

## *Experimental Gingivitis in Man*

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INCREASING evidence from different fields of dental research has indicated that oral deposits play a major role in the development and maintenance of periodontal disease.

Epidemiological data have shown that there is a close correlation between periodontal destruction and oral debris (Lövdal et al. 1958, Schei et al. 1959). Microscopy (Waerhaug 1952) and electron microscopy (Theilade 1960) have demonstrated that an intimate anatomical relationship exists between the micro-organisms of the deposit and the gingival tissues. Clinical experiments (Hine 1950) have shown that accumulation of debris leads to gingival inflammation. Other investigations have corroborated the common clinical observation that as soon as bacterial deposits are removed from the area, gingival inflammation subsides (Ramfjord and Kiester 1954, Waerhaug 1955). Biochemical and microbiological research (Schultz-Haudt 1960) has suggested that periodontal disease is the result of an interplay between bacterial activity and the host tissue, and offered some explanation of the mechanisms involved. Finally, bacteriological studies (Rosebury, MacDonald and Clark 1950, Schultz-Haudt, Bruce and Bibby 1954, and Socransky et al. 1963) have indicated that the difference between the microbial flora of the healthy gingivae as compared to inflamed gingivae is mainly quantitative, although minor differences in the relative composition of the flora have been observed.

The purpose of this investigation was to attempt to produce gingivitis in patients with healthy gingivae by withdrawing all active efforts directed towards oral cleanliness, and to study the sequence of changes in the microbial flora and in the gingivae thus produced.

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## MATERIAL AND METHODS

The subjects were nine first-year clinical students, one teacher in periodontology and two laboratory technicians, who were all healthy individuals living on an adequate diet. There were two women and ten men, and the mean age of the group was twenty-three years.

## CLINICAL EXAMINATION

*Gingivae:* At the start of the experimental period the participants were scored for their periodontal condition according to the Gingival Index (GI) system (Löe & Silness 1963):

## Criteria for the Gingival Index System

- |   |
|---|
| 0 = Absence of inflammation.  |
| 1 = Mild inflammation—slight change in color and little change in texture.                            |
| 2 = Moderate inflammation—moderate glazing, redness, oedema, and hypertrophy. Bleeding on pressure.   |
| 3 = Severe inflammation—marked redness and hypertrophy. Tendency to spontaneous bleeding. Ulceration. |

In this system, each of three gingival units (buccal, mesial, lingual) of the tooth was given a score from 0-3, called the *GI for the area*. The scores from the three areas of the tooth were added and divided by three to give the *GI for the tooth*. The scores for the individual teeth (incisors, premolars and molars) were grouped to designate the *GI for the group of teeth*. Finally, by adding the indices for the teeth and dividing by the number of teeth examined, the *GI for the patient* was obtained. The index for the subject is thus an average score for the areas examined. Each gingival area was also checked for pathological pocket formation.

Subjects with mild inflammation usually scored from 0.1 - 1.0, those with moderate inflammation from 1.1 - 2.0, and an average score between 2.1 - 3.0 signified severe inflammation. The subjects were also scored for periodontal disease by means of the Periodontal Index (PI) system (Russell 1956), based upon the clinical signs of marginal periodontitis.

*Oral hygiene:* Assessments of soft deposits were made according to the Plaque Index system (Silness & Löe 1964):

## The Plaque Index System

Scores	Criteria
0	No plaque.
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen <i>in situ</i> only after application of disclosing solution or by using the probe on the tooth surface.
2	Moderate accumulation of soft deposits within the gingival pocket, or on the tooth and gingival margin which can be seen with the naked eye.
3	Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.

Each of the following three areas of all teeth (buccal, mesial, lingual) was given a score from 0-3, the *plaque index for the area*. The scores from the three areas of the tooth were divided by three in order to give the *plaque index of the tooth*. The indices of the teeth (incisors, premolars and molars) were grouped to designate the *index for the group of teeth*. By adding the indices of the teeth and dividing by the number of teeth examined the *index for the subject* was obtained. The index for the subject is thus an average score of the number of areas examined.

No systematic registration of calculus was carried out. In the few areas where mineralized deposits were observed, the plaque formation was assessed as usual.

Prior to clinical examination the gingivae and the teeth were dried by a blast of air. No cotton was used in order not to interfere with the soft deposits.

Subsequent to the first examination the subjects were given instructions not to brush their teeth and not to use any other measure of oral hygiene. The subjects were rechecked at varying time intervals, and a full assessment of their plaque and gingival status was carried out each time using the same criteria as at the introductory examination.

TABLE 1

Mean Plaque Index at the start of the experiment, at the end of the period of no-cleansing and after re-commencement of tooth cleansing.

Patient No.	1	2	3	4	5	6	7	8	9	10	11	12	Mean
Start	0.76	0.95	0.54	0.23	0.58	0.17	0.60	0.33	0.35	0.11	0.00	0.52	0.43
End of no-cleansing period	2.00	1.99	1.82	1.46	1.60	1.42	1.64	1.40	1.58	1.79	1.64	1.81	1.67
Final	0.17	0.11	0.21	0.06	0.18	0.02	0.19	0.28	0.10	0.05	0.50	0.14	0.17

As soon as inflammatory changes were observed and a complete index and bacteriological assessment had been made, the patients were given detailed instructions in oral hygiene methods using brush and wood massage sticks. This was begun the same afternoon and continued once in the morning and once at night during the duration of the experiment. Assessment of plaque and gingival condition continued during this "hygiene" period. At a point where the GI and PI scores approached zero, the experiment was terminated. The clinical examination was carried out by one examiner.

#### BACTERIOLOGICAL EXAMINATION

The bacterial flora at the gingival margin was examined at intervals in all twelve subjects from the start of the experiment until clinical gingivitis was diagnosed. A final examination was performed when healthy gingival conditions had been re-established. The number of bacteriological examinations varied from 6 to 10 according to individual variation in the length of the experimental period.

The bacteriological data are based on microscopic examination of impression preparations and conventional bacterial smears.

The impression-technique was originally developed by Gins & Mattig (1941) and later slightly modified by Jensen (1958). A piece of thin transparent plastic film, 0.02 - 0.05 mm. thick is gently but firmly pressed against the area of marginal gingiva to be examined. Bacterial plaque, desquamated epithelial cells and a certain amount of leukocyte-containing pocket ex-

udate will adhere to the film when removed. This material is stained with gentian violet for twenty seconds and the film thoroughly rinsed in running tap water and air dried. The film is then embedded in castor oil, a coverglass is applied and the preparation sealed with paraffin. It is possible under the microscope to follow the curvature of the gingival margin and to localize the bacterial accumulations to specific points at the gingiva.

The areas selected for bacteriological examination were the buccal gingival margins of both premolars and the mesial part of the first molar in the left maxilla. The bacteriological findings in each preparation were recorded as the average of 5 separate observations. Three observations were made corresponding to the gingival margin of maxillary 4,5,6, and 2 observations corresponding to the papillary areas between maxillary 4,5 and maxillary 5,6. At each site the number of bacteria colonizing the area was registered using an 0 to ++ score. Bacterial types were registered according to morphological criteria: Cocco forms, short rods, filaments, fusobacteria, vibrios and spirochetes. The presence or absence of leukocytes and the approximate

TABLE 2

Mean Plaque Index for groups of teeth in the upper and lower jaws at the end of the no-cleansing period.

Groups of teeth	Maxilla	Mandible
Incisors	1.70	1.66
Premolars	1.65	1.70
Molars	1.73	1.62
Total	1.70	1.65

TABLE 3

Mean Plaque Index for the *different areas* of maxillary and mandibular incisors, premolars and molars at the end of the no-cleansing period.

		<i>Incisors</i>	<i>Premolars</i>	<i>Molars</i>	<i>Total</i>
Buccal areas	Maxilla	1.89	1.85	1.88	1.87
	Mandible	1.76	1.70	1.59	1.69
	Total	1.82	1.77	1.73	1.77
Inter-prox. areas	Maxilla	2.16	2.16	2.07	2.14
	Mandible	2.02	1.91	1.75	1.90
	Total	2.10	2.03	1.92	2.03
Lingual areas	Maxilla	1.07	0.96	1.23	1.07
	Mandible	1.22	1.45	1.52	1.36
	Total	1.15	1.19	1.38	1.23

size of leukocyte accumulations were noted at each observation.

Bacterial smears were prepared as follows: Samples of plaque were collected with a scaling instrument from the buccal gingival margins of the teeth previously mentioned. At each examination scrapings were collected immediately after taking the impression. Approximately the same amount of debris was collected at each sampling and suspended at once in 0.5 ml. sterile saline. The suspension was kept in ice water and ground in a tissue-homogenizer at high speed for one minute. Smears were made from this homogenized suspension, air dried and stained with Gram stain. With the aid of a microscope 200 microorganisms were counted in each smear and the percentages of different microbial types were calculated.

#### RESULTS

*Oral hygiene:* The oral hygiene status at the commencement of the experiment was good. The mean Plaque Index for individual subjects is shown in Table 1. During the period of no-cleansing all participants accumulated soft debris in large quantities, which was expressed by an increase in mean Plaque Index from 0.43 to 1.67.

Taken as a whole, no major difference in the tendency to form plaque was no-

ticed between the upper and lower jaw or between different groups of teeth (incisors, premolars, molars, Table 2). Also when mean scores for the different surfaces of the teeth were compared (Table 3) no marked variations seemed to exist in the interproximal and buccal areas of the teeth. The lingual surfaces, on the other hand, accumulated less debris. Of all areas in both jaws the lingual areas of the upper premolars showed the smallest amount of plaque.

All participants were well informed as to the technicalities of tooth cleansing and consequently the scores dropped rapidly as soon as oral hygiene measures were reinstated. The soft debris did not mature into clinically detectable calculus and could be removed by the subjects themselves. At the end of the experiment, the mean Plaque Index for the whole group was somewhat lower than that at the beginning (Table 1).

*Gingival condition:* Gingival conditions at the start of the experiment were in general very good. The Gingival Index for each of the twelve subjects is shown in Table 4. The mean Gingival Index for the group of subjects was 0.27 and the mean Periodontal Index was 0.19. Out of approximately one thousand gingival units examined one pathological pocket was found.

TABLE 4

Mean Gingival Index at the start of the experiment, at the end of the period of no-cleansing and after recommencement of tooth cleansing.

Patient No.	1	2	3	4	5	6	7	8	9	10	11	12	Mean
Start	0.49	0.41	0.15	0.02	0.24	0.17	0.43	0.15	0.08	0.69	0.38	0.07	0.27
End of no-cleansing period	1.23	1.23	0.92	0.96	0.90	1.05	1.12	0.99	0.90	1.12	0.98	1.23	1.05
Final	0.11	0.09	0.13	0.02	0.12	0.07	0.14	0.16	0.06	0.02	0.29	0.10	0.11

In the course of the "no-brushing" part of the experiment all the subjects developed gingivitis. The mean Gingival Index increased from 0.27 to 1.05. Three subjects developed gingivitis within ten days, whereas nine subjects took between fifteen and twenty-one days. No subjective symptoms related to pain or bleeding were reported by the subjects, and objectively no acute stage was observed during the development from normal to chronically inflamed gingiva.

The gingival changes were dispersed throughout the dentition. No marked differences were found between teeth of the upper and lower jaws. Nor did any one group of teeth (incisors, premolars, molars) seem to be more susceptible than others (Table 5). However, when the scores for the different gingival areas of the teeth were compared, it was seen (Table 6) that the interproximal areas tended to score higher than the buccal and that the lingual surfaces had a markedly lower index.

When all surfaces of the different groups of teeth were considered, it appeared that the interdental areas of upper molar teeth were most affected and that the lingual

area of the lower premolars regularly showed the best gingival condition.

After recommencement of tooth cleansing, gingival inflammation resolved in about a week, during which the mean GI for the whole group dropped from 1.05 to 0.11 (Table 4). The corresponding figures for the Periodontal Index were 0.93 and 0.01.

#### BACTERIOLOGY

The first preparations obtained from areas of clinically healthy gingivae gave evidence of an extremely sparse bacterial flora at the gingival margin. The main features were the presence of a few desquamated epithelial cells, some widely scattered leukocytes and small groups of bacteria—mainly cocci and short rods. When tooth cleansing was stopped the bacterial flora increased enormously, and the increase followed a uniform pattern in all except one of the twelve persons examined.

It was possible to recognize three distinct phases in this new bacterial colonization of the gingival margin. The first phase starting immediately after the beginning of the experiment was characterized by a drastic increase of the coccal flora. Masses of desquamated epithelial cells completely overgrown with cocci, and large "mats" of cocci, probably loosened from the underlying epithelial tissue were dominant in these preparations (Fig. 1A). At the same time small accumulations of leukocytes were observed along the gingival margin.

The second phase of bacterial proliferation usually started two to four days after

TABLE 5

Mean Gingival Index for groups of teeth in the upper and lower jaws at the end of the no-cleansing period.

Groups of teeth	Maxilla	Mandible
Incisors	0.99	1.08
Premolars	1.01	1.01
Molars	1.10	1.13
Total	1.03	1.07

tooth cleansing had been abolished. It was characterized by the preponderance of filamentous forms and slender rods, although cocci were still present in fairly large numbers (Figs. 1B and 1C). According to morphological criteria the filamentous bacteria were predominantly leptotrichia and fusobacteria were found in varying numbers. Leukocyte accumulations increased in size. In one of the twelve subjects examined this type of microflora was present from the start of the experiment.

While the transition from the first to the second phase of bacterial colonization was rather easily observed, the transition from second to third phase was more gradual and somewhat difficult to time. The bacterial flora in the last stage was characterized by the presence of vibrios and spirochetes (Figs. 1D and 1E). At first these organisms were often seen strictly localized to a single papilla or to the gingival margin of a single tooth, but later they spread out to cover the total area examined. Cocci, rods and filamentous organisms were still numerous. In two cases spirochetes were not found by microscopic examination of impression preparations or smears, but large numbers of vibrios were present. On an average the transition from second to third phase took place six to ten days after tooth cleansing had ceased. Leu-

kocyte accumulations were usually very heavy during this phase which persisted for varying intervals of time until clinical gingivitis was diagnosed.

The final bacteriological examination was made when regular habits of oral hygiene had been resumed and healthy gingival conditions re-established. In ten of the twelve subjects the gingival flora at this time consisted predominantly of cocci and short rods. In two cases filamentous bacteria were found in rather large numbers, but in no case were vibrios or spirochetes observed.

Microscopic examination of the smears indicated corresponding changes in the relative composition of the bacterial flora during the experimental period. Smears taken at the start of the experiment showed that the bacterial population in the very small amounts of plaque which could be collected was dominated by gram-positive cocci and short rods. These organisms accounted for 90 - 100 per cent of the total organisms counted in nine out of twelve subjects. In two subjects the gram-positive cocci and short rods made up 80 per cent, and in one subject only 50 per cent of the bacteria counted.

By the time clinical gingivitis had developed this composition of the gingival flora had altered radically. In all subjects

TABLE 6  
Mean Gingival Index for the *different areas* of maxillary and mandibular incisors, premolars and molars at the end of the no-cleansing period.

		Incisors	Premolars	Molars	Total
Buccal areas	Maxilla	1.16	1.22	1.31	1.23
	Mandible	1.34	0.93	1.05	1.13
	Total	1.25	1.07	1.15	1.17
Inter-prox. areas	Maxilla	1.33	1.51	1.54	1.44
	Mandible	1.38	1.11	1.05	1.20
	Total	1.37	1.31	1.30	1.33
Lingual areas	Maxilla	0.53	0.30	0.46	0.46
	Mandible	0.57	1.00	1.25	0.89
	Total	0.56	0.66	0.86	0.67

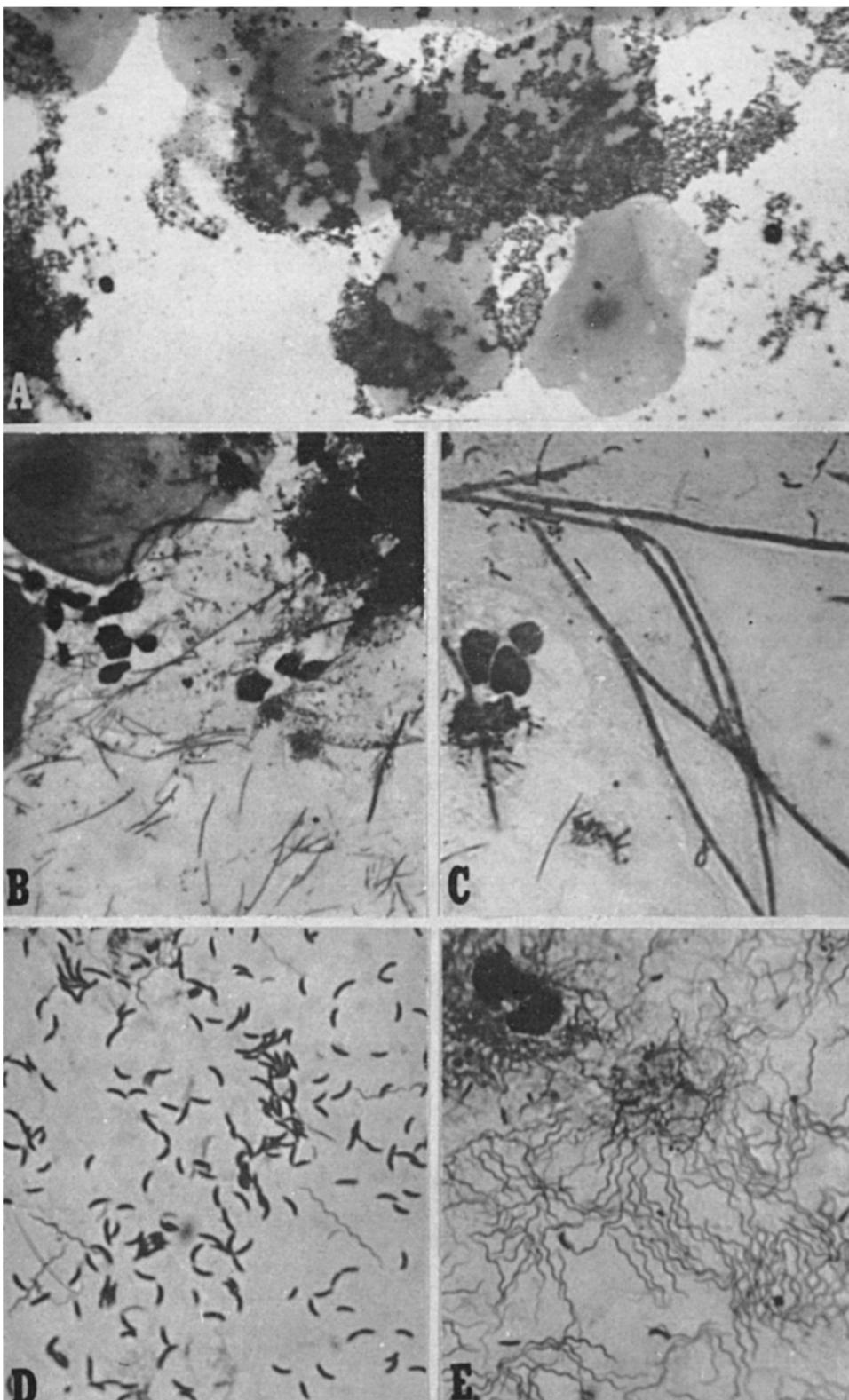


Fig. 1. Microphotographs of impression preparations from the gingival margin during the period of no hygiene. Gentian violet. A: Predominantly cocciform microflora and desquamated epithelial cells in early phase of no hygiene. (x 460). B: Filamentous organisms and leukocyte accumulations seven days after withdrawal of toothbrushing. (x 730). C: Higher magnification of filaments and fusobacteria from preparation shown in B. (x 1150). D: Concentration of vibrios. Same preparation as E. (x 1150). E: Spirochetes and vibrios predominate after two weeks of no hygiene and three days before clinical gingivitis could be diagnosed (x 1150).

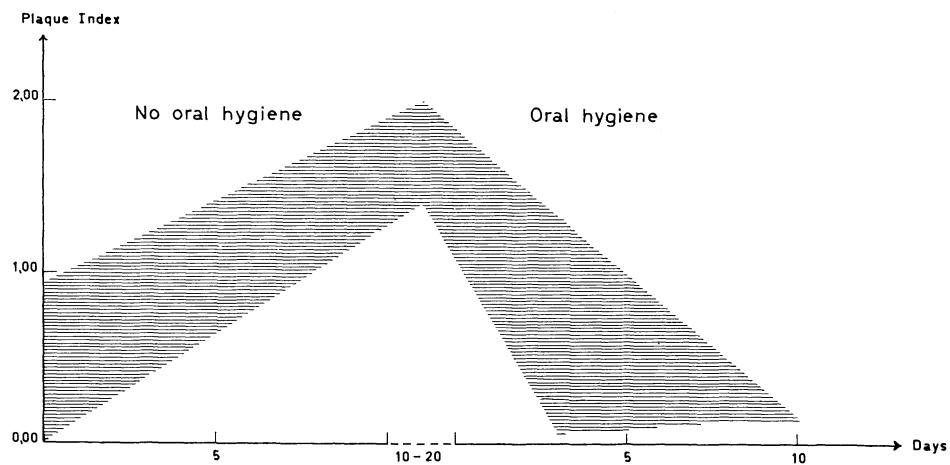


Fig. 2. Trends in the accumulation of soft debris during the periods of no oral hygiene and oral hygiene.

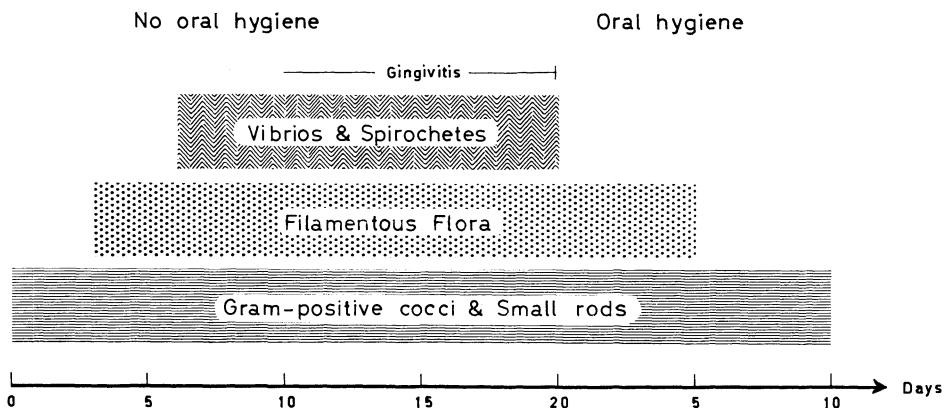


Fig. 3. Trends in the changes in the microflora of the gingival margin during the periods of no oral hygiene and oral hygiene.

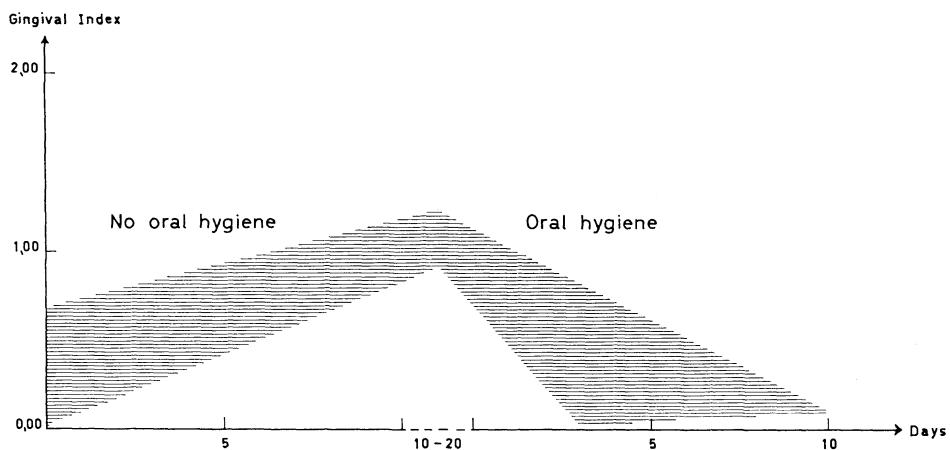


Fig. 4. Trends in the gingival changes during the periods of no oral hygiene and oral hygiene.

gram-positive cocci and short rods now only accounted for 45 - 60 per cent of the microorganisms in the plaque along the buccal gingiva. The distribution of other bacteria constituting the remaining 40 - 55 per cent of the flora, was as follows:

- Gram-negative cocci and short rods:  
22% (range 11 - 31%)
- Gram-positive filaments: 10% (range 5 - 16%)
- Fusobacteria: 10% (range 4 - 15%)
- Vibrios: 6% (range 1 - 12%)
- Spirochetes: 1% (range 0 - 2%)

It is interesting to note that the originally predominating flora of gram-positive cocci and short rods was reduced to 50 - 70 per cent of the total flora during the first four - seven days of plaque formation and remained fairly constant at 45 - 60 per cent throughout the rest of the experimental period. Gram-positive filaments, fusobacteria, and gram-negative cocci and small rods were conspicuous in the smears after two - four days, while vibrios and spirochetes generally were found a few days later.

#### DISCUSSION

An evaluation of the results of the present study must take into account that although the twelve subjects who participated in the experiment made a rather homogenous group the gingival condition at the start of the experiment varied to some degree. Moreover, the study was hampered by the fact that the clinical and bacteriological examinations were not always made on the same day and that the length of the interval between examinations varied. Therefore, no statistical analyses have been made, and the tables and diagrams accordingly are intended more to show trends than specific data.

In spite of these obvious shortcomings the present investigation has shown that the abolishing of tooth cleansing procedures results in a rapid increase of oral debris. Nearly all areas of all teeth investigated

showed plaque formation shortly after the toothbrushing had been withdrawn, and the accumulation increased steadily during the experimental period (Fig. 2). With reference to the fact that all participants lived on a standard Scandinavian diet which includes coarse bread and ample amounts of fresh fruit, this observation would appear to indicate that the concept of self cleansing with this type of diet is highly questionable.

The lingual surfaces of maxillary premolars were the only gingival areas that occasionally were free of plaque formation. This finding and the observation that lingual surfaces of all maxillary teeth scored lower for soft deposits than other areas can most likely be explained by the cleansing effect of tongue movements.

The bacteriological examinations have clearly shown that essential changes occur in the bacterial flora of the gingival margin during the period of plaque development. The number of microorganisms colonizing a clean and healthy gingiva is low and the flora consists almost entirely of gram-positive cocci and short rods. During plaque formation a general increase in the number of microorganisms takes place, and in the course of a few days a definite change in the composition of the flora occurs. From a predominance of coccal forms the microflora changes to a more complex population in which first filamentous bacteria, and later vibrios, spirochetes and gram-negative cocci are prominent (Fig. 3). A similar sequence in bacterial colonization has been repeatedly observed in studies of early calculus formation (Mandel, Levy & Wassermann 1957, Mühlemann & Schneider 1959, Turesky, Renstrup & Glickman 1961). This shift cannot be entirely explained by the increase in the amount of plaque. It is more reasonable to assume that the increasing age of the plaque causes alterations in the local environment which favors the growth of certain bacterial types.

The present study has further demonstrated that gingivitis is produced simply

by withdrawing all measures of oral hygiene (Fig. 4). Thus, to some extent, this investigation has confirmed the observations made by Hine (1950). However, whereas the majority of his cases did not show differences in the gingival condition, all participants in the current study developed clinically observable gingival inflammation. The time required to develop clinical gingivitis varied considerably. Some had gingivitis after ten days, but the majority of the subjects required from fifteen to twenty-one days. It is likely, therefore, that those who did not show gingival changes at the termination of the two weeks' experiment (Hine 1950) would have developed gingivitis if the experimental period had been extended.

Judged by clinical inspection of the gingivae and by microscopic count of leukocytes in the preparations the severity of the gingival changes increased steadily throughout the no-brushing period. A definite acute clinical stage did not seem to occur in the progression from a healthy to a chronically inflamed gingiva.

There is no doubt that the bacterial plaque is essential in the production of gingival inflammation. However, it is still an open question, which factors within the plaque are directly responsible. The present investigation has pointed out that significant changes in the microflora invariably took place during the aging of the plaque. The fact that normal gingiva did not harbor these bacteria and that the change in the microflora occurred before gingivitis was clinically diagnosed, may indicate that these microorganisms play a role in the initiation of periodontal inflammation. The observation that the time necessary to develop clinical gingivitis varied between individuals could then be a reflection of individual defense mechanism variability.

Finally, this experiment has corroborated the well known clinical experience that removal of bacterial plaque causes resolution of gingival inflammation. Within a few days after oral hygiene procedures were re-

instituted, all participants demonstrated gingivae which clinically appeared more healthy than at the beginning of the experiment.

With reference to the suggested variations in response to gingival irritation it is worthwhile to note that irrespective of any individual difference in resistance the removal of plaque resulted in clinically normal gingivae.

#### SUMMARY

Withdrawal of all measures of oral hygiene in twelve healthy persons with clinically normal gingivae resulted in gross accumulations of soft debris and the development of marginal gingivitis in all subjects. The time necessary to develop gingivitis varied from ten to twenty-one days. Concurrent bacteriological examinations showed that the number of microorganisms in the gingival area increased and that distinct changes in the relative composition of the flora occurred. Reinstatement of oral hygiene resulted in healthy gingival conditions and re-establishment of the original bacterial flora.

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