Decrease of Acid Phosphatase Activity in the Epithelial Cells from Inflamed Gingivae

Periodontitis is characterized by a progressive destruction of the tissues supporting the teeth. The inflammation starts at the gingival margin, and is caused by the presence of excessive amounts of bacteria colonizing on the tooth surface close to the gingiva, the bacterial 'plaque'[1]. The exact mechanism by which such plaques cause gingivitis and periodontitis is, however, poorly understood.

It is possible that lysosomal enzymes, liberated from the exfoliating epithelial cells and migrating leucocytes, contribute to the tissue destruction along the tooth surface in the so-called 'sulcular' region.

High levels of acid phosphatase[2], β-glucuronidase[3] and cathepsin D[4] have been demonstrated in the inflammatory exudate appearing in the sulcular region in concentrations positively correlated with the gravity of the periodontal lesion. The purpose of this communication is to give evidence that desquamating gingival epithelial cells collected from cases of increasingly severe gingivitis show a decreasing degree of intracellular acid phosphatase activity.

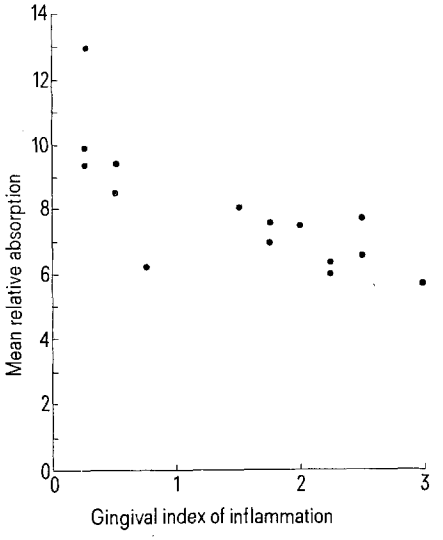

Fig. 1. Negative correlation between the microdensitometric readings of relative absorption in the epithelial cells (intracellular acid phosphatase activity) and the gingival index of inflammation in 15 individuals. The correlation coefficient ($r = -0.68$) is statistically significant.

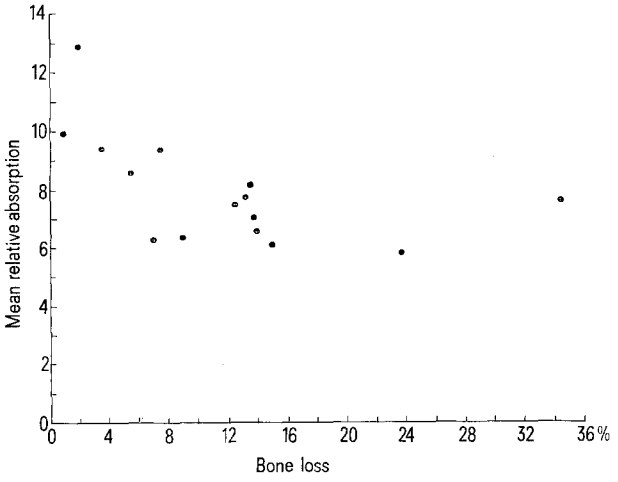

Fig. 2. Negative correlation between the mean relative absorption in the epithelial cells (acid phosphatase activity) and the mean percentage of periodontal bone loss measured on radiographs for the 15 patients ($r = 0.55$).

Materials and methods. Epithelial cells were harvested by means of a platinum loop from the sulcular areas of the upper incisors and canine teeth in 15 patients. They were transferred into microtubes containing α-naphthyl-phosphate and Garnet GBC salt in 0.1 *M* acetate buffer pH 5.05[5]. After 30 min incubation at 37 °C, the contents of the tubes were centrifuged and the supernatant discarded. Smears were prepared from the sedimented pellet after repeated washing followed by fixation with formol. 8 to 10 isolated epithelial cells were chosen in each slide for the microphotometric determination of relative absorption. These were performed by means of a BARR and STROUD integrating microdensitometer, type GN 2, using a wave length of 5500 Ä and a ×100 N.A. 1.3 oil-immersion objective; with a high magnification projection lens this gave a standard field size of 60 µm diameter. The relative absorption in arbitrary units was measured 3 times for each cell, a field outside the cell serving as the blank. 6 specimens and 6 smears were prepared from each patient, so that an average relative absorption could be computed for each case, thus representing the mean of between 300 and 360 measurements.

The degree of gingival inflammation and periodontal destruction was assessed along the same upper anterior teeth by measuring the gingival index of inflammation[6], the flow of gingival exudate[7], the mean depth of the periodontal pockets[3,4] and the mean percentage of bone loss as assessed from radiographs[3,4,8].

Results. A negative and statistically significant correlation ($r = -0.68$) was found between the amount of acid phosphatase activity in the epithelial cells and the gingival index of inflammation (Figure 1). The acid phosphatase activity was also negatively correlated with the flow of gingival fluid, but in this case the degree of correlation was above the 5% level of significance ($r = -0.45$).

As for the parameters measuring the destruction of the deeper periodontal structures, both the values of mean bone loss and pocket depth were negatively correlated with the relative absorption of the acid phosphatase activity measured in the smears: the coefficients of correlation were, respectively, -0.55 ($p < 0.02$, Figure 2) and -0.48 ($p < 0.1$).

Discussion. The incubation time of 30 min has been chosen after some preliminary kinetic observations: a series of smears were incubated for 3 to 60 min, and the microdensitometric readings showed that the relative absorption increased linearly during this time.

[1] D. F. G. POOLE and H. N. NEWMAN, Nature, Lond. *234*, 329 (1971).
[2] T. SUEDA and G. CIMASONI, Archs oral Biol. *13*, 553 (1968).
[3] J. BANG, G. CIMASONI and A. J. HELD, Archs oral Biol. *15*, 445 (1970).
[4] J. ISHIKAWA, G. CIMASONI and C. AHMAD-ZADEH, Archs oral Biol. *17*, 111 (1972).
[5] A. G. E. PEARSE, *Theoretical and Applied Histochemistry* (Churchill, London 1960), p. 882.
[6] H. LÖE and J. SILNESS, Acta odont. scand. *21*, 535 (1963).
[7] A. VALAZZA, J. MATTER, A. OGILVIE and G. CIMASONI, Rev. mens. suisse odonto-stomatol. *82*, 824 (1972).
[8] C. D. MARSHALL-DAY and K. C. SHOURIE, J. Am. dent. Ass. *39*, 573 (1949).
[9] C. DE DUVE, Scient. Am. *208*, 64 (1963).

It is generally admitted that the discharge of lysosomal enzymes from dying cells can cause a destruction of the surrounding tissues[9]. The hypothesis of a labilization of the lysosomal membranes through the inflammatory process seems to be supported by our results. Since most of the acid phosphatase activity of cells is found within the lysosomes, it is possible that the permeability of the lysosomal membranes has become increased as a consequence of the gingival inflammation, with a liberation of the 'bound' fraction of the enzyme and consecutive diminution of the total intracellular activity.

Accumulation of lysosomal enzymes in the sulcular region could represent a possible mechanism for the formation of the periodontal pocket[10].

Résumé. Les cellules épithéliales qui desquament au niveau de la gencive en contact avec la surface dentaire contiennent des concentrations de phosphatase acide plus faibles en présence d'inflammation et de destruction des tissus parodontaux. Il est possible que la libération d'enzymes lysosomaux au niveau du sillon gingivodentaire soit une des causes de la formation de la poche parodontale.

A. CORNAZ[11], G. CIMASONI[11] and A. PATAKI[12]

Dental School, University of Geneva, 30, rue Lombard, CH-1211 Genève 4 (Switzerland); and Department of Toxicology, Sandoz Ltd., CH-4133 Basel (Switzerland), 16 July 1973.

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[11] Dental School, University of Geneva, 30, rue Lombard, CH-1211 Genève 4 (Switzerland).

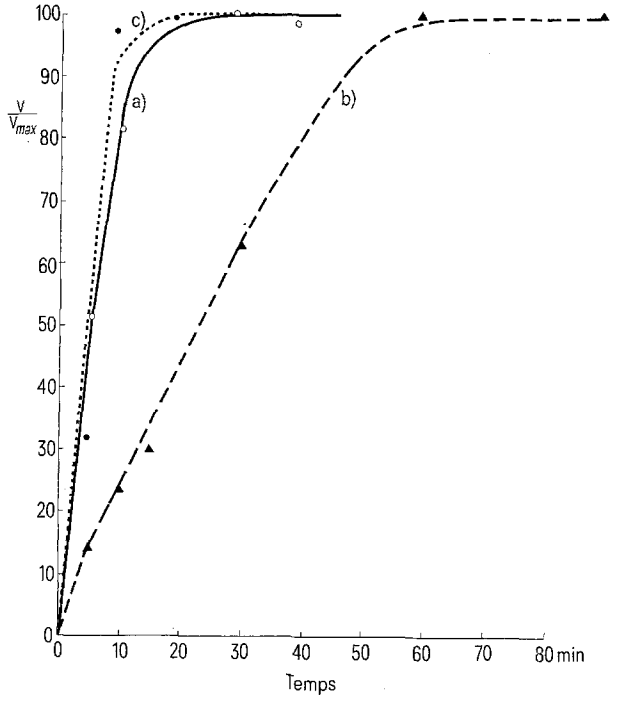
[12] Department of Toxicology, Sandoz S.A., CH-4133 Basel (Switzerland).

Action de la synthèse protéique sur le transfert de la N-acetylglucosamine dans la muqueuse intestinale de rat

Nous avons montré dans de précédents travaux que les microsomes des cellules épithéliales de la muqueuse intestinale de rat renfermaient une activité de transfert de la N-acetylglucosamine sur des accepteurs glycoprotéiques endogènes[1].

Lorsque l'on recherche la localisation submicrosomique de cette N-acetylglucosaminyltransférase[2], on observe qu'elle apparaît principalement dans la fraction des membranes agranulaires préparées selon la méthode de DALLNER[3]; la très faible activité rencontrée dans la fraction des membranes granulaires pouvait être soit le résultat d'une contamination par les membranes agranulaires, soit la manifestation discrète d'une N-acetylglucosaminyltransférase responsable de l'incorporation de la première glucosamine qui joue le rôle de trait d'union entre les chaînes peptidique et polysaccharidique, et dont l'activité pourrait être accrue grâce à une synthèse protéique active.

C'est cette dernière hypothèse que nous avons essayé de vérifier dans le travail que nous décrivons ici.



Etude cinétique de l'incorporation a) d'acides aminés (^{14}C) en milieu acellulaire de biosynthèse protéique. b) de N-acetylglucosamine en l'absence de synthèse protéique. c) de N-acetylglucosamine en présence de synthèse protéique. L'activité d'incorporation est exprimée en pourcentage de l'activité maximale Vo: a) $3{,}5 \times 10^4$ cpm/mg de protéines; b) 40 cpm/mg de protéines; c) 400 cpm/mg de protéines.

Matériel et méthodes. Les animaux sont des rats mâles de souche wistar pesant de 200 à 300 g, normalement nourris.

Fractionnement cellulaire: Les membranes granulaires ont été séparées des membranes agranulaires par la méthode de DALLNER[3] modifiée par KIM et al.[4] dans le tampon de LITTELFIELD et KELLER[5] contenant en plus 50 µg/ml de polyvinylsulfate de potassium.

Techniques analytiques: Les milieux acellulaires de synthèse protéique[6] comprennent pour un volume final de 0,450 ml, 100 µg de protéines des membranes granulaires, 750 µg de protéines de la fraction «pH 5 enzyme» préparée selon KELLER et ZAMECNIK[7], 9 µmoles de phosphoenolpyruvate, 20 µg de pyruvate kinase (Boehringer), 5 µmoles de $Mg\,Cl_2$, 30 µmoles de KCl, 0,5 µmole d'ATP, 0,05 µmole de GTP, 1 µmole de mercaptoéthanol, 1 µCi d'acides aminés ^{14}C (OB 4 (F) PR 80/68. 560 µCi/mg - CEA) ou 5 µg de chacun des 19 aminoacides froids et 0,05 µCi d'UDP-N-acetylglucosamine (NEN-Lot 483 156.)

Les incubations sont effectuées à 37 °C et la radioactivité est mesurée sur des précipités par l'acide trichloracétique (concentration finale 10 %) recueillis sur filtre de verre whatman (GF/B), lavés par l'eau distillée puis par

[1] J. FROT-COUTAZ et R. GOT, Biochimie *53*, 595 (1971).

[2] J. FROT-COUTAZ, P. DUBOIS, G. BERTHILLIER et R. GOT, Expl. Cell Res. *77*, 223 (1973).

[3] G. DALLNER, Acta path. microbiol. scand. Suppl. *166*, 1 (1963).

[4] Y. S. KIM, J. PERDOMO et J. NORDBERG, J. biol. Chem. *246*, (17) 5466 (1971).

[5] J. W. LITTELFIELD et E. E. KELLER, J. biol. Chem. *224*, 13 (1975).

[6] D. H. ALPERS et J. K. ISSELBCHER, J. biol. Chem. *242*, 5617 (1967).

[7] E. B. KELLER et P. C. ZAMECNIK, J. biol. Chem. *221*, 45 (1956).