sprechende L-Aminosäure als Gegenkomponente anbietet. Die Messungen im Kreuztest erbrachten folgende Daten:

Peptid:L-Aminosäure	Verhältnis
L-Arg-D-Ala-L-Phe:L-Ala	= 1:1
L-Arg-D-Val-L-Phe:L-Val	= 1:1
L-Arg-D-Leu-L-Phe:L-Leu	= 1:2
L-Arg-D-Tyr-L-Phe:L-Tyr	= 1:2
L-Arg-D-Phe-L-Phe:L-Phe	= 1:6

Die erhaltenen Ergebnisse zeigen eine deutliche Übereinstimmung mit den Resultaten, die LÖFFLER et al.[1] bei Experimenten mit dem von ihnen isolierten Peptid erhalten haben. Darüber hinaus wird aus der vorliegenden Arbeit deutlich, dass D-allo-Thr auch durch andere D-Aminosäuren in der obigen Sequenz ersetzt werden kann. Weiter wird augenfällig, dass die antibiotische Aktivität der Peptide desto grösser wird je hydrophober die eingesetzten D-Aminosäuren sind. Trifunktionelle, aliphatische D-Aminosäuren sind unwirksam, obwohl das natürliche Peptid (mit D-allo-Thr) antibiotisch aktiv ist. Die Kreuztestergebnisse lassen darauf schliessen, dass die mittelständige D-Aminosäure ein wirksames Prinzip der Tripeptide darstellt.

Die antibiotische Aktivität der 5 wirksamen, synthetischen Peptide erstreckt sich auf folgende Mikroorganismen: *Paecilomyces varioti, Mucor miehei, Candida albicans, Candida pseudotropic, Geotrichum candidum, Nematospora corylii* und *Torulopsis glabrata.*

Summary. Five tripeptides with the sequence L-Arg-D-X-L-Phe showed antibiotic activity on fungi and on some pathogenic moulds. The action of the peptides could be neutralized in the cross-strip test by the L-amino acid corresponding to the D-amino acid in the middle position.

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7 Frau Dr. A. M. FRETZDORFF danke ich für die Ausführung aller Analysen, Frau S. WALKER für die technische Assistenz.

Inflamed Gingivae Contain more free Lysosomal Enzyme

Evidence that lysosomes could play a role in chronic inflammatory processes comes especially from studies on joint rheumatoid diseases[1-3] and from in vitro experiments[4-8]. High levels of acid phosphatase and cathepsin D, apparently independent of increased cellularity, were found in rheumatoid synovial membranes when compared with normal synovial tissue[2-3]. Furthermore, in model experiments, cathepsin D has been shown to be largely responsible for the degradation of articular cartilage[4].

We now present evidence that a very common inflammatory process, periodontal inflammation (gingivitis and periodontitis), is accompanied by a significant increase in free lysosomal hydrolases.

Periodontal inflammation is responsible for the loss of about one half of the total number of teeth extracted throughout the world[9]. The inflammation starts at the gingival margin (gingivitis) and is caused by excessive amounts of bacteria colonizing on the tooth surface close to the gingiva, the bacterial 'plaque'[10]. Probably in association with immunological mechanisms[11], the disease leads to a progressive destruction of the tissues supporting the teeth. The severity of the disease can be assessed clinically by various types of indices. Among these, the gingival index of inflammation[12], and the amount of crevicular exudate[13,14], are measures of the degree of inflammation of the gingiva itself, while the depth of the pockets is a criterion for the destruction of the deeper periodontal structures (connective and bone tissues).

The release of lysosomal enzymes has been studied in vitro using cultures of leucocytes[5,6] and macrophages[7,8]. In vivo, however, with the exception of what has been observed in experimentally induced inflammatory lesions[15,16], a clear demonstration of an extra-lysosomal liberation of enzymes in a naturally occurring pathological situation is still lacking.

Material and methods. Samples of marginal gingiva were obtained during tooth extraction and periodontal surgery from 35 patients after measuring in the biopsy area the degree of gingival inflammation, the flow of gingival exudate and the depth of the periodontal pockets (average of 3 measurements). The biopsies were chilled in ice-cold 0.25 *M* sucrose and washed in the same solution to remove blood. After weighing, the gingiva was finely minced with scissors and homogenized with a motor-driven, loose-clearance, all-glass homogenizer. The homoge-

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Correlations between the free activities of the 3 hydrolases (as percentages of total) and the parameters measuring the severity of gingival inflammation

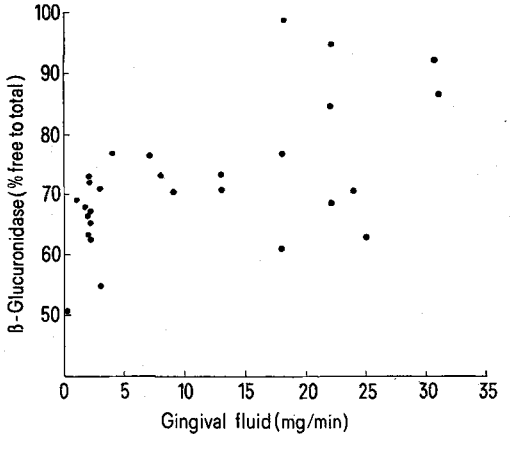
Clinical parameters	Degree of multiple (R^2) or canonical (^{C}can) correlation	P [a]
Gingival index of inflammation	$R^2 = 0.126$	0.25
Gingival fluid flow	$R^2 = 0.433$	0.01
Pocket depth	$R^2 = 0.323$	0.05
Gingival index and gingival fluid	$^C\text{can} = 0.691$	0.05
Gingival index, gingival fluid and pocket depth	$^C\text{can} = 0.461$	0.05

[a] Probability as determined from the correlation tables.

nization was performed under standardized conditions for 2 min with one up-and-down stroke every 15 sec[17]. The homogenate was centrifuged at 600 g for 10 min yielding a cell-free supernatant (cytoplasmic extract) and a sediment[17]. Free to total activities of acid phosphatase, β-glucuronidase and cathepsin D were determined in the cytoplasmic extract by assaying the enzymes in the absence and in the presence of 0.1% Triton X-100 (w/v). 4-Methylumbelliferyl dihydrogen phosphate and 4-methylmubelliferyl-β-D-glucuronide were used as substrate for acid phosphatase[18] and β-glucuronidase[19], respectively, while cathepsin D was determined by a modification of the method of ANSON[20].

Results. The free activities of β-glucuronidase and cathepsin D were found to be on average 69.7% and 77.8%, respectively. A similar value (73.8%) was found for acid methylumbelliferyl phosphatase.

When the biochemical data were compared with the clinical findings, positive correlations were found between the relative free activity of the 3 hydrolases and the index of inflammation, the intensity of gingival exudate flow, and the depth of the pockets. The Table shows these correlations. They were calculated using either the multiple or the canonical coefficient[21]. The degree of positive correlation was, with one exception, always significant with a probability level higher than 95%. The Figure represents the correlation ($r = +0.559$, $p < 0.01$) found between the free activity of β-glucuronidase, expressed as percent of the total activity, and the intensity of gingival fluid flow.



Relationship between the free β-glucuronidase (as percentage of total) found in the gingival biopsies, and the degree of gingival inflammation, measured by the intensity of gingival fluid flow. The fluid was collected by filter paper strips placed in the gingival margin during a given time [13,14]

Discussion. The data presented show that chronic gingival inflammation is accompanied by an increase in the amount of free lysosomal hydrolases. This is evidently not a consequence of increased cellularity in the inflamed area, since the severity of inflammation is shown to correlate with the ratio of free to total hydrolase activities.

Several mechanisms could account for the extracellular release of acid hydrolases in the inflamed gingiva. Macrophages, a major cellular component of chronically inflamed tissues, are known to release lysosomal enzymes during phagocytosis of bacterial products or immune complexes[22]. In vitro, it has been shown that macrophages can extrude lysosomal hydrolases without loss of viability when they are exposed to extracts of bacterial plaque[7]. Similar phenomena are known to occur in polymorphonuclear leucocytes which release part of their granule contents in the attempt of phagocytosing large particles such as immune complexes[22]. Finally, since cellular immune reactions are probably implicated in periodontitis[11], it should be kept in mind that lectin-stimulated lymphocytes were found to contain an increased amount of lysosomal enzymes[1].

It should be pointed out that polymorphonuclear leucocytes contain a non-lysosomal acid phosphatase which splits aromatic substrates like methylumbelliferyl phosphate[23,24]. This may lessen the significance of the data obtained with this particular enzyme. On the other hand, as discussed above, there are various sources of lysosomal enzymes in chronically inflamed tissue, and the relative contribution of polymorphonuclear leucocytes is almost certainly small.

Résumé. Les activités libre et totale des enzymes phosphatase acide, β-glucuronidase et cathepsine D ont été mesurées dans 35 biopsies gingivales aprés homogénisation et isolation de l'extrait cytoplasmique. Les résultats semblent prouver une libération extralysosomale d'enzymes pendant un processus inflammatoire trés fréquent chez l'homme.

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