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THE BACTERIOLOGY OF PHYSIOLOGICAL GINGIVAL POCKETS

by

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The oral cavity contains a varied and numerous microbial flora. The numbers of microbes in saliva has been estimated at 43—5500 mill./ml (1). In deposits along the marginal gingiva and in plaques the concentration presumably is still higher (2).

Clinical experience shows that marginal periodontal disease is closely related to accumulation of deposits along the gingival margin. The role played by microorganisms in periodontal disease is, however, not settled (3—5), but it has been shown (6) that some oral bacteria contain enzymes which degrade the gingival epithelium and connective tissue (6, 7).

The presence or not of microbes in the so-called physiological gingival pocket and the role played by these microbes has been much discussed. While some investigators (8, 9, 10) find that physiological pockets contain ordinary oral microbes, others (11, 12) claim that as a rule they are sterile.

The present paper is concerned with a new technique which makes it possible to investigate the bacteriology of pockets without contamination from the surface of the tooth or gingiva.

I. MATERIAL AND METHODS

The experiments were performed on 17—20 year-old pupils of the School for Dental Nurses at the Odontological Institute. All had clinically healthy gingiva, pockets of 1—2 mm depth, and no visible deposits on their teeth.

The collection of material was performed with a 0.2 mm thick bacteriological loop, diameter 2 mm and length of the handle 130 mm. The material was cultivated at 37° C in 5 ml of sulphite medium (13) in 12×80 mm tubes. To permit both aerobic and anaerobic growth the loop was moved to the bottom of the tubes several times during the inoculation. Growth was recorded as positive or negative after incubation for 5 days.

Before sampling, the free gingiva and the tooth surface were disinfected with an iodine varnish as follows:

Colophonium	25.4 %	97.5 g
Copal from Manilla	11.3 %	
Etanol 96 %	63.3 %	
Iodine crystals		2.5 g

When this iodine varnish is applied on the gingiva and the tooth surface, a membrane forms within 3 min. of drying in the air. It is very adhesive and can only be removed by scratching or by washing with alcohol. The varnish adheres to the gingiva and the tooth surface even if they are not completely dry, because small amounts of moisture are easily taken up by the varnish.

The experiments were performed in the following manner: The lips and cheeks were lifted aside by cheekhooks, and the area to be investigated was dried in the air. With sterile cotton wool pellets the iodine varnish was applied to the gingiva and the crown of the tooth. Within 3 minutes of drying in the air the membrane formed. The loop, moistened in hydrosulphite medium, was now pushed through the membrane to the bottom of the pocket and moved along the labial surface of the tooth. The material was at once incubated at 37° C and readings were taken after 1, 2, 3, 4, and 5 days.

Results

Material from 60 clinically healthy pockets in 20 individuals was examined. 57 of the pockets showed growth after 24 hours. 2 of 3 pockets, which were negative in the first experiment, gave growth by new sampling. On the third "negative" pocket no new experiments were performed.

II. CONTROL OF THE IODINE VARNISH METHOD

After application of the iodine varnish, the situation is like that shown in Fig. 1.

To make the method reliable the following requirements must be fulfilled: –

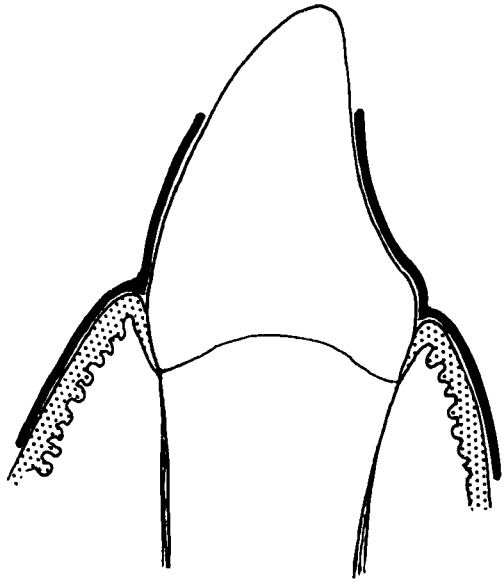


Fig. 1.

(1) The surface of the membrane must not show any growth of microbes.

(2) The surface of the tooth or the gingiva must be effectively disinfected by the membrane.

(3) The disinfectant must not penetrate into the physiological pocket.

The following control experiments were performed:

The iodine varnish was applied to the free gingiva and the tooth surface around 105 physiological gingival pockets of 20 nurses. 105 samples were taken from the surface of the membrane and cultivated in hydrosulphite medium.

On 5 other persons the iodine varnish was applied to the

moistened mucosa on the inside of the cheeks. After 3 minutes of drying in the air 10 samples were taken as described above.

In order to examine the disinfecting ability of the membrane the gingiva and the tooth crowns in the upper jaw of 5 persons were covered with iodine varnish. After 3 minutes of air drying the membrane was peeled off and 30 samples were taken from the gingiva and 30 from the tooth surfaces. On the same individuals the iodine varnish was applied to the moistened mucosa on both sides of the cheeks. After removal of the membranes 10 samples were taken from the mucosa.

Table 1

Bacterial examination after disinfection with iodine varnish.

	No. of samples	Growth	No growth
Oral mucosa	10	0	10
Gingiva	30	0	30
Tooth surface	30	0	30

The results of these experiments are shown in Table 1.

Further experiments were performed to test the ability of the iodine varnish to disinfect the contact surface between the membrane and the underlying tissue.

A suspension of Serratia marcescens, concentration 1,000 mill./ml, was applied with sterile cotton wool pellets on the mucosa of the cheeks of 5 persons. Immediately afterwards iodine varnish was applied and dried in the air for 3 minutes. After removal of the membranes 10 samples were taken from the mucosa.

Table 2

Disinfection with iodine varnish.

Test microbe: Serratia marcescens.

	No. of samples	Growth of S. m.	No growth of S. m.
Oral mucosa	10	0	10
Pockets	30	0	30

a b

Fig. 2.

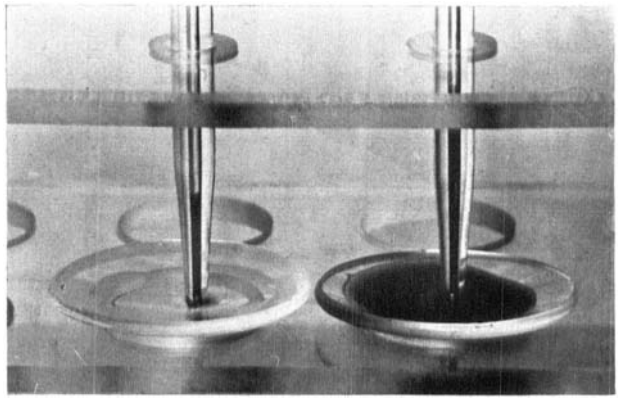
On 5 other persons the Serratia suspension was applied to the gingiva and the teeth in the upper jaw. Immediately afterwards iodine varnish was applied. After drying, the loop moistened in hydrosulphite medium was pushed through the membrane and moved along the inside of the pocket. 30 samples were taken from the pockets. The results are shown in Table 2.

It is of great importance that the disinfectant is fixed to those areas which we want to disinfect and not allowed to spread.

The iodine varnish hardens rapidly in a moist environment and prevents the iodine from leaking out. This is shown in Figs. 2a, 3a, 4a.

An ordinary agar plate was inoculated with Serratia marcescens. Circular areas were punched out and the holes filled with iodine varnish. No inhibition of the bacterial growth was observed after 24 hours (Fig. 2a).

The ability of iodine varnish not to leak out was investigated with capillary tubes filled with iodine varnish. When such tubes were dipped into 1 % amylum solubile a clot was formed in the tubes on the surface between the iodine varnish and the starch



a b

Fig. 3.

solution, preventing the iodine from leaking out. Even after days no blue colouring of the starch was observed. (Fig. 3a).

The ability of iodine varnish not to penetrate into fluid-filled capillary spaces was shown by means of slides:

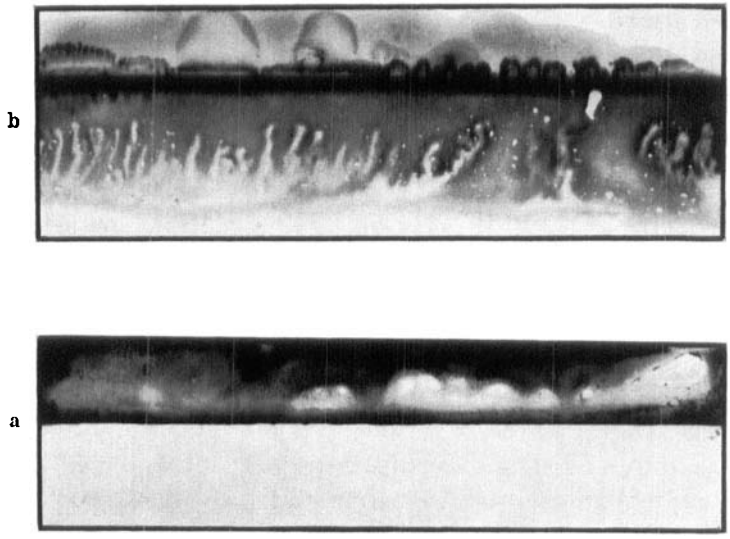


Fig. 4.

2 slides were put together so that their contact surface corresponded to about $^2/_3$ of the height of the slides. The capillary space was filled with 1 % amylum solubile. If now a few drops of iodine varnish were dripped along the edge of the top slide a dark membrane formed after 2 –3 minutes along the opening of the split, while no reaction between the iodine and the starch was seen between the slides even after several days (Fig. 4a).

The following experiment was performed *in vivo* to demonstrate that the iodine in the varnish does not diffuse:

A 1,000 mill./ml suspension of S. marcescens was placed at the bottom of 10 physiological pockets of 5 persons. With the same technique as before 50 samples were taken and cultivated on hydrosulphite medium at room temperature (20° C).

Results of the controls

(1) None of the 115 samples from the surface of the membrane showed bacterial growth.

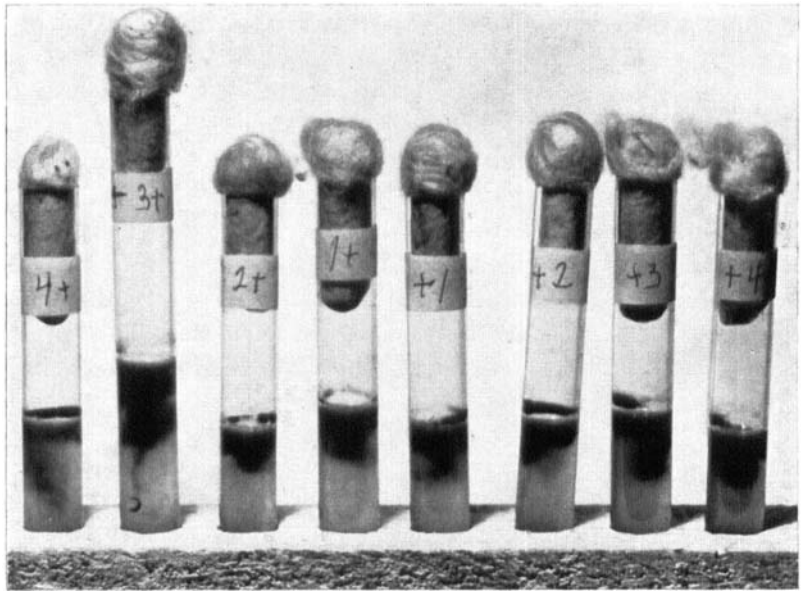


Fig. 5.

(2) Growth was neither shown of oral nor of test microbes from the surfaces of the teeth or the gingiva after disinfection with iodine varnish.

(3) Diffusion tests with agar plates, capillary tubes and capillary spaces showed that the iodine in the varnish did not diffuse.

50 tests *in vivo* all showed good growth of the test microbe, indicating that the iodine in the varnish did not diffuse into physiological pockets (Fig. 5).

III. CONTROL OF THE IODINE GLYCERINE METHOD

In the literature on the bacteriology of physiological gingival pockets only *Wærhaug & Steen* (11) maintain that the pockets are sterile. Their technique was therefore examined, especially with regard to the requirements necessary for a disinfectant to be used in such experiments.

The iodine glycerine does not harden and it is not possible to remove it by air-blowing. Even after heavy blowing *without* iodine glycerine in the balloon, an easily discernible strip of glistening iodine glycerine is left along the edge of the gingiva. Some of this material might be carried into the pockets by the instrument used for sampling, some might be blown into them while trying to dry up the area. Finally, the iodine glycerine from the rubber balloon used in the experiments, might be blown into the pockets. The balloon is filled with warm air drawn in through a flame. When blown out again this air will contain iodine vapour and small drops of iodine glycerine. This vapour mixture is strongly bactericidal as shown in the following control experiments:

Agar plates were inoculated with S. marcescens or C. albicans. An ordinary air blower was filled with 2 ml of iodine glycerine and then with air sucked up through a flame. The air-blow was directed against the centers of the plates which were then incubated at 20° C and 35° C, respectively, and controlled after 24 and 48 hours. Distinct zones free of microbes were

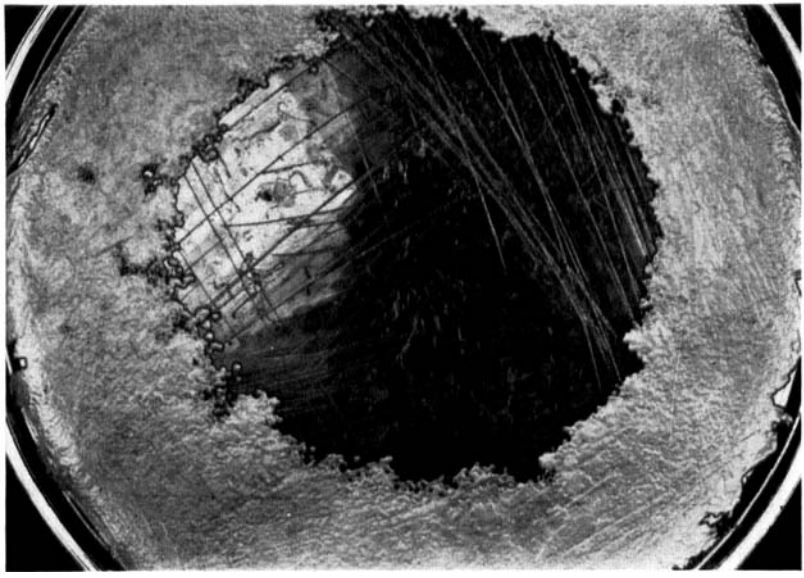


Fig. 6.

shown in the areas which had been subjected to the air-blow while heavy growth was observed in the peripheral areas (Fig. 6).

Diffusion tests carried out on agar plates in the same way as for iodine varnish showed distinct microbe-free zones around the holes (Fig. 2b).

Diffusion tests with capillary tubes and slides showed a marked iodine-starch reaction after a short while (Figs. 3b and 4b).

The ability of the iodine glycerine to diffuse *in vivo* was shown in the following way: A 1,000 mill./ml suspension of S. marcescens was placed at the bottom of 4 physiological pockets in one person. Iodine glycerine was applied on the gingiva and the teeth. With a rubber balloon, filled with 2 ml of iodine glycerine, it was attempted to dry up the area. Samples from the pockets taken after 3 minutes showed neither growth of oral nor of test microbes (Fig. 7).

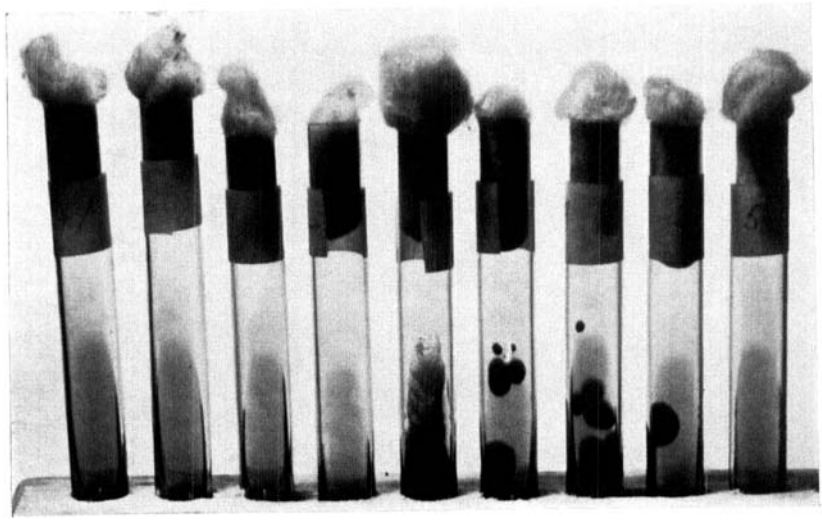


Fig. 7.

—2, —3, 4: Growth of Serratia after 1 hour in the pockets.
+1, +2, +3, +4: No growth of Serratia after disinfection with iodine glycerine.

IV. COMPARATIVE STUDIES ON THE ACTION OF IODINE VARNISH AND IODINE GLYCERINE

Iodine varnish was applied to the gingiva and the teeth on the right side of the upper jaw of 3 persons. On the left side the same procedure was carried out with iodine glycerine.

Result: All the 15 samples from the right side showed growth after 24 hours.

None of the 15 tests from the left side showed growth after 5 days (Fig. 8).

On two other persons the iodine varnish was applied to the gingiva and the teeth of the entire upper jaw. All samples from the right and left sides, 20 in all, showed growth.

On the same persons 4 days later the iodine varnish was applied to the gingiva and the teeth of the right side of the upper jaw and iodine glycerine to the left side. 10 samples from the pockets of the right side showed growth while none of the 10 samples from the left side showed growth.

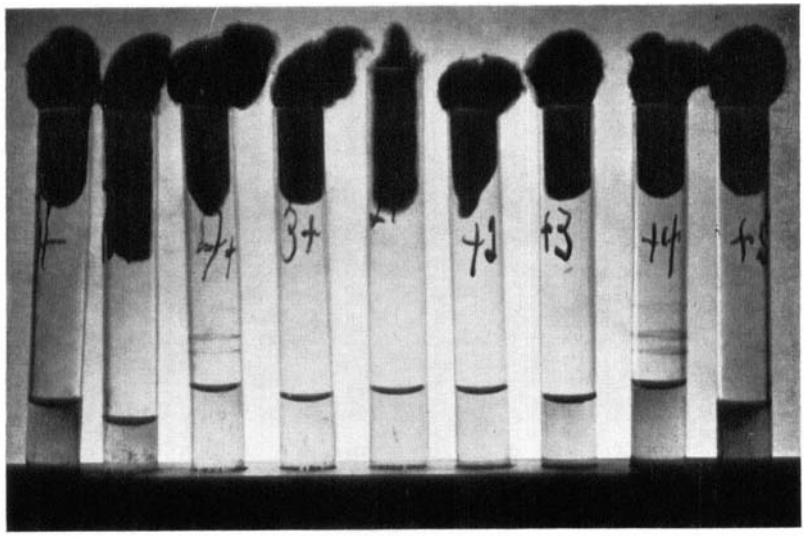


Fig. 8.

On the left side of the upper jaw: iodine glycerine.
On the right side: iodine varnish.

DISCUSSION

During bacteriological investigations of small areas such as physiological gingival pockets, there is always a risk of contamination from the adjacent tooth surface or the gingival margin. The methods which can be used in such investigations are the following:

(1) Direct tests after cauterization. Growth from the pockets is usually obtained by sampling after cauterization of the gingival margin and the adjacent tooth surface. This method is, however, uncertain, because an undesired sterilization of the pockets may occur.

(2)The direct sampling with fine instruments is not reliable due to the risk of contamination from the gingival margin and the tooth surface. This method rests on the assumption that the flora in the pockets is of the same type as that found at the entrance of the pockets (8, 9, 10).

(3) It is possible to obtain material by puncture of the gingiva from the outside followed by aspiration.

The physiological pocket is very narrow and its content of fluid is minimal and difficult to aspirate. There is always a danger of sucking material from the gingival margin as well. It is possible to press some fluid into the pocket before aspiration, as done by *Wilkinson* (16). The pocket may, however, be overfilled and the aspirated fluid may be contaminated from the gingival margin.

In a control of this method the pockets were sealed with iodine varnish before aspiration. The results of these aspiration tests varied greatly. Absence of growth with this method does not necessarily mean that the pockets are sterile, but might be due to insufficient material having been aspirated.

(4) Sampling after disinfection of the gingiva and the teeth seems to be the most suitable method for use in routine investigation.

Usually, iodine is used as the disinfectant. During a systematic investigation by *Wærhaug & Steen* (11) »equal quantities of 5 per cent iodine and glycerine» were used. This solution disinfects the mucosa completely (14, 15). It has a disadvantage in that the disinfectant is not fixed to those areas which one wants to disinfect, i.e. the gingival margin and the tooth surface, but floats around uncontrolled. There is always a possibility of leakage into the pockets.

During a control of the method *Boyd & Rosenthal* (10) obtained growth in 25 % of 16 tests while 75 % were sterile. "After obtaining the cultures, a supersaturated starch solution was introduced into the sterilized sulcus. The telltale blue iodine-starch reaction could be seen under the magnifying lens."

The controls of the method referred to in the present paper, show that the iodine glycerine method has a great deal of disadvantages:

(1) The vapour mixture from the rubber balloon is very bactericidal (Fig. 6).

(2) Diffusion tests on agar plates (Fig. 2b), with capillary tubes (Fig. 3b), and with slides (Fig. 4b), show that the iodine glycerine easily leaks out.

(3) Experiments *in vivo* indicate that iodine glycerine leaks into the pockets and kills the test microbes placed there (Fig. 7).

Most likely other microbes, present in the pocket beforehand, are also killed by the iodine glycerine.

The weakness of the technique is due to the failure of the iodine glycerine to harden thus allowing the disinfectant to spread. Especially the ability of the iodine glycerine to diffuse into a narrow fluid-filled space makes the method rather doubtful.

The iodine varnish used in the present experiments hardens within 2—3 minutes and forms a membrane over the entrance of the pocket. This membrane effectively disinfects the gingiva and the tooth surface. The iodine in the varnish does not penetrate into the physiological pocket. The iodine varnish presumably meets the requirements to a disinfectant in bacteriological investigations of physiological gingival pockets.

The different action of iodine varnish and iodine glycerine is demonstrated in the comparative studies of the methods. Quite illustrating is one experiment where growth was obtained from all pockets when iodine varnish was used, while 4 days later no growth was shown from the same pockets after disinfection with iodine glycerine.

The results with the new technique described in this paper seem to establish that physiological pockets contain bacteria.

It also seems unreasonable that the gingival pocket should remain sterile when its entrance is heavily contaminated by the salivary flora.

Clinically healthy gingiva is firmly pressed on to the tooth surface. This relation is, however, not permanent. It changes during talking, chewing and swallowing, toothbrushing etc. and allows the saliva to pass in and out of the pockets.

This concept is confirmed by the results of the experiments described in the present paper.

SUMMARY

1. A survery and a critical evaluation is given of the methods available for bacteriological examinations of physiological pockets.

2. A new method for this purpose is presented.

3. As disinfectant is used an iodine varnish of the following composition:

Colophonium	25.4 %	97.5 g
Copal from Manilla	11.3 %	
Etanol, 96 %	63.3 %	
Iodine cryst.		2.5 g

4. The experiments were performed on dental nurses 17—20 years of age, with clinically healthy gingivae, pocket depths of 1—2 mm and without visible deposits on their teeth.

5. Iodine varnish was applied to the area to be examined and dried in the air for 2—3 minutes until a membrane was formed.

6. The samples were taken with a bacteriological loop, diameter 2 mm, thread thickness 0.2 mm.

7. The samples were cultivated in hydrosulphite medium at 37° C and read after 1, 2, 3, 4, and 5 days.

8. 60 healthy pockets were examined. 57 of them showed growth after 1 day, 3 showed no growth after 5 days. 2 of the "negative" pockets were re-examined later and showed growth after 1 day. The third "negative" pocket was not re-examined.

9. The conclusion of these experiments is that physiological gingival pockets are always infected.

RÉSUMÉ

BACTÉRIOLOGIE DES CULS-DE-SAC GINGIVO-DENTAIRES PHYSIOLOGIQUES

1. Aperçu et évaluation critique des méthodes en usage pour l'examen bactériologique des culs-de-sac physiologiques.

2. Indication d'une nouvelle méthode.

3. Le désinfectant employé est un vernis à l'iode de la composition suivante:

Colophane	25,4 %	97,5 g
Copal de Manille	11,3 %	
Alcool éthylique à 96%	63,3 %	
Iode cristallisé		2,5 g

4. Les expériences sont faites sur des assistantes dentaires âgées de 17 à 20 ans, présentant à l'examen clinique une gencive

saine et des culs-de-sac gingivo-dentaires de 1 à 2 mm, sans dépôts visibles sur les dents.

5. Le vernis à l'iode est appliqué à la zone devant être examinée et séché à l'air pendant 2 à 3 minutes jusqu'à formation d'une membrane.

6. Les spécimens sont prélevés au moyen d'une anse bactériologique (diamètre: 2 mm, épaisseur du fil: 0,2 mm).

7. Les spécimens sont mis en culture sur un milieu à l'hydrosulfite à 37° C et contrôlés après 1, 2, 3, 4 et 5 jours.

8. 60 culs-de-sac sains ont été examinés. 57 d'entre eux avaient une culture positive au bout d'un jour, 3 d'entre eux avaient une culture négative au bout de 5 jours. 2 des culs-de-sac »négatifs» ont été réexaminés plus tard et avaient alors une culture positive au bout d'un jour. Le troisième cul-de-sac »négatif» n'a pas été réexaminé.

9. La conclusion de ces examens est que les culs-de-sac gingivo-dentaires physiologiques sont toujours infectés.

ZUSAMMENFASSUNG

DIE BAKTERIOLOGIE PHYSIOLOGISCHER ZAHNFLEISCHTASCHEN

1. Eine Übersicht und eine Kritik der Methoden, die für bakteriologische Untersuchungen von physiologischen Zahnfleischtaschen angewandt werden, wird gegeben.

2. Eine neue Methode für die Erhaltung von bakteriologischen Proben aus den Zahnfleischtaschen wird vorgeschlagen.

3. Um eine Infektion von der Oberfläche des Zahnes und Zahnfleisches zu vermeiden wurde diese Gebiete mit einem desinfizierenden Jod-Lack-Lösung mit folgender Zusammensetzung gedeckt:

Colophonium	25,4 %	97,5 g
Manilla Copal	11,3 %	
Etanol, 96 %	63,3 %	
Jodkristallen		2,5 g

4. Die Untersuchungen wurden an 17—20jährigen Klinikschwestern mit klinisch frischem Zahnfleisch, Taschentiefe 1—2 mm und keinen sichtbaren Zahnbelag vorgenommen.

5. Der Jod-Lack wurde über das Zahnfleisch und die Zähne in der Region, wo die Untersuchung vorgenommen werden sollte, gepinselt und für 2—3 Minuten in der Luft getrocknet bis ein Häutchen gebildet wurde.

6. Proben wurden mit einer bakteriologischen Öse von 2 mm Weite und Drahtdicke 0,2 mm genommen.

7. Das Probematerial wurde bei 37° C in Hydrosulfit-Medium inkubiert und die Gläser nach 1, 2, 3, 4 und 5 Tagen abgelesen.

8. Proben aus 60 frischen Taschen wurden untersucht. Von diesen erwiesen 57 nach 1 Tage Wachstum, während 3 keinen Wachstum nach 5 Tagen aufwiesen. Neue Proben von den 2 „negativen" Taschen zeigten Wachstum. Aus der letzten negativen Tasche wurde keine erneute Probe genommen.

9. Die Schlussfolgerung dieser Untersuchungen ist, dass physiologische Zahnfleischtaschen immer infiziert sind.

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