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REMOVAL OF PARTICLES AND BACTERIA FROM GINGIVAL POCKETS BY TISSUE FLUID

by

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Wærhaug (1952) has shown that when India ink is deposited in gingival pockets, an exudate with a pronounced content of polymorphonuclear leukocytes is found in the pockets twenty-four hours later, and the India ink has disappeared. Similarly *Wærhaug & Steen* (1952) presented evidence indicating that pure cultures of pathogenic bacteria introduced into bacteria-free gingival pockets produce necrosis of the epithelial cuff and inflammation in the subjacent connective tissue. In this case also, an inflammatory exudate rich in polymorphonuclear leukocytes is formed. The exudate pervades the epithelium. The net result of the inflammatory reaction is a reversion after forty-eight hours to the previous sterile condition of the pockets. The authors suggest that the secretion, which they observed coming from the pockets during the first hours, either kills the bacteria on the spot or removes them.

Brill & Krasse (1958) and *Brill & Björn* (1959) have shown that ordinarily a stream of tissue fluid passes from subepithelial spaces through the epithelial cuff into gingival pockets and thence into the oral cavity. They suggest that this stream of fluid is

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able to eliminate bacteria and particulate matter from gingival pockets. From this, one may deduce that an inflammatory exudate is not necessarily required to achieve this effect. Their suggestion, however, has not been substantiated experimentally, but evidence might accrue from studies of particles injected into gingival pockets and observed to leave these sites shortly afterwards.

Studying the circulation of foreign particles and bacteria in the mouth *Bloomfield* (1921, 1922) was able to show that currents of saliva washed away charcoal particles and bacteria from the site of introduction, e.g. the tip of the tongue. The material introduced was efficiently swept towards the base of the tongue. It was therefore decided that these two kinds of material would also be suitable in studying a similar effect of the tissue fluid passing through gingival pockets.

Although *Wærhaug* (1952) used India ink, charcoal particles are preferred in the present study, mainly because particles of the former substance are submicroscopic (*Venuto*, 1953), whereas a preparation of charcoal contains particles, which can be measured in an ordinary light microscope. If it could be shown that charcoal particles of a known size and introduced into gingival pockets were removed by the stream of fluid, it might be inferred that other particles of similar and smaller size suspended in the fluid of gingival pockets were also removed. Such other particles and bacteria might incidentally gain entrance to gingival pockets during chewing or dental treatment etc. The present study was undertaken to gain information about the flushing action on non-vital particles and living bacteria by tissue fluid, when the fluid passes through gingival pockets on its way into the oral cavity.

MATERIAL AND METHODS

The charcoal preparation used was made by May and Baker Ltd. and labelled Charcoal Decolourising. Two gr of the preparation were allowed to settle for fifteen minutes in 50 ml of a physiological salt solution. The supernatant still containing some small but visible particles was discarded. From the remaining suspension, containing larger particles a sample was taken and

spread on a glass slide for microscopic examination. Particles discernible in the light microscope, ranged in size from 0.5 μ to 50 μ. Most of these particles were between 2 μ and 10 μ and looking more or less like needles with irregular contours.

Also from the same charcoal suspension containing the larger particles, a syringe was filled. The syringe was provided with a blunted hypodermic needle. By means of the syringe some of the suspension was deposited in gingival pockets of two dogs, approximately ten months old. Their gingival health was good. Thirty-four pockets of first molars, premolars, canines and third incisors in the upper and lower jaws were studied. During the experiments the dogs were anæsthetized by intravenously injected "Pentothal" ® (Thiopentone Sodium, B.P. or Thiopental Sodium, U.S.P.). The narcosis was reinforced every half hour by an additional injection. The experiments lasted about two and a half hours on each occasion.

Injection of the charcoal suspension was performed in such a manner that interference with the epithelial cuff was reduced to a minimum. During insertion of the needle, the point of the needle rested against enamel and pointed towards the cemento-enamel junction. From a position almost parallel to the buccal surface of the tooth and at an acute angle to the gingival margin, the needle was gently drawn along an oblique path towards the bottom of the pocket. When the needle came to rest 1—2 mm from the gingival margin, the charcoal suspension was gently ejected, and the syringe immediately withdrawn. The pockets were always filled to overflowing. This procedure lasted two to three seconds per pocket, and no visible bleedings occurred.

When the pockets in one quadrant were filled, the overflow of charcoal was washed away with surgical sponges thoroughly soaked in tap water. Particles still remaining on the teeth and the marginal gingivae including the gingival crevices were blotted off by means of strips of filter paper. Usually three to five strips were used in each instance before a strip could be removed without particles of charcoal clinging to it. This last strip was used as a control.

Experimental strips, also made of filter paper were then placed on the gingivae and the teeth, bridging the entrance to the gin-

gival pockets. (Fig. 1). They were thus in a position to take up fluid oozing out of the pockets. The absorbed fluid made the filter paper transparent. Owing to this transperancy it was in all cases possible to observe through the paper, when a certain amount of charcoal particles had settled on the side of the strips facing the teeth. Soon after the formation of visible black spots on the strips, the latter were removed and examined microscopically. The time interval between application and removal of each of the thirty-four experimental strips varied between six and twenty minutes.

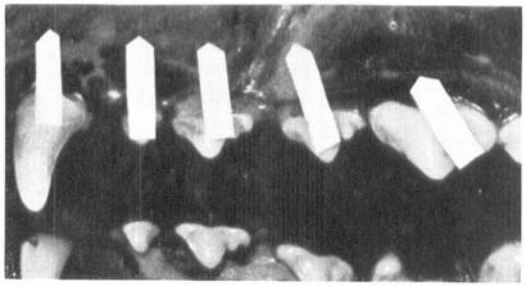

Fig. 1. Strips of filter paper bridging the entrances to gingival pockets.

One of the dogs was used in still another experiment, in which the removal of bacteria was studied. Essentially, this experiment is like the former. The experiment with bacteria was performed on two separate occasions seven days apart.

The actual experiments were preceded by the intravenous injection of "Pentothal", and the anæsthesia was reinforced as previously. The gingivæ were carefully dried with cotton sponges, before and after bacteria were deposited in the gingival pockets.

The method applied in depositing the bacteria was similar to the one described above. The pockets were filled to overflowing, and the surplus was blotted off by means of cotton sponges. No disinfection of the pockets or their adjacent structures was performed, neither before injection of the bacteria nor at later stages of the experiment.

The bacterial suspension used, was prepared from the species *Serratia marcescens*. Ten ml of an eighteen hour old broth cul-

ture was centrifugated. After centrifugation, the packed cells were resuspended in five ml of physiological saline. In each pocket of first molars, premolars, canines and a third incisor in the upper jaw 0.5 ml of this suspension was ejected. Two pockets were used twice. Before injection of bacteria five pockets were stimulated by toothbrushing twenty-five times applying the modified method described earlier (*Brill & Krasse*, 1959).

Seven samples were taken from each pocket. Number one was taken immediately before and number two immediately after the injection of bacteria. The remaining five samples were taken at intervals of increasing length: Three, six, twelve, twenty-four and forty-eight minutes after injection.

The samples were taken by means of filter paper fabricated in squares of 2 by 2 mm. A piece bridging the entrance to the pocket was placed on the gingiva and the tooth of each pocket. Samples number one and two of each pocket remained *in situ* for one to three minutes, the minimal period required for a sufficient amount of fluid to be absorbed. In the time intervals between collection of the remaining five samples, so much fluid had accumulated outside the pockets that the paper squares were instantly filled.

The samples with tissue fluid were transferred to 10 ml physiological saline in glass tubes containing a few glass pearls. One tube was used for each sample, and after prolonged shaking, 0.1 ml of its contents was spread on endo-agar in a Petri dish. The dishes were incubated for eighteen hours, whereafter the colonies of *Serratia* of each dish were counted.

RESULTS

Typical results from the charcoal experiments are reproduced in Fig. 2. It presents from left to right the samples from the third incisor, canine, second, third and fourth premolars and the first molar in the left side of the lower jaw of one of the dogs. The strips in the upper row are the controls, taken immediately after the charcoal had been introduced, the overflow washed away and blotted off. The lower row shows strips removed eight to twelve minutes after removal of the controls. These latter strips carry black spots, which on microscopic examination were found to

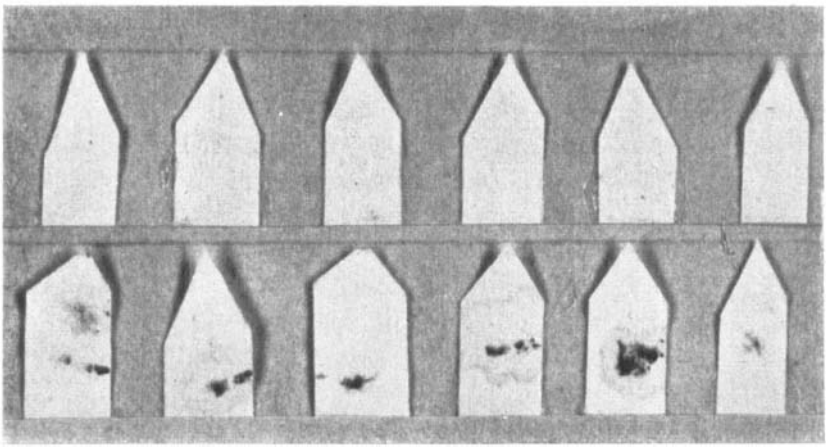

Fig. 2. In the lower row strips with samples of charcoal particles recovered from gingival pockets. The strips in the upper row are controls.

consist of charcoal particles. Similar results were obtained from the remaining twenty-eight pockets.

It was found that in the samples from pockets yielding small amounts of charcoal, the shape, size and distribution of particles were similar to the shape, size and distribution of particles in the freshly prepared suspension; i.e. the particles varied in size from 0.5 μ to 50 μ, and most particles were between 2 μ and 10 μ. On the strips from pockets yielding great amounts of charcoal, the particles were mostly heaped together in great masses of a highly irregular outline. The biggest conglomerate had a diameter of about 200 μ. Usually, however, the masses were between 30 μ and 100 μ. A reduced number of separate particles were scattered in between the conglomerates. It was a general observation that these latter particles seldom were of the smallest sizes. Usually they were from 5 μ and upwards.

Results from the bacterial experiments are entered in Table I, which shows that bacterial colonies had grown in varying numbers. Apart from the controls, those samples which were removed initially from unstimulated pockets yielded growth of many colonies, whereas those removed at the later periods, mainly showed growth of colonies in decreasing numbers. The same tendency is found in the samples from stimulated pockets.

Table 1. Numbers of colonies of *Serratia marcescens* grown from samples taken at varying intervals after the introduction of the *Serratiae* into gingival pockets.

	Pockets without stimulation*				Pockets with stimulation*					
	M_1	P_2	I_3	C	C	C	P_2	P_2	P_3	M_1
Before bacterial injection	0	0	0	0	0	0	1**	0	0	0
Immediately after bacter. inj.	540	42	16	260	288	34	1	144	145	34
3 minutes after bacter. inj.	138	9	3	25	74	14	16	19	25	5
6 minutes after bacter. inj.	4	1	3	5	14	7	7	6	4	7
12 minutes after bacter. inj.	1	1	1	0	0	8	10	4	11	1
24 minutes after bacter. inj.	0	0	1	0	0	2	6	0	3	8
48 minutes after bacter. inj.	0	0	2	0	1	0	0	0	0	0

* Stimulation = toothbrushing

** Contamination

DISCUSSION

The two experimental series in this study disclose that, when non-vital particles varying in size from 0.5 μ to 50 μ are deposited in gingival pockets, or when living bacteria are introduced, the flow of tissue fluid coming from such pockets will soon remove at least some of the material introduced.

Apparently these results confirm the suggestion made by *Brill & Krasse* (1958) and *Brill & Björn* (1959) that, on its way into the oral cavity the flow of tissue fluid, which ordinarily passes from subepithelial compartments through the epithelial cuff, will wash away particulate matter, having incidentally gained entrance to gingival pockets.

Although the experimental procedure was designed to inflict the least possible irritation upon the structures concerned, the possibility cannot be disregarded that the procedure may have

resulted in a stimulation of the epithelial cuff and subjacent structures. This stimulation may have caused an increase in the flow of fluid from subepithelial spaces. Such a view then would entail the conclusion that the increased flow of tissue fluid, which can be produced by chewing (*Brill,* 1959 b) or toothbrushing (*Brill & Krasse,* 1959) is able to remove particulate matter from gingival pockets. These latter kinds of stimuli are definitely more powerful in provoking an increased flow of tissue fluid than the present experimental method, which was cautiously applied.

On the assumption that the numbers of bacterial colonies counted in the Petri dishes, reflect the concentration of bacteria in the gingival pocket fluid, the reduction of the numbers reflects an elimination of bacteria from gingival pockets. It is believed that this elimination, like the removal of charcoal particles, is caused mainly by the flushing action of the tissue fluid. The results from the charcoal experiment lead to the conclusion that the impressive results, which are achieved within three minutes in the bacteria experiment cannot be ascribed exclusively to a bactericidal or bacteriostatic action by the tissue fluid. This does not mean, however, that such action does not take place. On the contrary, an antimicrobial effect should be expected, especially when bacteria are exposed for longer periods of time, as suggested by *Wærhaug & Steen* (1952). Their suggestion is supported by the observation that the fluid coming from gingival pockets is of serumal origin (*Brill,* 1959 a), and therefore may contain antibodies.

SUMMARY

Suspended charcoal particles were introduced into thirty-four clinically healthy gingival pockets in two young dogs. Six to twenty minutes later some of the particles had settled on strips of filter paper bridging the entrances to the pockets. Similarly, bacteria ejected in ten gingival pockets were afterwards deposited on pieces of filter paper. It is concluded that the flow of tissue fluid from subepithelial structures of marginal gingivæ is able to remove particulate matter, including bacteria, from gingival pockets.

RÉSUMÉ

ELIMINATION DE PARTICULES PAR UN ECOULEMENT DE FLUIDES TISSULAIRES DANS LES CULS-DE-SAC GINGIVAUX

Des particules de charbon de bois en suspension ont été placées dans 34 culs-de-sac gingivaux cliniquement sains de 2 jeunes chiens.

Au bout de 6 à 20 minutes, quelques unes des particules s'étaient déposées sur des bandes de papier filtre formant pont sur l'entrée des culs-de-sac. De même, des bactéries introduites dans 10 culs-de-sac gingivaux ont été déposées peu à peu sur des morceaux de papier filtre. On en conclut qu'un écoulement de fluides tissulaire venant des régions sous-épithéliales de la gencive marginale, peut éliminer des culs-de-sac gingivaux les particules diverses, entre autres les bactéries.

ZUSAMMENFASSUNG

ENTFERNUNG VON STOFFEN UND BAKTERIEN AUS DER GINGIVALEN TASCHE DURCH GEWEBSFLÜSSIGKEIT

Holzkohleteilchen in Suspension wurden in 34 klinisch gesunde Zahnfleischtaschen zweier junger Hunde hineingeführt. Sechs bis zwanzig Minuten später hatten sich einige Teilchen an Filterpapierstreifen, die den Eingang zur Tasche sperrten, niedergeschlagen. Auf ähnliche Weise hatten sich Bakterien, die in zehn Zahnfleischtaschen gespritzt waren, auf Filterpapierstückchen abgelagert. Hieraus wird geschlossen, dass ein Strom von Gewebsflüssigkeit aus der subepithelialen Struktur der marginalen Gingiva dazu fähig ist, gewisse Stoffe einschliesslich Bakterien aus der Zahnfleischtasche zu entfernen.

REFERENCES

Bloomfield, A. L.: The Circulation of Foreign Particles in the Mouth. Amer. Rev. Tuberc. *5:* 903—914, 1921/1922.

—»— The Relation of Bacteria to the Mucous Membrane. Bull. Johns Hopkins Hosp. *33:* 252—256, 1922.

Brill, N.: Influence of Capillary Permeability on Flow of Tissue Fluid into Gingival Pockets. Acta odont. scandinav. *17:* 23—33, 1959a.

—»— Effect of Chewing on Flow of Tissue Fluid into Gingival Pockets. Acta odont. scandinav. *17:* 277—284, 1959b.

Brill, N. & B. Krasse: The Passage of Tissue Fluid into the Clinically Healthy Gingival Pocket. Acta odont. scandinav. *16:* 233—245, 1958.

Brill, N. & H. Björn: Passage of Tissue Fluid into Human Gingival Pockets. Acta odont. scandinav. *17:* 11—21, 1959.

Brill, N. & B. Krasse: Effect of Mechanical Stimulation on Flow of Tissue Fluid Through Gingival Pocket Epithelium. Acta odont. scandinav. *17:* 115—130, 1959.

Venuto, L. J.: Carbon Blacks. New York, Binney & Smith Co. 1953. p. 3.

Wærhaug, J.: The Gingival Pocket. Thesis. Odont. Tidskr. *60:* suppl. 1., 1952.

Wærhaug, J. & E. Steen: The Presence or Absence of Bacteria in Gingival Pockets and the Reaction in Healthy Pockets to Certain Pure Cultures. Odont. Tidskr. *60:* 1—24, 1952.

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