation found between dental caries and the number of lactobacilli in the oral cavity is discussed and a tentative explanation is offered.

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