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DIFFUSION OF HISTAMINE INTO THE GINGIVAL CREVICE AND THROUGH THE CREVICULAR EPITHELIUM

by

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Brill and co-workers (*Brill & Krasse* 1958, *Brill & Björn* 1959, *Brill* 1959 a, *Brill & Krasse* 1959) demonstrated the passage of tissue fluid from sub-epithelial blood vessels into clinically healthy gingival crevices. The flow of fluid is increased when the permeability of the capillaries is increased as in gingivitis, after mechanical stimulation of the gingivae and after intravenous injections of histamine. If bacteria or carbon particles are introduced into the crevice they are removed with the fluid (*Brill* 1959 b). It has been suggested that the gingival fluid may form an important part of the defence mechanism of the gingival crevice, due partly to its mechanical rinsing properties and partly to the presence of anti-bacterial factors in the fluid (*Brill & Brönnestam* 1960).

Waerhaug (1955) using human and dog material found that solutions of different dyes did not penetrate into gingival crevices or pockets from deposits placed round the tooth and the gingival border. He suggested that a flow of fluid in the opposite direction prevented the dyes from diffusing into the pockets.

It seems possible, from the results of the investigations cited above, that the gingival fluid may form a protective seal in the crevicular region and hinder the penetration of any substance

into the crevice and further into the sub-epithelial tissues. On the other hand, diffusion of various substances into the gingival crevice should not be considered impossible. This problem was studied in the present investigation. The substance used was histamine which is known to affect blood capillaries, causing an increase of the permeability of the capillary walls (*Zweifach* 1954).

The present study was undertaken to determine whether topical application of histamine has any effect on

(1) the amount of gingival fluid and

(2) the accumulation of carbon particles in gingival capillaries.

An increase in the permeability of these capillaries and an increased flow of gingival fluid would mean that the histamine had affected the blood vessels after having penetrated into the sub-epithelial tissues from the deposits outside the crevice.

MATERIAL AND METHODS

1. Effect of topical application of histamine on the amount of gingival fluid

Two groups of persons were studied, viz., one experimental group and one control group. The experimental group contained 21 persons, the control group 16 persons. They were nurses and patients from the Dental School, their ages ranging from 11 to 70 years. Some of them had clinically healthy gingivae and some of them chronically inflamed gingivae.

Gingival pockets of molars, premolars, canines, and incisors of the upper jaw were used for the study. Previous to and immediately after the application of a 10 mg/ml histamine solution in the experimental group and a 0.9 % saline solution in the control group, samples of fluid were recovered from the pockets by means of strips of filter paper. The method used was that described by *Brill* (1959 c).

The field of operation was carefully dried with absorbant rolls and compressed air. A strip of filter paper, 1.5 mm wide and 10 mm long, was inserted into the gingival pocket mesial to each tooth until resistance was felt. The strip was placed almost parallel to the long axis of the tooth and as far approximally as

the contact point permitted. It was left in place for three minutes and allowed to absorb the fluid entering the pocket. After the strip had been removed, a minute drop of the test solution was placed on the gingival margin where the strip had been inserted, using a small pipette. The solution was allowed to act for one minute. The gingiva was then dried, first with cotton and afterwards with compressed air, care being taken to avoid blowing into the pocket. A second strip was then inserted at the same place for a further 3 minutes in order to absorb the resultant fluid.

After their removal, the strips were moistened with a 0.2 % ninhydrin solution and were allowed to dry. Since ninhydrin reacts with the α-amino acids in the fluid to give a blue colour, the areas of the strips which had taken up tissue fluid were stained blue. The lengths of the coloured parts of the strips were measured to an accuracy of half a millimeter and thus represent the amount of gingival fluid. Any differences recorded before and after the drug application were believed to have been a result of the latter.

2. Effect of topical application of histamine on the accumulation of carbon particles in gingival capillaries

Two young dogs with clinically healthy gingivae were used. The dogs were given intravenous injections of India ink (Pelikan Perltusche, Günter Wagner). It is known that carbon particles circulating in the blood stream have a marked tendency to adhere to the endothelial lining of capillaries which have an increased permeability (*Kusnetzowsky* 1929, *Zweifach* 1954). This leads to the accumulation of carbon in such capillaries.

The dogs were anesthetized by intravenous injection of Mebumal-Sodium 6 %. A 10 mg/ml solution of histamine was applied to the marginal and attached gingivae of molars, premolars, and canine of the right side of the lower jaw. The left side of the lower jaw was used as a control. Immediately after the histamine had been applied, the intravenous injection of India ink was started. The ink had been diluted with an equal amount of 0.9 % saline and centrifuged several times to remove most of the large particles from the suspension. 40 ml of the suspension were given to the dog weighing 10 kg and 60 ml to

the dog weighing 12 kg. Corresponding amounts of blood had been withdrawn previously. The ink was given slowly during 15—20 minutes. The histamine was re-applied twice during this period. The dogs were then sacrificed with an overdose of Mebumal. Their jaws were removed and fixed in 10 % formalin for 24 hours. Pieces of gingivae were then carefully taken from the fixed jaws and examined in the microscope using incident light. The pieces of gingivae were first studied from the "tooth aspect" in order to observe the capillaries under the crevicular epithelium. Later, thick frozen buccal-lingual sections were cut from these specimens and observed under the microscope in an unstained state.

RESULTS

1. Effect of topical application of histamine on the amount of gingival fluid

The effect of topical application of a 10 mg/ml solution of histamine is seen in Table 1. A total of 202 healthy and pathological gingival pockets from 21 persons were investigated. There was a mean increase by a factor of 2.7 in the amounts of fluid recovered after, when compared to the amounts recovered before, the histamine application. A statistical analysis showed the difference to be highly significant ($t = 4.00$ and $P < 0.001$).

The effect of 0.9 % saline is given in Table 2. In the control group of 16 persons there was an increase in 10, a decrease in 4 and no change in 2. The mean values showed an increase by a factor of 1.1 which was statistically probable ($t = 2.93$ and $0.01 < P < 0.02$).

The effect of histamine was compared with that of saline by means of variance analysis between the two series of differences. It was then found that the difference in effect is significant ($t = 3.46$ and $0.001 < P < 0.002$).

In Table 3 the subjects in the histamine group are assigned to one of three groups depending on the mean amount of gingival fluid recovered before application. The flow of gingival fluid is known to be increased in gingivitis and it has been shown that there is a relationship between the amounts of fluid and different histological features of inflammation (*Brill* 1960). The persons in the first group having a small amount of fluid

Table 1.

Effect of topical application of histamine on the amount of gingival fluid.

Subject	Number of pockets	Mean amount per pocket before	after	Difference	Increase
1	10	1.1	1.6	0.5	1.5×
2	10	1.1	1.6	0.5	1.5×
3	10	1.1	1.6	0.5	1.5×
4	10	1.2	1.7	0.5	1.4×
5	12	1.2	1.4	0.2	1.2×
6	12	1.2	2.0	0.8	1.7×
7	7	1.3	2.1	0.6	1.4×
8	10	1.5	2.1	0.6	1.4×
9	12	1.5	3.0	1.5	2.0×
10	12	1.5	7.3	5.8	4.9×
11	8	1.7	2.8	1.1	1.6×
12	9	1.7	4.9	3.2	2.9×
13	12	1.8	3.0	1.2	1.7×
14	10	1.9	3.2	1.3	1.7×
15	7	2.1	8.7	6.6	4.1×
16	9	2.3	2.6	0.3	1.1×
17	5	2.9	15.2	12.3	5.2×
18	5	3.7	11.0	7.3	3.0×
19	8	4.1	12.3	8.2	3.0×
20	12	4.3	14.7	10.4	3.4×
21	12	4.8	15.1	10.3	3.1×
Total	202	44.0	117.0		
Mean		2.1	5.6	3.5	2.7×
s				0.88	
t				4.00	
p				$P < 0.001$	

thus had healthy gingivae while the other groups with greater amounts were persons with inflamed gingivae. It can be seen from Table 3 that histamine seems to have less effect on persons with healthy, than on persons with inflamed gingivae.

The concentration of the histamine solution which was used (10 mg/ml) is ten times the concentration known to be able to produce dermal wheal and flare reactions after subcutaneous injections (1 mg/ml). This lower concentration was used in pilot studies. In 56 pockets from 10 persons with an average amount

Table 2.

Effect of topical application of 0.9 % NaCl on the amount of gingival fluid

Subject	Number of pockets	Mean amount per pocket before	Mean amount per pocket after	Difference
1	8	0.9	0.8	—0.1
2	12	1.0	0.9	—0.1
3	11	1.3	1.0	—0.3
4	12	1.4	1.0	—0.4
5	12	1.5	1.5	±0
6	10	1.8	3.5	+1.7
7	12	2.0	3.1	+0.5
8	12	2.5	2.5	+0.6
9	12	2.5	2.8	+0.3
10	7	2.8	3.4	+0.6
11	6	3.1	3.5	+0.4
12	6	4.1	5.3	+0.8
13	7	5.6	6.3	+0.7
14	8	5.9	5.9	±0
15	6	6.0	7.5	+1.5
16	11	6.1	6.9	+0.8
Total	152	48.5	54.9	
Mean		3.0	3.4	0.4
ε				0.15
t				2.93
p				$0.01<P<0.02$

Table 3.

Effect of topical application of histamine on the amount of gingival fluid. The patients are grouped after the mean amount recovered before application.

Amount per pocket before application	1.0—1.4	1.5—1.9	2.0—4.8
Increase following application	1.5×	2.3×	3.3×
Number of subjects	7	7	7

of gingival fluid before application of 1.5 there was a mean increase of 2.0 times. This concentration is therefore sufficient to produce a capillary response with the method used in these experiments. The higher concentration, however, was chosen because of its greater effect.

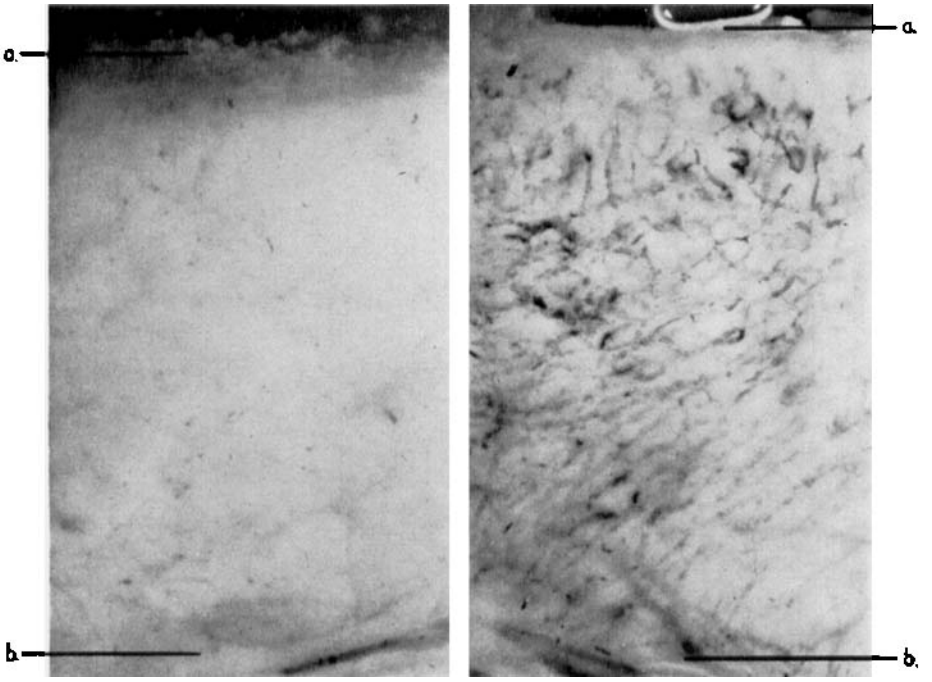

Fig. 1. Accumulation of ink particles in the blood vessels inside the crevicular epithelium after topical application of histamine. The vessels are viewed directly through the crevicular epithelium. Left: —M_2, control side. Right: M_2—, experimental side. a: gingival margin. b: base of the crevicular epithelium.

2. Effect of topical application of histamine on the accumulation of carbon particles in gingival capillaries

The results from the dog experiments after topical application of histamine combined with intravenous injection of India ink showed a consistent pattern. When the capillaries inside the crevicular epithelium were studied directly by means of pieces of gingivae, an obvious difference between the control side and the experimental side could be seen. While only small amounts of ink were observed in the capillaries on the control side, large amounts were seen in the corresponding capillaries on the experimental side. The ink accumulations were more pronounced

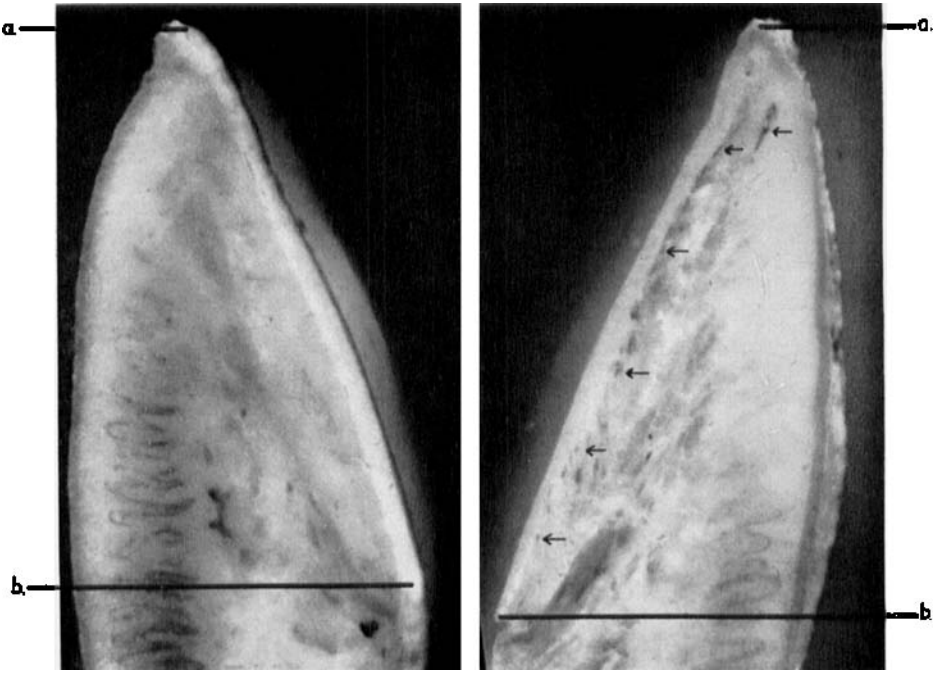

Fig. 2. Accumulation of ink particles in the blood vessels inside the crevicular epithelium after topical application of histamine. Buccal-lingual section. No ink is seen in vessels under the attached epithelium. The arrows are pointing to some of the blood vessels containing ink. Thick, unstained frozen sections, incident light. Left: —M_2, control side. Right: M_2—, experimental side. a: gingival margin. b: base of the crevicular epithelium.

in capillaries under the marginal half of the crevicular epithelium than under the cervical half of this epithelium (Figure 1).

In buccal-lingual sections of the same gingival pieces no ink could, as a rule, be observed in the blood vessels inside the crevicular epithelium on the control side. In the sections from the experimental side, however, ink had accumulated in the corresponding vessels. In spite of the fact that histamine had been applied not only on the marginal but also on the attached gingivae, no ink was observed in the blood vessels under the attached gingivae (Figure 2).

DISCUSSION

Application for one minute of small quantities of histamine to the gingival margin produced a highly significant increase in the flow of gingival fluid. Since it has been shown that mechanical stimulation of the crevicular epithelium increases this flow (*Brill & Krasse* 1959), it is possible that the irritation caused by inserting the paper strips into the crevice was responsible for this increase. Using the same method, but substituting 0.9 % saline for histamine, there was a small increase in the flow of fluid indicating that the paper strips had caused slight irritation. The difference registered between the effect of histamine and saline, however, was statistically significant. Most of the increased flow of gingival fluid can thus be attributed to the effect of topically applied histamine.

After the histamine application, the gingivae were carefully dried with cotton and afterwards with compressed air. It is possible that some histamine was forced into the pockets, although great care was taken during the drying. Any possibility for this to occur was eliminated in control experiments where the patients were allowed to rinse before the drying was carried out. In 28 pockets from 4 persons there was a mean increase of 2.4 times in the amount of gingival fluid after histamine application. This result is similar to that registered in the main experiment.

The effect of topical application of histamine on the gingival capillaries was also demonstrated in the dog experiments. The increase in permeability leading to accumulation of ink particles on the capillary walls was found in the capillaries inside the crevicular epithelium only. Accumulations could be seen beneath the entire length of the crevicular epithelium although they were heavier in the marginal region. Under the epithelium of the attached gingivae no ink could be observed, demonstrating that the histamine had penetrated into the connective tissue solely through the crevicular epithelium. The epithelium of the attached gingivae did not appear to be permeable to histamine molecules under the conditions of these experiments. This is in agreement with the results obtained by *Takata & Kawamura* (1962) using isolated sublingual dog mucosa.

The findings in the present investigation, thus, suggest that histamine, which has a low molecular weight (M. W.: 111) can penetrate into the gingival crevice through the crevicular epithelium and into the connective tissue. The oozing outwards of tissue fluid does not seem to render diffusion in the opposite direction impossible. This is in contradiction to the observations of *Waerhaug* (1955) who used dyes with molecular weights ranging from 240 to 374. The discrepancy between the results of these two experiments might, in part, be explained by the difference in size of molecules, by differences in concentration and physical state of the solutions and by variation in methods.

Further studies are needed to clarify the problem of diffusion in the crevicular region. It is suggested that larger molecules comparable to bacterial enzymes and toxins could be used and might give information concerning the possibility for such substances to pass into the gingival tissue.

SUMMARY

The results of previous investigations (*Waerhaug* 1955, *Brill* 1959 b) suggest that the gingival fluid may form a protective seal in the crevicular region thus hindering the penetration of different substances into the gingival crevice. The results of the present study, however, suggest that a histamine solution can penetrate into the gingival crevice, through the crevicular epithelium and into the connective tissue. Increased capillary permeability could be demonstrated in the blood vessels inside the crevicular epithelium after topical application of histamine along the gingival margin. Increased permeability was demonstrated by the increased amounts of gingival fluid recovered and also by the accumulation of India ink in the capillaries after intravenous injection of the ink.

RÉSUMÉ

DIFFUSION DE L'HISTAMINE VERS L'INTÉRIEUR DU SILLON GINGIVO-DENTAIRE ET À TRAVERS L'ÉPITHÉLIUM DU CUL-DE-SAC

Les résultats d'études faites précédemment (*Waerhaug* 1955, *Brill* 1959 b) semblent indiquer que le liquide gingival formerait une barrière protectrice dans la région du sillon gingivo-dentaire,

entravant ainsi la pénétration de diverses substances dans le sillon gingivo-dentaire. Les résultats de la présente étude semblent cependant indiquer qu'une solution d'histamine peut pénétrer dans le sillon gingivo-dentaire, à travers l'épithélium de ce sillon et dans le tissu conjonctif. Une augmentation de la perméabilité capillaire a pu être mise en évidence dans les vaisseaux sanguins à l'intérieur de l'épithélium du cul-de-sac après application locale d'histamine le long du bord gingival. L'augmentation de la perméabilité a été mise en évidence par l'augmentation des quantités de liquide gingival recueilles ainsi que par l'accumulation d'encre de Chine dans les capillaires parès injection intra-veineuse de cette encre.

ZUSAMMENFASSUNG

DIE DIFFUSION VON HISTAMIN IN DIE ZAHNFLEISCHTASCHE UND DURCH DAS EPITHEL DER ZAHNFLEISCHTASCHE

Das Ergebnis früheren Untersuchungen (*Waerhaug* 1955, *Brill* 1959 b) deuten an, dass angenommen werden kann, dass die Gingivalflüssigkeit dazu beiträgt ein schützendes Medium (gleich einer Verriegelung) in der Zahnfleischtasche zu bilden und dadurch eine Penetration von verschiedenen Substanzen in die Zahnfleischtasche zu verhindern. Das Ergebnis der vorliegenden Untersuchung deutet doch an, dass eine Histaminlösung in die Zahnfleischtasche, durch das Epithel der Zahnfleischtasche und in das Bindegewebe eindringen kann. Erhöhte Kapillarpermeabilität in den Blutgefässen innerhalb des Epithels der Zahnfleischtasche nach einer lokalen Applikation von Histamin entlang des Gingivalrandes konnte gezeigt werden. Die erhöhte Permeabilität wurde dadurch gezeigt, dass grössere Mengen Gingivalflüssigkeit erhalten wurden sowie durch eine Ansammlung von Perltusche in den Kapillaren nach intravenöser Injektion von Perltusche.

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