Female sex hormones and lysosomal stability in gingival polymorphonuclear leucocytes[1]

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Summary. In a group of 5 women taking oral contraceptives, the concentration of polymorphonuclear leucocytes along the gingival margin and the activities of cathepsin D, β-glucuronidase and elastase were found to increase significantly during the intermenstrual phases.

Gingival inflammation is usually caused by excessive amounts of bacteria colonizing on the tooth surface close to the gingiva, the bacterial plaque[2]. The intensity of this inflammation varies, however, in different individuals and can also be modulated by pharmacological[3] or hormonal[4] factors: it is known, for instance, that the gingiva of pregnant women and of women taking hormonal contraceptives is particularly sensitive to bacterial aggression[4].

Previous work from our laboratory has shown the probable involvement of lysosomal enzymes in tissue damage associated with gingival inflammation and periodontitis: we have shown that acid phosphatase, β-glucuronidase and cathepsin D are largerly latent in homogenates of human gingiva[5,6] and that the free activities of these enzymes increase significantly in gingivitis[7]. Female sex hormones and their synthetic derivatives can induce a labilization of the lysosomal membrane and thus facilitate the release of lysosomal enzymes[8,9].

The purpose of the present longitudinal study was to investigate the release of lysosomal enzymes in the gingival crevice in a group of women taking oral contraceptives.

Material and methods. 5 women, 25–28 years old, with healthy gingiva and taking regularly hormonal contraceptives participated in this longitudinal investigation. Cellular and enzymatic analysis were performed in gingival washings of the upper crevicular region for 12 menstrual cycles in one of the volunteers and for 4–6 cycles in the others. The washings were performed twice during the menstruations, and 2–3 times during the intermenstrual period by circulating 5 ml of physiological saline containing 0.25 M sucrose for 15 min along the buccal and palatal marginal region, using acrylic individual appliances connected to a peristaltic pump[10,11].

Intact polymorphonuclear leucocytes (PMNs) and epithelial cells were counted in the washings after concentration, using a Neubauer haematocytometer (American optical Corp. Buffalo, N.Y.). Free activities of cathepsin D, elastase and β-glucuronidase were determined in the cell-free supernatant after centrifuging the gingival washing at 180×g for 10 min. Total activities of these enzymes were determined after adding 0.1% Triton X-100 (w/v) to the samples of washings. The intracellular specific activity of the enzymes was estimated by the difference between total and free activities divided by the number of cells in 1 ml of washing. Cathepsin D and elastase were both determined by modifications of the method of Anson[12], as previously described[13], while β-glucuronidase was determined fluorometrically, by using 4-methylumbelliferyl-β-D-glucuronide as substrate[14].

For the statistical analysis, the average of the 2 values obtained for a given parameter during menstruation and that of the 2 (or 3) values obtained during the intermenstrual period were determined for all the cycles, which allowed the determination of a mean difference with its SE. 2 confidence limits (Ls and Li) of the mean (M) were then established according to the equations: $Ls = M + t\,(SE)$ and $Li = M - t\,(SE)$, where t represents the value of the distribution of Student with a degree of freedom of $n-1$ and a probability of 5%. The increase (or decrease) of a parameter during the intermenstrual period was therefore statistically significant when the lower confidence limit was positive ($Li > 0$), which can be clearly shown graphically[15].

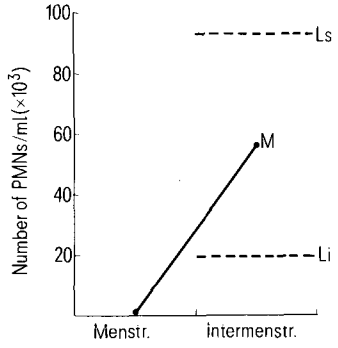

Fig. 1. Mean increase of the number of PMNs in 5 patients. The ordinate axis shows the number of PMNs per ml of gingival washing and the abcissa indicates the phases of the menstrual cycle: menstruation (*m*enstr.) and intermenstrual period (*inter*menstr.). The confidence limits are indicated by the dotted lines Ls and Li: the increase is statistically significant ($Li > 0$; $p < 0.05$).

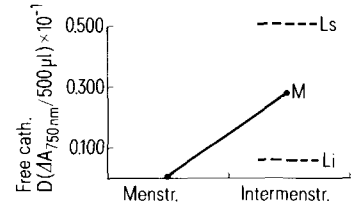

Fig. 2. The mean free activity of cathepsin D increased significantly in 5 patients during the intermenstrual period ($Li > 0$; $p < 0.05$).

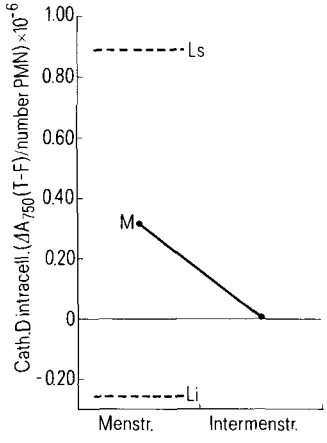

Fig. 3. Mean decrease of the intracellular activity of cathepsin D during the intermenstrual phases (Intermenstr.). The ordinate axis shows the intracellular activity of the enzyme, estimated by the difference between the total and free activities, divided by the number of PMNs in 1 ml of washing ($Li < 0$; $p > 0{,}05$).

Results. The concentration of PMNs in the washings increased for all the patients during the intermenstrual period. For the volunteer who had been followed over the longest period (12 cycles), the average concentration of PMNs at menstruation was of 46,900 and reached the peak of 118,000 during the intermenstrual period. The mean increase (M in figure 1) for the 5 patients amounted to 56,000 PMNs/ml.

The number of epithelial cells/ml of washing also increased significantly during the intermenstrual period ($M = 4900$ cells/ml; $Li > 0$).

Parallel to the increase in the number of cells, all the free and total activities of cathepsin D, elastase and β-glucuronidase showed a significant rise during the intermenstrual period. As an example, figure 2 shows the significant increase of the free activity of cathepsin D.

As for the intracellular specific activity (total - free activity/N° of cells) this parameter was found, for the 3 enzymes, to be maximum at menstruation and to decrease during the intermenstrual period. This is illustrated for cathepsin D in figure 3.

Discussion. The cellular and enzymatic analysis presented here show that the free and total activities of cathepsin D, elastase and β-glucuronidase, having a well-known intracellular distribution[16], followed the same pattern of cyclic variations as the number of PMNs, with a low activity during menstruation and an increase during the intermenstrual periods.

A bacterial origin of the 3 enzymes cannot be entirely excluded; however it should be emphasized that the hygiene of the volunteers was excellent (very little bacterial plaque). Moreover, recent investigations from our laboratory have shown the absence of bacterial isoenzymes of β-glucuronidase in gingival washings collected from healthy gingiva[17].

Gingiva can be considered as a target tissue for the female sex hormones. In ovariectomized rats, it has been observed that gingiva can quickly accumulate oestradiol-17β, a derivative of oestrone, having a marked hyperemic action[18]. Investigations in vitro have shown that gingiva, particularly when inflamed, possesses an enzymatic system capable of converting oestrone into oestradiol-17β[19]. Female sex hormones are known to increase circulating PMNs[20] and to induce a labilization of lysosomal membranes[8,9]: the increase in the free activities of the 3 lysosomal enzymes found in this study could be the result of both the increase in the number of PMNs and the increased fragility of the lysosomal membranes within these cells. As shown by the results concerning the intracellular concentration of enzymes, the PMNs indeed tended to lose more enzyme during the intermenstrual phase, when the amount of circulating (and probably gingival) sex hormones is at its maximum.

A high concentration of extracellular lysosomal enzymes could sensitize the gingival tissue and contribute to the aggravation of periodontal inflammation when bacterial plaque is present.

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Purification of 2-deoxy-2-dansylamido-D-glucose by affinity chromatography on a lectin-loaded agarose column

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Summary. A method is demonstrated to purify 2-substituted derivatives of glucose and sterically related sugars by affinity chromatography on Con A-sepharose. The method seems to be of rather general applicability.

Lectins are proteids mainly occurring in the seeds of plants, especially legumes, which have the property of binding specifically to mono- and oligosaccharides[1]. Lectins can be coupled to carrier materials, e.g. agarose; these matrix-bound lectins are powerful tools for separating sugar derivatives by affinity chromatography[2]. Agarose-bound lectins specific for mannose and glucose residues (Glycosylex, Miles; Con A-sepharose, Pharmacia), agarose and fucose (Fucosylex, Miles), or N-acetyl-D-galactosamine (agarose-soy bean agglutinin, Miles) are commercially available.

Fluorogenic or fluorescent sugar derivatives are very useful for the characterization of sugar transport systems and sugar carrier proteins in *Escherichia coli*[3,4]. Being interested in the transport mechanism of N-acyl-D-glucosamines, we synthesized the non-metabolizable fluorescent dansylated analogue of D-glucosamine (DansAGlc) by the reaction of dansyl chloride with glucosamine[5]. Looking for a method to purify the dansylated sugar from the reaction mixture, we found affinity chromatography on Con A-sepharose to be a new, powerful tool.