

TRADUZIONE GIURATA DALL'ITALIANO IN INGLESE - SWORN
TRANSLATION

HYALURONIC ACID AND PERIODONTAL DISEASE

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Introduction

Knowledge of the structure of collagen and the structural variations it undergoes in the various areas of the body is a prerequisite for understanding its nature and morphological and etiological characteristics and the reason why tissue is liable to "collagen disease", a term which does not indicate a well-defined clinical picture but is now commonly used to indicate a complex of functional and structural alterations of the intercellular substance of the mesenchyme which is not yet fully understood.

According to Ricci (1959), the term "mesenchymal disease" should replace "collagen disease" because of a largely histopathological criterion, ie. the fact that this particular disorder does not affect collagen properly so called, but all of the ground substance of the connective tissue (or mesenchyme).

The connective tissue consists of a set of mobile cells with multi-directional potential, fibrous structures and an amorphous ground substance; the relative proportions, physiochemical properties and spatial relationships of these constituents of the connective tissue vary according to the

site, species and function of the tissue.

The fibrous structures comprise collagen, reticulin and elastin, while the ground substance consists of a set of acid polysaccharides and proteins; this amorphous substance, which is insinuated between the fibrils and cements them together, is believed to consist of six different mucopolysaccharides, the main one being hyaluronic acid, together with chondroitinsulphuric acid A, B and C, chondroitin and ketosulphates; a 7th constituent is believed to be represented by heparin (Curri, 1961-62).

Recent studies of the biology and physiopathology of the ground substance of the periodontal connective tissues indicate that an alteration in the ground substance underlies the processes characterising periodontal disease; its depolymerisation by stresses of various natures and origins (bacterial, metabolic or hormonal) causes the loss of the specific selectivity whose function is to let useful substances through and prevent access by harmful substances (Prayer-Galletti, 1965).

The periodontium represents the anatomical outcome of a histogenetic process which affects a particular mesodermal unit (the follicle); its structural maturation is highly representative of the varied architectural specialisations specific to the different types of connective tissue.

Amici (1964) was convinced that both primary and secondary modifications in the static/dynamic attitude of the oral

cavity and any inflammatory processes of bacterial origin must be considered wholly ancillary phenomena, and not always secondary to the functional prime mover of a degenerative lesion of the periodontal connective tissue. According to this author, the syndrome described as "periodontosis" should be classified as an essential lesion of the periodontal connective tissue and defined by the term "periodontal connective-tissue disease".

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three phases are functionally interdependent during the life cycle of the organ.

In the cellular phase of the periodontium there are three main types of mesenchymal cells - the fibroblast, osteoblast and cementoblast.

The acellular phase comprises a fibrous system (collagen fibres, reticular, elastic and oxytalan fibres) and an amorphous interfibrillary system, the ground substance.

The mineral phase is a physical complex consisting of hydroxyapatite crystals and hydroxyapatite isomorphs, arranged in a stereotactic configuration.

From the chemical standpoint the ground substance is a non-fibrous, optically empty macromolecular extracellular aggregate which extends continuously through the intercellular spaces; its best identified constituent is high molecular weight carbohydrates which may be neutral (like glycogen) or

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acid (like chondroitinsulphuric acids, heparin, ketosulphate and hyaluronic acid, and non-sulphurated mucopolysaccharides containing anionic carboxyl groups). There are also non-collagen proteins and carbohydrate-protein complexes, otherwise known as glycoproteins.

The oral bacterial flora consists of micro-organisms which produce substances like collagenases, aminopeptidases, proteinases and hyaluronidases which can act selectively on the substrata contained in the organic phase of the periodontium.

Amici (1964) states that essential lesions of the periodontium, called periodontoses, present the most suitable physiopathological characteristics for the syndrome to be included among the complete connective-tissue diseases. He also suggests that connective-tissue disease of the periodontium could be considered a special response by a tissue system in unstable equilibrium to the non-replicable physiological conditions of the oral cavity.

The question therefore arises as to whether it is possible to develop substances able to re-establish the biological order in connective tissue in which the vital processes of synthesis and reduced repair capacity have been modified to the extent that they are almost irreversible.

In the economy of the ground substance of periodontal connective tissue, particular attention should be given to the

functional relationships between the "amorphous" ground substance of the connective tissue and the genesis of the ible

to develop substances able to re-establish the biological order in connective tissue in which the vital processes of synthesis and reduced repair capacity have been modified to the extent that they are almost irreversible.

In the economy of the ground substance of periodontal connective tissue, particular attention should be given to the functional relationships between the "amorphous" ground substance of the connective tissue and the genesis of the morphological elements constituting its structured part, both under physiological conditions and, in particular, in the presence of alterations of the normal organisational processes of the mesenchymal stroma.

Grossfeld, Meyer and Godman (1955) demonstrated that fibroblasts cultured in vitro produce mucopolysaccharides, and Gross (1951-57) showed the importance of some constituents of ground substance in the organisational process of the stromal tissues.

Among these constituents hyaluronic acid is of particular importance, and it can be considered, together with chondroitinsulphuric acid, the main representative of the mucopolysaccharides.

The conventional term "hyaluronic acid" is used to mean "acid mucopolysaccharides electively split from hyaluron-

ase". A definition which contemplates the multiple aspects of its chemical nature and its functions in the animal organism does not seem possible at present, because of the lack of certain knowledge of its molecular structure and its actual participation in the morphogenesis of collagen and its relationships with the other polysaccharides in the ground substance.

According to Favilli (1949), hyaluronic acid "is a highly viscous polysaccharide resulting from acetyl glucosamine and a hexuronic acid (very probably glycuronic acid) which is a normal constituent of the protein-carbohydrate complexes (also incorrectly called mucins) of mesenchymal origin, namely those of the synovial fluid, the umbilical cord and the vitreous humour, and those constituting the ground substance of collagen connective tissue".

Meyer (1947) considered it to be "an acid polysaccharide which in animal tissues bonds the water in the interstitial spaces and holds together the cells in a gel type matrix, also serving as lubricator and joint shock absorber".

According to Stacey (1946), while hyaluronic acid allows the passage of metabolites, it also has the important function of putting up a barrier against penetration by foreign substances, including germs which cause infectious diseases.

By definition, hyaluronic acid is an asulphurated acid polysaccharide consisting of a longer or shorter set of chains of polymers or homologues containing molecules of various

lengths whose molecular cleavage, induced by enzymatic hydrolysis (hyaluronidase) or by the action of strong acids, produces two monomers, namely D-glycuronic acid and D-glucosamine.

The structure of the hyaluronic acid extracted from the human umbilical cord by the Weissman method has been defined as follows: 3-O-beta-glucopyranurosyl (2-acetonamide-2-dioxy-3-O-beta-D-glucopyranurosyl)-2 acetamide-2-dioxy-D-glucose.

From the practical standpoint, the best sources of hyaluronic acid are the umbilical cord and the vitreous humour.

Extractive hyaluronic acid presents as a fine white powder, soluble in water and insoluble in alcohol and acetone; in aqueous solution its pH varies around ± 4 , and the pH of aqueous solutions of its salts ranges between 7 and 8.5.

Aqueous solutions of hyaluronic acid present relatively high viscosity, which gives the various extractive hyaluronic acids a special characteristic; this viscosity falls to a more or less marked extent at each attempt at isolation, and is higher in hyaluronic acid in the native state.

Under normal conditions hyaluronic acid is a ubiquitous constituent of the tissues and the parenchymal organs. It is found in human and animal skin, the ground substance of connective tissue, Wharton's jelly, the vitreous humour and the synovial fluid cells; it is also found in various pro-

portions in the tendinous tissue, the nucleus pulposus of the intervertebral disc, the lungs, thyroidal colloid substance and the heart valves.

Hyaluronic acid can also be found in pathologically altered or diseased tissues: in some tumours of mesenchymal origin, in cases of malignant synovioma and in sarcomas communicable by viruses.

Campani (1942) drew attention to the problem of the chemical composition of the exudates and transudates, and stated that hyaluronic acid is probably responsible for the positive Rivalta reaction in fluids of an inflammatory nature.

Branchini (1961) observed a marked increase in Hotchkiss-positivity in the dental pulp of patients suffering from periodontal disease compared with the pulp of normal teeth. This variation in colour is interpreted by the author as being due to the qualitative variation in the mucopolysaccharides, as it is known that where neutral mucopolysaccharides exist, greater affinity for Hotchkiss prevails (red colour), whereas when an issue which was particularly accentuated in the fibre bundles; high concentrations were also found in the vascular parietal endothelium of the gingival chorion, where Alcian-positivity is marked and evident, both under normal conditions and in the presence of slight, sub-pathological infiltrates.

As regards the cementum, Baratieri found that the pro-

portions of acid mucopolysaccharides are particularly high in the nucleus and periphery of the cell and at the outer limit of the cementum of the upper and middle third of the root length. Finally, study of the mucopolysaccharide locations in the gingiva, cementum, alveolar cortex and periodontal membrane under pathological conditions led that author to state that "the result of stimulation of the repair processes found in periodontal disease during chronic inflammation seems to be characterised by an increase in visualisation of acid mucopolysaccharides and an increase in reticular fibres; the mucopolysaccharides detected probably consist of mucoproteins and hyaluronic acid".

Various techniques are used to detect hyaluronic acid in the tissues and organic fluids; the most common are the Meyer and Palmer turbidimetric technique (1936) modified in 1948 by Dorfmann and Ott, the colorimetric method devised by Greif in 1948 and perfected by Pierce, Steel and White in 1953, and the more recent techniques of electrophoresis and assaying the oligosaccharides participating in the structure of the hyaluronic acid molecule.

The effects of administering hyaluronic acid in its various combinations to the animal organism are many and varied, and not yet fully known.

Hyaluronic acid can be considered the main constituent of the ground substance of connective tissue, which contains substances in transit between the vascular system and cell

elements; hyaluronic acid gives the connective tissue elasticity, tensile strength and hydrophilia, and forms a well-defined chemico-physical system together with the collagen fibrils (Prayer-Galletti, 1965).

Experiments and clinical trials have established that hyaluronic acid has the following biological functions:

1) Fibrillogenetic activity: on the basis of morphological and histochemical observations of the evolution of the connective tissue's organisational processes in varicose ulcers subjected to local infiltrations with hyaluronic acid, Czemely and Curri (1958) suggested that the mucopolysaccharides may interfere with the genesis of granulation tissue. Curri (1958) states that the administration of hyaluronic acid influences the tissue reaction to neogenesis of rapidly proliferating granulation tissue. Hyaluronic acid is of particular importance as a totally new drug and as biochemical substrate of mesenchymal reactivity in disorders involving poor tissue regeneration capacity (Barbieri, Curri and Pecile, 1959); this is due to the intense fibrillogenetic effect and accelerated differentiation into fibroblasts of the primitive mesenchymal elements (Curri, 1963). It is also believed to govern differentiation of the primitive mesenchymal elements into fibroblasts and chondroblasts (Curri and Maschio, 1959) and osteoblasts (Lunardi and Curri, 1958; Barbieri, Curri and Pecile, 1959; Curri a involving poor tissue regeneration capacity (Barbieri, Curri and Pecile, 1959); this is due to the intense fibrillo-

genetic effect and accelerated differentiation into fibroblasts of the primitive mesenchymal elements (Curri, 1963). It is also believed to govern differentiation of the primitive mesenchymal elements into fibroblasts and chondroblasts (Curri and Maschio, 1959) and osteoblasts (Lunardi and Curri, 1958; Barbieri, Curri and Pecile, 1959; Curri and Castiglioni, 1963) through early development of granulation tissue which proliferates rapidly and is differentiated into osteoblasts, with rapid chondroid evolution and subsequent bone transformation.

2) Anti-inflammatory activity is evidenced by the scarcity of the inflammatory component in granulation tissue, because an excess of hyaluronic acid inactivates bacterial hyaluronidase; it circumscribes the inflammatory process and aids local defences by activating the reticuloendothelial system (Cucurachi, Portioli and Curri, 1961).

3) Anti-exudative activity by normalisation of increased capillary permeability, anti-hyaluronidase action performed by hyaluronic acid, anti-diffusion and plasma loss inhibition factor (Confortini et al., 1959).

4) Hydropexic activity: hyaluronic acid performs an evident, rapid anti-diffusion effect evidenced by inhibition of the inflammatory process, increases tissue hydrophilia and restores interstitial exchanges to normal (Curri, 1963; D'Aroma, 1964).

Under pathological conditions such as those causing trophic

disorders based on a circulatory malfunction of the skin and inflammatory processes in general, the hyaluronic acid normally existing in the ground substance is split into its constituents by a specific enzyme, bacterial hyaluronidase, and torpidity of the connective-tissue repair processes may in some cases be associated with a local hyaluronic acid deficiency (Czermely and Curri, 1958) which reduces the ability to constitute a protective barrier, maintain the tissue fluid balance and perform an anti-diffusion, anti-inflammatory effect.

In the elderly, the cicatrising and bone-forming repair processes are performed particularly slowly, because in old age the connective tissue gradually loses its mucopolysaccharide constituent. According to Prayer-Galletti (1965), sclerosis, hyalinosis, water loss and reduced turnover between circulating blood and tissue cells are associated with physiological involution of the ground substance and a reduction in the percentage of interstitial hyaluronic acid.

It therefore appears evident that a relationship exists between the local proportions of mucopolysaccharides and glycoproteins and the normal connective-tissue organisation processes.

Purpose of study

The purpose of this research was to establish whether, by

increasing the amount of one of the mucopolysaccharide constituents of ground substance (hyaluronic acid), it is possible to improve the clinical and histological evolution of the inflammatory and dystrophic processes affecting the periodontium, ie. those systemic-progressive diseases which damage both individual elements of the organ supporting the tooth and the organ as a whole, prejudicing the intactness and static-dynamic function of the tooth.

Personal contribution

Technique

The substance used for this research was a hyaluronic-acid-based preparation of high purity free of protein residues, consisting of sodium hyaluronidate in the proportion of 4 mg to each 2 cc vial.

The forms of periodontal disease examined were diagnosed as follows:

- 1) hyperplastic gingivitis
- 2) diffuse marginal periodontitis
- 3) mainly dystrophic periodontal disease.

To avoid any nosological uncertainty we should specify that:

- a) By hyperplastic gingivitis we mean hyperplasia of the gingiva interproximally, labially, and to a lesser extent buccolingually and, where appropriate, chronic productive gingival inflammation. Gingival hyperplasia provides favourable conditions for the accumulation of food residues because it increases the depth of the gingival crevices and



interferes with effective hygiene and cleaning by food, by deflecting the normal food flow routes during chewing.

In conclusion, it is made up of two constituents: primary hyperplasia of the connective tissue and epithelium, whose origin is unrelated to the inflammation, and a secondary inflammatory component that complicates the first condition, which can sometimes be concealed by it.

b) The term diffuse marginal periodontitis describes cases in which the disease event, which originated with an inflammatory alteration of the gingiva, subsequently spreads to a greater or lesser extent to all the periodontal elements, from the gingiva to the underlying alveolar bone and the periodontal membrane, leading to structural modifications and gradual destruction of these tissues, with consequent loss of the tissue supporting the tooth.

c) The forms of mainly dystrophic periodontal disease are characterised by the fact that dystrophic/involucional symptoms prevail over inflammatory symptoms. In the pure forms there is a non-inflammatory reduction in the height of the alveolar bone, accompanied by retraction of the gingiva without the formation of an actual pocket. However, these forms are often complicated by supervening secondary inflammation of the periodontal tissues, with additional loss of alveolar bone.

30 patients of both sexes were treated, namely 13 men and 17

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women aged 18 to 73, suffering from forms of periodontal disease of varying degrees of severity but always diffuse, broken down as follows: 7 cases of hyperplastic gingivitis, 1 case of mainly dystrophic periodontal disease and 22 cases of marginal periodontitis.

None of the 30 patients had undergone previous treatment, except that prior to the trial thorough scaling was performed in order to prevent the possible onset of local complications due to the presence of an irritant stimulus.

The preparation tested was administered by submucosal injection, after disinfection of the injection site, of 2 cc for each upper and lower hemiarch, injected at the boundary between adherent gingiva and free gingiva. The contents of two control vials each containing 1 mg of Lidocaine in 2cc of saline solution were injected into the other two hemiarches as placebo (Table 1).

Clinical results

In all cases the infiltrations were well tolerated; apart from a slight pain reaction lasting for a few hours after the first infiltration, all the subsequent infiltrations produced lesser disturbances. At the same time further injections of the drug became gradually more difficult to administer because of the connective tissue's reaction to them.

The only exception was case 10, a patient with erethistic

characteristics, who discontinued treatment after the injection of 6 vials complaining of persistent pain at the infiltration site.

Patient 15, however, was given 16 infiltrations, all tolerated without any particular pain reaction.

In all cases, as from the first infiltrations, the patients reported a subjective feeling of improvement which became increasingly evident as the treatment progressed. This improvement was clinically characterised by attenuation of the inflammatory symptoms in the tissues affected, together with considerable reduction in the exudate formed in the pockets. The reduction in fermentation of food residues, stagnating in lesser amounts in the gingival pockets, probably contributed to this attenuation of the symptoms, and in some cases in which fetor oris was constantly present it gradually disappeared.

Objectively, in addition to the above-mentioned cessation of pus formation, the gingiva ceased to bleed readily, the size of the periodontal pockets reduced and a reasonable improvement was observed in loosening of the teeth, where present. The result was an improvement in masticatory efficiency (Figs. 1, 2, 3 and 4). These results were observed in all cases treated, though to different extents (Tables 1 and 2). It should be noted that these results were obtained with the local treatment tested alone, even in the case of patients who presented systemic factors in association with the local

present pathological alterations of the outermost layers, in other areas signs of necrosis were observed, with loss of the outermost layers. The base of the solution of continuity appeared to be covered by a thin layer of weakly Schiff-positive fibrinoid substance; numerous red corpuscles were present between the meshes of the fibrin lattice.

The epithelium deepened in fingerlike projections towards the sub-mucosa. These projections were arranged in columns at some points, anastomosing together to enclose islets of a connective tissue generally containing few cells and consisting of a sclero-hyalinotic framework.

Large connective-tissue frameworks arranged parallel to that of the mucosa are observed in the innermost layers.

At other points the epithelium projected in papillae towards a connectival stroma whose outermost layers contained few cells, while in the innermost layers it appeared to be invaded by numerous lymphocyte-like elements and rare histioid and monocytoïd elements (plate IA).

These areas had a rounded or irregular shape and were surrounded by septa of sclero-hyalinotic connective tissue containing few cells. At other points the epithelium of the mucosa deepened, forming characteristic nest-like structures in which individual rounded areas of connective tissue appeared to be surrounded by the epithelium; numerous capillary vessels with swollen, projecting endothelium were ob-

served in these areas (plate Ia A).

In the innermost areas of the gingival stroma the cell infiltrate, consisting of elements with a large, rounded or oval nucleus with a loose chromatinic meshwork and lymphocytoid elements, was very abundant (plate I C).

The vessels were dilated; no alterations in the wall of the small arteries were observed, except for tumefaction of the endothelia. The venous vessels were full of blood, but presented no pathological alterations.

Second biopsy 13/1/1967. After treatment.

The entire area of the mucosal epithelium presented a regular arrangement, without solutions of continuity in the outermost layers (plate I B); it projected into the sub-mucosa forming regular projections and islets of various shapes and sizes, sometimes arranged in columns and sometimes circular, surrounding areas of connective tissue entirely free of inflammatory infiltrates (plate I D).

The connectival stroma of the sub-mucosa consisted of fibre bundles which in some areas contained few cells and in others contained numerous chunky fibroblasts, with cytoplasm packed with fine, Schiff-positive granules (plate Ia B). No inflammatory infiltrates were found in the areas from which the biopsy was taken, except in a limited area situated at the apex of papillae projecting into the stroma. An accumulation of lymphocytoid elements and occasional granulocytes was observed in this area.

Case 21: Elena R.

First biopsy 17/11/1966. Before treatment.


The continuity of the mucosa was interrupted in many places; in some areas the epithelium was totally absent and the break was invaded by an infiltrate consisting of lymphomonocyte elements in the necrotic tissue, which presented numerous blood-filled areas. The epithelium of the papillary projections appeared to have proliferated at the edges of the break, forming a kind of columns which partially circumscribed the necrotic zone. The mucosa presented no particular alterations at other points of the biopsy specimen.

The connectival stroma consisted of frameworks of intensely eosinophilic, sclero-hyalinotic fibres containing few cells.

Parvicellular infiltrates were observed around some small vessels.

Second biopsy 29/11/1966. After treatment.

The outermost layers of the lining epithelium consisted of elements with a pale oval nucleus and a large, leaflike cytoplasm halo packed with intensely Schiff-positive granules which almost entirely occupied the element. The arrangement of the cells was highly irregular; as they deepened towards the sub-mucosa they presented considerable modifications in both nucleus shape and the volume of the element. A column arrangement of the basal layers could not be recognised; they projected in a disorderly manner towards



the connectival stroma of the innermost layers. It appeared to be abundantly infiltrated by closely-packed lymphomonocyte and histiocyte elements; no haemorrhagic infiltrates were detected. The architecture of the sub-mucosal stroma appeared to have been totally subverted; the tissue consisted of thin Alcian-blue-positive fibrils, indicating intense neofibrillogenesis. Areas in which the connective tissue was loose and rich in fibroblasts were embedded in the existing connective-tissue framework with closely and irregularly interwoven sclero-hyalinotic fibres.

Case 22: Iole C.

First biopsy 1/12/1966. Before treatment.

The biopsy specimen was taken from areas in which neither the mucosa nor the sub-mucosa presented pathological alterations. The gradual thinning of the epithelium, whose basal layer projected towards the underlying layers with smaller and smaller papillae, could only be seen in a limited area. The gingival epithelium had been lost in a small area. The solution of continuity was covered in necrotic material, and the base consisted of inflammatory tissue, rich in inflammatory elements and newly-formed vessels, which reached the innermost layers of the stroma. It consisted of connectival shoots containing few fibroblasts which were dissociated from the inflammatory infiltrate.

On the opposite side, the epithelium was still intact, and rested on a sclero-hyalinotic stroma containing few vessels.

At some points of the innermost layers the sub-mucosa appeared to be intensely infiltrated (plate II A).

Second biopsy 3/1/1967. After treatment.

The epithelium was perfectly preserved, hyperplastic and projected regularly as far as the innermost layers of the stroma (plate IIB). It appeared to consist of connective tissue rich in fibroblasts and histioid elements, with numerous newly-formed capillaries (plate II C). No diffuse inflammatory infiltrates were observed; lymphomonocytoid cells and some rare monocytes were only found in some limited areas, irregularly scattered in the connectival stroma.

Case 25: Giorgio Z.

First biopsy 24/11/1966. Before treatment.

The epithelium appeared thin at many points, with atrophic papillae which only deepened for short stretches in the connectival stroma of the gingiva. The connective-tissue frameworks were disordered and invaded by an inflammatory infiltrate consisting of lymphomonocyte elements, histocytes and occasional plasma cells (plate III A).

In some zones, areas entirely packed with haemorrhagic extravasation were observed, especially sub-epithelially. The vessels were dilated, with hyperplastic endothelium projecting into the lumen (plates III C, III A and IIIa).

Second biopsy 28/11/1966. After treatment.

No alterations were observed in the epithelial lining, which did not present erosion or solutions of continuity (plate III B).

It proliferated towards the innermost layers of the gingival connectival stroma with rather thin but regularly arranged papillae, enclosing areas of the gingival stroma which consisted of connective tissue rich in fibroblasts and histiocyte elements (plate III D).

The connectival stroma of the gingiva appeared far less infiltrated by inflammatory elements than the previous specimen, and these elements were scattered irregularly without any relationship of juxtaposition with the vessels; the infiltrate consisted of lymphocytes mixed with large histiocyte elements. The vessel walls presented no pathological alterations, and were only blood-filled in some areas.

Case 26: Nicola M.

First biopsy 7/12/1966. Before treatment.

A large area of the gingival margin appeared completely devoid of epithelium; the base of the ulcer consisted of sub-mucosa densely infiltrated by inflammatory elements, with areas presenting more or less manifest necrosis. At many points the connectival stroma presented areas densely infiltrated by irregularly scattered lymphomonocyte elements. The connective tissue was formed by large sclero-

hyalinotic frameworks containing few fibroblasts.

Second biopsy 13/1/1967. After treatment.

Compared with the picture previously described, although no breaks in the lining epithelium were found in the biopsy fragment, the inflammatory component remained in the connectival stroma, especially in the innermost areas, supported by abundant parvicellular infiltrate which invaded and dissociated the connective-tissue frameworks.

Case 27: Maria F.

First biopsy 17/3/1966. Before treatment.

Only limited areas in which the epithelium was preserved were found in the biopsy fragment. At the gingival pocket the mucosa projected deeply towards a connectival stroma rich in cells, with the characteristics of irritable tissue, infiltrated by parvicellular elements and relatively rich in vessels.

The symptoms of myositis were observed in the muscles of the deep layers, with more or less evident degenerative phenomena and fragmentation of the fibrils.

Second biopsy 6/5/1966. After treatment.

The mucosa was well preserved, but the outermost layers were very thin. The sub-mucosa, however, appeared to be abundantly infiltrated by inflammatory elements represented by lymphocytes and polymorphs.

Case 28: Rosanna M.

First biopsy 11/3/1966. Before treatment.

Extremely thin, atrophic mucosa with slender papillae projecting moderately into a stroma with an abundant inflammatory component. Numerous perivascular infiltrates in the innermost layers. Dilated, blood-filled vessels (plate IV A).

Second biopsy 5/5/1966. After treatment.

The innermost layers of the connectival stroma were less involved in the inflammatory process than before treatment; however, numerous nests of inflammatory infiltrates, especially perivascular, remained. The connectival frameworks were more compact, and less dissociated and fragmented (plate IV B).

Case 29: Carla T.

First biopsy 9/3/1966. Before treatment.

Extensive inflammatory infiltrate in sub-mucosa, with zonal areas of necrosis. Acanthotic epithelium with free pearls in the stroma. Interstitial myositis.

Second biopsy 14/4/1966. After treatment.

The inflammatory components had considerably diminished, except in limited areas. The epithelium was thin but more regularly stratified. Although the connective-tissue bundles were often fragmented and subject to hyalinosis, in some areas they presented a particular wealth of cells (fibroblasts and histiocytes).

Case 30: Caterina G.

First biopsy 28/3/1966. Before treatment.

Epithelium regularly arranged but thin, with evident keratinisation of the outermost layers. At one point there was evident ulcerative erosion, with the base covered by necrotic material which reached the sub-mucosa.

The stroma appeared to be infiltrated by mainly lymphomonocyte infiltratory elements.

Second biopsy 24/4/1966. After treatment.


A stromal inflammatory component still remained, though to a lesser degree. In one area there was a large cavity full of necrobiotic material with haemorrhagic extravasations.

Intercapillary sub-mucosa relatively intact and free of infiltrates.

Evident movement of neofibrillogenesis, with active fibroblast and histiocyte proliferation in the innermost layers.

Discussion

It is impossible to comment on the arrest of osteolysis phenomena and the possible restoration of destroyed bone tissue because of the short period which elapsed between the first X-rays and the end of the trial; however, the radiological evidence will be presented and discussed in a subsequent note after the necessary tests have been performed at a longer interval.



It should be noted that although the clinical improvements obtained at the site of hyaluronic acid infiltration were far more evident, a reasonable though modest improvement was also observed in the hemiarches where the control injections were performed. This may be due to the more effective dental hygiene practised by the patient; we do not propose to consider here whether the action of hyaluronic acid may be more than purely local.

We will now move on to an overall evaluation of the results obtained with the various disorders examined (Table 2). It will be noted that in the half the cases of hyperplastic gingivitis observed in male patients the alterations were associated with malocclusion or dental malpositions complicated by malocclusion and occlusal imbalances, whereas in the case of the female patients hormonal imbalances and general factors conducive to the disease, particularly during puberty, were always present.

In the case of mainly dystrophic periodontal disease, disorders attributable to occlusal trauma such as pain affecting both temporomandibular joints (chronic arthritis, presumably of traumatic origin) were evident in addition to the occlusal trauma.

In 15 patients suffering from diffuse marginal periodontitis, secondary disturbances induced by occlusal trauma which were able to modify and increase the destruction of

the tissues supporting the tooth were constantly evident, and two female patients belonging to the same group presented pain, clicking and crepitation of the temporomandibular joints.

The results as a whole were excellent in 20 cases and good in a further 10 cases.

An excellent result was even obtained in case 18, a patient suffering from slight diabetes, a systemic disorder which, by means of metabolic alterations, accelerates the loss of alveolar bone in periodontal disease; the patient's diabetes was under control, but it should be borne in mind that it is always necessary to eliminate all the other etiological factors of periodontal disease in order to obtain a healthy periodontium, even in patients with controlled diabetes.

Conclusions

The above considerations highlight the therapeutic characteristics of the drug tested, and the following properties justify its being described as worthy of interest:

- 1) it accelerates the fibrillo-plastic differentiation of the primitive mesenchymal elements, leading to the formation of tissue characterised by considerable neofibrillopoiesis and abundant formation of connective-tissue frameworks which sometimes even invade the leiomyogenic layer
- 2) it circumscribes and subsequently eliminates the inflammatory process and aids the local defences, probably by

activating the reticulohistiocyte system, deactivating bacterial hyaluronidase and maintaining the tissue fluid balance, thus performing an anti-diffusion and anti-inflammatory effect and promoting the normal connective-tissue organisation processes.

Preparations which exploit the action of hyaluronic acid are of particular importance in this respect, as they are capable of effectively treating disorders featuring poor tissue regeneration capacity.

Fig. 1. Case no. 6, B.G.

A: before treatment

B: after treatment $\frac{C|c}{C|c}$ (C = connective [sic] c = placebo)

Fig. 2. Case no. 15, S.T.

A: before treatment

B: after treatment $\frac{C|c}{C|c}$

Fig. 3. Case no. 16, T.C.

A: before treatment

B: after treatment $\frac{C|c}{C|c}$

Fig. 4. Case no. 24, T.L.

A: before treatment

B: after treatment $\frac{C|c}{C|c}$

TABLE I

Case no.	Name	Sex	Age	Diagnosis	Local factors	General factors	Dose (vials)	Infiltration site quadrants	Results
1)	B.P.	M	18	Hyperplastic gingivitis	Dysgnathia	-	10	$\frac{c}{c}$ $\frac{c}{c}$	+++
2)	M.M	F	33	Marginal periodontitis	-	-	10	$\frac{c}{c}$ $\frac{c}{c}$	++
3)	B.A.	F	42	Marginal periodontitis	Occlusal trauma	Arthritism	10	$\frac{c}{c}$ $\frac{c}{c}$	+++
4)	R.P.	M	26	Hyperplastic gingivitis	Occlusal trauma	-	10	$\frac{c}{c}$ $\frac{c}{c}$	++
5)	P.D.	M	45	Marginal periodontitis	Extensive tartar deposits. Occlusal trauma	-	10	$\frac{c}{c}$ $\frac{c}{c}$	+++
6)	B.G.	M	45	Marginal periodontitis	Extensive tartar deposits. Occlusal trauma	-	10	$\frac{c}{c}$ $\frac{c}{c}$	+++
7)	V.A.	F	18	Hyperplastic gingivitis	Occlusal trauma	Dysendo- crinia	10	$\frac{c}{c}$ $\frac{c}{c}$	++
8)	D.A.	F	40	Marginal periodontitis	Occlusal trauma	-	10	$\frac{c}{c}$ $\frac{c}{c}$	++
9)	B.M.	M	21	Marginal periodontitis	-	-	10	$\frac{c}{c}$ $\frac{c}{c}$	++
10)	F.M.	F	38	Marginal periodontitis	Occlusal trauma	-	10	$\frac{c}{c}$ $\frac{c}{c}$	+++
11)	F.C.	M	27	Marginal periodontitis	Occlusal trauma	-	6	$\frac{c}{c}$ $\frac{c}{c}$	++
12)	F.L.	M	18	Hyperplastic gingivitis	Dysgnathia	-	10	$\frac{c}{c}$ $\frac{c}{c}$	+++
13)	G.C.	M	43	Marginal periodontitis	Occlusal trauma	-	10	$\frac{c}{c}$ $\frac{c}{c}$	++
14)	M.T.	F	73	Mainly dys-trophic periodontal disease	Occlusal trauma	Arthritism	10	$\frac{c}{c}$ $\frac{c}{c}$	+++
15)	S.T.	F	35	Marginal periodontitis	Occlusal trauma	-	16	$\frac{c}{c}$ $\frac{c}{c}$	+++

(Table I cont.)

Case no.	Name	Sex	Age	Diagnosis	Local factors	General factors	Dose (vials)	Infiltration site quadrants	Results
	F.A.	F	42	Marginal periodontitis	Occlusal trauma	-	10	$\frac{c c}{c c}$	++
17)	F.A.	F	19	Hyperplastic gingivitis	-	Dysendocrinia	10	$\frac{c c}{c c}$	+++
18)	O.G.	M	52	Marginal periodontitis	Occlusal trauma	Slight diabetes	10	$\frac{c c}{c c}$	+++
19)	T.G.	M	22	Hyperplastic gingivitis	-	-	10	$\frac{c c}{c c}$	+++
20)	I.F.	F	55	Marginal periodontitis	-	Arthritism	10	$\frac{c c}{c c}$	+++
21)	R.E.	F	37	Marginal periodontitis	Occlusal trauma	-	10	$\frac{c c}{c c}$	+++
22)	C.I.	F	40	Marginal periodontitis	-	-	10	$\frac{c c}{c c}$	+++
23)	B.M.	F	36	Marginal periodontitis	Occlusal trauma	-	10	$\frac{c c}{c c}$	+++
24)	T.L.	M	45	Marginal periodontitis	Extensive tartar deposits	-	10	$\frac{c c}{c c}$	+++
25)	Z.G.	M	27	Marginal periodontitis	-	-	10	$\frac{c c}{c c}$	++
26)	M.N.	M	26	Marginal periodontitis	Occlusal trauma	-	10	$\frac{c c}{c c}$	+++
27)	F.M.	F	36	Marginal periodontitis	-	-	10	$\frac{c c}{c c}$	+++
28)	M.R.	F	28	Hyperplastic gingivitis	Occlusal trauma	Dysendocrinia	10	$\frac{c c}{c c}$	+++
29)	T.C.	F	40	Marginal periodontitis	Occlusal trauma	-	10	$\frac{c c}{c c}$	+++
30)	G.C.	F	34	Marginal periodontitis	Occlusal trauma	-	10	$\frac{c c}{c c}$	++

c = connectivin; c = control

TABLE II

Diagnosis	No. of cases	Age	Sex	No.	Local factors	General factors	Average dose	Result
Hyperplastic gingivitis	7	18-26	M	4	Dysgnathia: 2, occlusal trauma: 1		10	In 5 cases +++ In 2 cases ++
			F	3	Occlusal trauma: 1	Dysendo- crinia: 3		
Mainly dys- trophic periodontal disease	1	73	F	1	Occlusal trauma	Arthritism	10	+++
Marginal perio- dontitis	22	21-55	M	9	Abundant tartar deposits: 3 Occlusal trauma: 6	Slight diabetes: 1	10	In 14 cases +++ In 8 cases ++
			F	13	Occlusal trauma: 9	Arthritism: 2		

Plate 1

F.A.: gingival margin biopsy, A and C: before treatment. Enlargement 10 x 1.25 x 8; 40 x 1.25 x 8. B and D: after treatment. Enlargement 6.3 x 1.25 x 8; 10 x 1.25 x 8. Fixed with 10% formalin, stained with Alcian-Blue-Pas.

Plate 1a

F.A.: gingival margin biopsy, A: before treatment. Enlargement 10 x 1.25 x 8. B: after treatment. Enlargement 40 x 1.25 x 8. Fixed with 10% formalin, stained with Alcian-Blue-Pas.

Plate II

C.I.: gingival margin biopsy. A: before treatment. Enlargement 6.3 x 1.25 x 8. B: after treatment. Enlargement 6.3 x 1.25 x 8. Fixed with 10% formalin, stained with haematoxylin-eosin. C: after treatment. Enlargement 10 x 1.25 x 8. Fixed with 10% formalin, stained with haematoxylin-eosin.

Plate III

Z.G.: gingival margin biopsy. A and C: before treatment. Enlargement 6.3 x 1.25 x 8; 6.3 x 1.25 x 8. B and D: after treatment. Enlargement 6.3 x 1.25 x 8; 6.3 x 1.25 x 8. Fixed with 10% formalin, stained with haematoxylin-eosin.

Plate IIIa

Z.G.: gingival margin biopsy. A: before treatment. Enlargement -----. Fixed with 10% formalin, stained with haematoxylin-eosin.

Plate IV

M.R.: gingival margin biopsy. A: before treatment. Enlargement 6.3 x 1.25 x 8. B: after treatment. Enlargement 6.3 x 1.25 x 8. Fixed with 10% formalin, stained with haematoxylin-eosin.

SUMMARY

Department of Stomatology (Consultant: Prof. C. Ceria),
Maria Vittoria Hospital, Turin.

F. Brandimarte: Hyaluronic acid and periodontal diseases.
After a brief survey of previous studies on the biology and

clinical applications of hyaluronic acid, the author reports the results of research on the usefulness of local administration of this substance to patients with periodontal disease. On the basis of clinical and histological observations the results obtained demonstrated that the polysaccharide accelerated the processes of connective-tissue organisation in periodontal disease by means of its fibrillogenetic, anti-inflammatory and anti-exudative activities. ("Min. Stom.", 17, 140-156, 1968. F. Brandimarte: "Acido ialuronico e parodontopatie".)


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