HISTOLOGICAL INVESTIGATIONS OF "CARA INCHADA" IN CATTLE[1]

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SINOPSE.- Seifert K., Walter P., Döbereiner J. and Rüsse I. 1983. [**Estudos histológicos da doença peridentária "cara inchada" dos bovinos.**] Histological investigations of the periondontal disease "Cara inchada" in cattle. *Pesquisa Veterinária Brasileira 3(2): 67-70.* Institut f. Tieranatomie, Universität München, Veterinärstr. 13, D-8000 München 22, Alemanha Ocidental.

Foram realizados estudos histológicos da gengiva e do periodôncio interdentário de oito bezerros afetados pela "cara inchada" e de oito outros sadios, a fim de verificar possíveis alterações do colágeno nessa doença. As alterações observadas consistiram em degeneração e perda das camadas superficiais dos epitélios marginais externo e interno da gengiva, bem como invasão da lâmina própria, que estava infiltrada principalmente por granulócitos eosinófilos, pelo epitélio juncional. O colágeno nas áreas afetadas encontrava-se edemaciado, e o colágeno tipo I estava menos denso que o tecido conjuntivo circunvizinho, lembrando o das espécimes de controle. No osso alveolar foram observadas alterações osteofibróticas. As relações de causa e efeito, entre as alterações no epitélio, tecido conjuntivo e osso alveolar, são discutidas em relação às reações alérgicas, à integridade e ao metabolismo do colágeno e à osteodistrofia fibrosa. O fato de que o colágeno, nas partes não infiltradas, estava normal tanto estruturamente como em relação às propriedades tintoriais, leva a concluir que as alterações do colágeno, na "cara inchada", são de natureza secundária.

TERMOS DE INDEXAÇÃO: Doença peridentária, "cara inchada", bovinos, histologia, colágeno, gengiva periodôncio.

ABSTRACT.- With the aim of determining possible collagen changes in Cara inchada, investigations of gingiva and interdental periodontium of eight diseased and eight healthy calves were performed. Findings included degeneration and loss of superficial layers of marginal and crevicular epithelia and proliferation of the junctional epithelium into the lamina propria, which was infiltrated mainly by eosinophilic granulocytes. The collagen in affected areas was edematous and collagen I was less dense then the surrounding connective tissue, which resembled that of the control specimens. Osteofibrotic changes were observed in the alveolar bone. Cause and effect relationship between the changes in epithelium, connective tissues and alveolar bone are discussed with reference to allergic reactions, the integrity and metabolism of collagen and osteodystrophia fibrosa. The fact that collagen in non-infiltrated parts displayed normal structural and staining properties leads to the conclusion that the collagen changes in Cara inchada are of a secondary nature.

INDEX TERMS: Periodontal disease, Cara inchada, cattle, histology, collagen, gingiva, periodontium.

INTRODUCTION

Cara inchada is a periodontal disease occurring in pure bred and mixed Zebu cattle in West Central Brazil. Clinical, histopathological (Döbereiner et al 1974) and radiographical (Nunes et al. 1979), as well as other investigations, have not yet revealed the etiology of the disease. According to the results of some field experiments, alimentary factors seem to play a role (Döbereiner at al. 1975, 1976, Rosa et al. 1976). It appears very likely that the structure and composition of the soil are important (Döbereiner 1982, pers. comm.), the significance possibly lying in the specific population of microorganisms inhabiting the soil (Page & Schroeder 1982). The initial lesion takes place during eruption of teeth, possibly due to impaired physiologic regeneration of the periodontal connective tissue (Döbereiner et al. 1974). On the other hand, Nunes et al. (1979), based on the results of their investigations, suggested the disease to be an osteodystrophia fibrosa of nutritional origin.

The possibility exists that primary collagen changes play a pathogenic role in Cara inchada. Therefore histopathological studies of the connective tissue of the gingiva and periodontal ligament were performed.

MATERIALS AND METHODS

Material was obtained from eight Cara inchada diseased pure and mixed bred Zebu cattle between one and four months of age. Eight healthy

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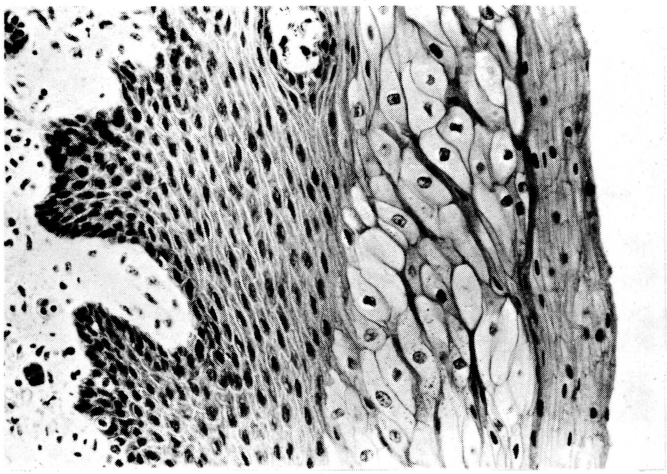

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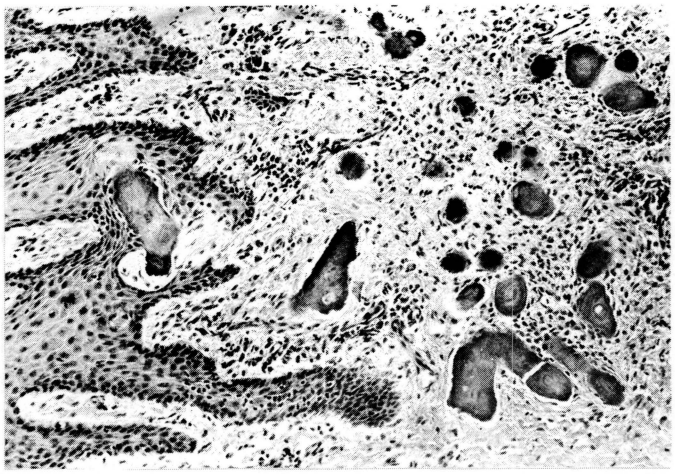

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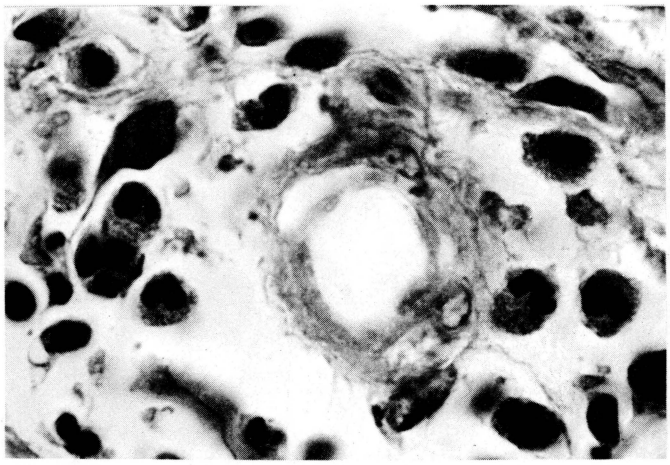

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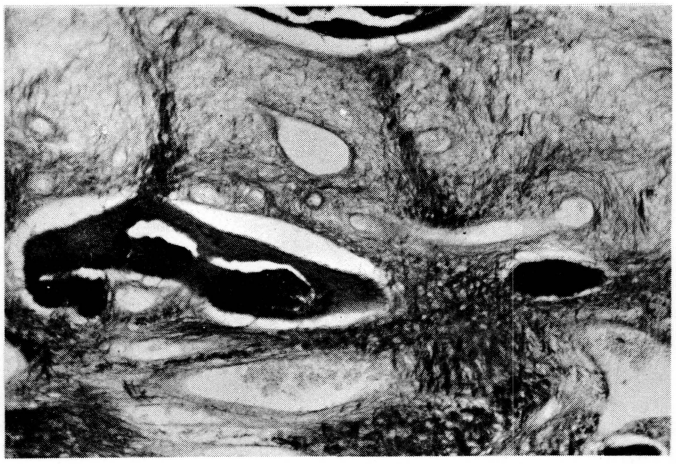

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Fig. 1. *Swelling of the prickle cell layer (stratum spinosum) in the marginal epithelium. H. E., Obj. 25.*

Fig. 2. *Calcifications in the proliferated basal epithelial cells and the infiltrated connective tissue. H. E., Obj. 10.*

Fig. 3. *Eosinophilic granulocytes in the infiltrated connective tissue. H. E., Obj. 100.*

Fig. 4. *Osteofibrotic changes in the interdental alveolar septum. Picrosirius Red, Obj. 10.*

calves were used as control animals. Investigations concerned mainly the interdental tissue between the second and third maxillar premolars (Pd_3 and Pd_4 resp.). In most cases the interdental papilla between Pd_2 and Pd_3 as well as that between Pd_4 and M_1 were also examined. Specimens were fixed in 10% formaldehyde or 6% glutardialdehyde (in 0,15 M Na-cacodylate buffer + 6% saccharose at pH 7,3). After washing in in - and decreasing concentrations of alcohol, the samples where the soft periodontal tissues remained attached to the tooth and alveolar bone were decalcified in 7% nitric acid. They were hung in lithium sulfate for 24 hours to prevent connective tissue swelling before being washed and dehydrated in alcohol. The decalcified samples and the gingival material was embedded in paraplast (Lancer) and cut at 7 - 8 μm, partially serially, and stained as follows: haemalaun-eosin (non-specific), light-green according to Goldner (connective tissue), resorcin-fuchsin (elastic fibres) (Romeis 1968) picrosirius-red (differentiation of collagen types) (Sweat et al. 1964) and peracetic acid-aldehyde-fuchsin-halmi (Fullmer & Lillie 1958).

After fixation with glutardialdehyde, the samples were washed in cacodylate buffer (as above), dehydrated in acetone, embedded in Araldyte^R (Serva) and polymerized before sectioning at 1-2 μm and staining with methylene blue. Details of the methods are described earlier (Seifert 1983).

RESULTS

In all specimens of the eight diseased calves pathological changes were found. The changes concerned the epithelium, the connective tissue and the alveolar bone. The prickle layer (stratum spinosum) of the oral and marginal epithelium showed swelling of the cells, the intercellular spaces and intercellular projections disappearing (Fig. 1). This occurred especially at the transition of the cornified oral epithelium to the non-cornified crevicular epithelium. In some cases, the superficial layers, seldom the entire epidermis, was missing while the adjacent junctional epithelium showed finger-like proliferations into the underlying connective, tissue. Here some calcifications (identified by Von Kossa's stain) were observed (Fig. 2). The lamina propria was loosely structured, edematous and infiltrated by predominantly eosinophilic granulocytes (Fig. 3). The alveolar bone was also pathologically altered. Rarefaction and fibrous repair of lamellae was observed in the central part of the interdental alveolar septum as well as the interradicular bone (Fig. 4). Thus osteolytic changes were not restricted to the bone adjacent to the inflammatory changes of the gingiva and marginal periodontal ligament (PDL). The periodontal space was not widened, the fibres of the PDL being of normal length and structure, penetrating the remaining alveolar bone.

The results of differential staining of collagen types I and III by the picrosirius plus polarization technique revealed

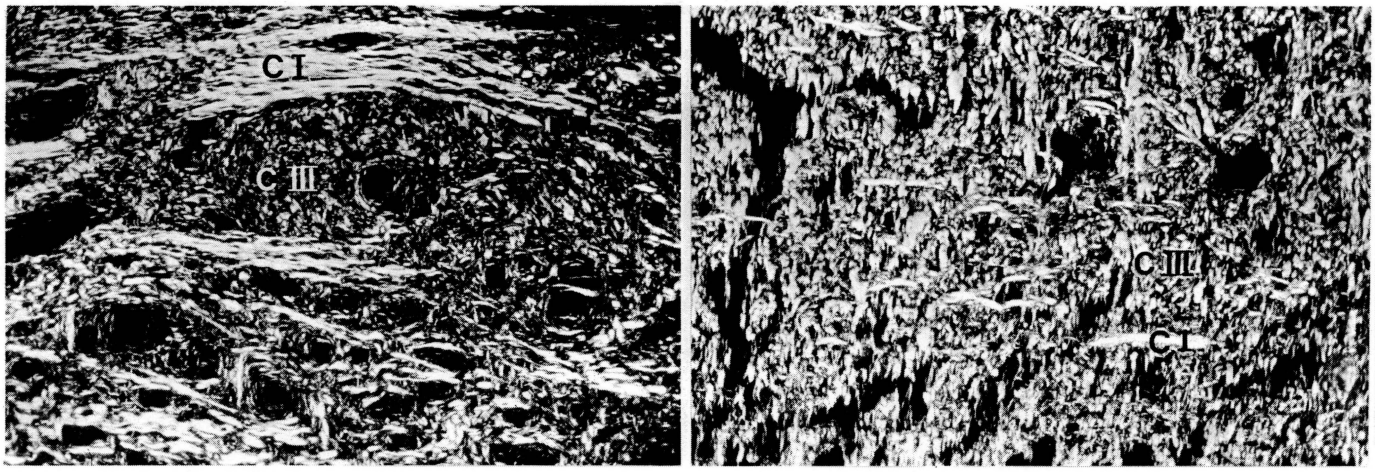

Fig. 5. *Collagen I fibres in gingiva. Picrosirius Red plus Polarization, Obj. 10.*

a) *Healthy gingiva: The fine network of collagen III (C III) fibres underly the thick bundles of bright collagen I (C I) fibres.*

b) *Collagen I fibres showing loss of integrity and discontinuity along their course in Cara inchada induced inflammation, collagen III fibres being more prominent.*

that collagen changes occurred in inflammatory regions. Here the integrity of collagen I, which was seen as thick bundles of strongly birefringent yellow, red or orange fibres, was lost: they were partly disintegrated and few bundles could be followed along their course. Due to the less dense structure of the usually predominating collagen type I, the network of fine, less birefringent green collagen III fibres could be more readily identified. In black and white photomicrographs these two fibre types could be differentiated by their differing diameters. Also, the thick bundles of collagen I fibres were more strongly birefringent and therefore showed up as thick white bundles in contrast to the less birefringent thinner collagen III fibres (Fig. 5a and b). It must be noted, that the non-infiltrated areas of diseased specimens resembled the control animals. There were differences in the transition between healthy and inflamed connective tissue. In some cases, the thick strongly birefringent collagen I fibres approached the aggregations of lymphocytes or granulocytes without showing looseness of structure or signs of edema in the vicinity of these leucocytic cell nests – in fact these seemed to be embedded in "healthy connective tissue". On the other hand, the transition of the normal connective tissue was very gradual when the cellular infiltrate was more diffuse and without aggregations. Here the thick bundles of collagen I were quite sparse and collagen III more prominent.

DISCUSSION

The histological features of healthy Brazilian and European cattle did not reveal a race disposition of Zebu cattle for the disease. Taking into account the gross anatomical and functional characteristics of ruminant dentition, it was found that the structure and arrangement of collagen, elastic and oxytalan fibres in the periodontium of calves were similar to that of man and other mammals (Seifert 1983).

The pathological findings concern the junctional and associated epithelia, signs of inflammation in the propria and osteofibrotic degeneration of alveolar bone. All of these are involved with the loss of periodontal attachment in periodontitis (Page & Schroeder 1982).

The swelling of the cells in the prickle cell layer of the marginal and crevicular epithelia probably leads to the loss of the superficial epithelial layers as observed in more advanced cases of Cara inchada. These changes are unlikely to be due to endogenous factors, as one would then expect a generalized involvement of the mucous membranes or the skin – these have not been found (Döbereiner et al. 1974, Döbereiner 1982, pers. comm.) The proliferation of the junctional epithelium into the infiltrated connective tissue can be interpreted as a reaction to the inflammatory stimulus (Müller & Kirk 1976). They took the form of finger-like projections. All these changes in the epithelium impede its effectiveness as a barrier to exogenous toxic and noxious agents (Müller & Kirk 1976), which could cause the inflammatory changes in the underlying connective tissue. Although polymorphonuclear neutrophils, macrophages and varying numbers of lymphocytes are usually found in healthy gingiva (Schumacher & Schmidt 1976, Stall & Slavkin 1972), the occurrence of aggregations of leucocytes and lymphfollicles as seen in Cara inchada is related to gingivitis and periodontitis (Attström et al. 1975, Soames & Davies 1977). The predominance of eosinophilic granulocytes in Cara inchada indicates an allergic reaction, the significance of which remains to be investigated. The role of specific microorganisms in the etiology of the disease is under investigation (Blobel & Döbereiner 1982, pers. comm.).

The intriguing question that arises when looking for the initial lesion in Cara inchada is why these are restricted to the interdental papilla between the 2nd and 3rd deciduous premolars (Pd_3 and Pd_4 resp., Schummer et al. 1982) in young calves and affect the erupting molars in older cattle (Döbereiner et al. 1974). This led to the hypothesis that primary connective tissue changes could cause loss of tooth attachment during eruption. A collagen-pathogenic factor such as β-amino proprionitrile

(a lathyrogen) leads to changes in the structure and organization of the PDL fibres as described by Thomas (1965) and Berkovitz (1972) in the rat. Morphological changes of the PDL were not observed in the calves investigated (Seifert 1983).

Collagen changes were observed in the inflamed gingiva using the picrosirius plus polarization technique, which allows the differentiation of collagen types I and III (Carrasco et al. 1981, Junqueira et al. 1978). The finding that collagen I fibres are less dense and show loss of integrity while collagen III fibres become more conspicuous in inflammatory regions, could be explained by the local control of collagenolysis (Rose & Robertson 1977) and by the specific collagenase I production by the granulocytic infiltrate (Kleinman et al. 1981). On the other hand, since collagen type III appears to be more rapidly synthesised than type I (Wang 1982), its predominance could indicate a phase of repair. A general loss of birefringence in inflamed connective tissue and a change in staining properties as described by Melcher (1967) were not observed. The local loss of fibre integrity could also be due to a deficiency in fibronectin (Baum & Wright 1980; for a review see Kleinman et al. 1981). Changes in the extracellular fluids and the presence of certain enzymes can cause a decrease in the fibronectin content (Garant et al. 1982). This could be explained by the defective epithelial barrier although the epidermal degenerative changes could, for their part, be a reaction to the alterations in the supporting tissue.

The involvement of the alveolar bone seemed to be of a generalized nature. Some investigators have obtained results indicating osteodystrophia fibrosa (Nunes et al. 1979) although the nutritional deficiency of the animals studied was possibly due to the pain and loss of teeth during a relatively long period of illness. In this case, the osteodystrophic changes would be secondary. We observed osteoclastic processes in the central region of the alveolar septum, while the tooth orientated bone lamellae which are penetrated by the fibres of the PDL remained intact; the periodontal space was therefore not widened. In earlier investigations it was found that an osteoclastic process at inflammatory site was "compensated" for by an osteoblastic process on the buccal or lingual aspect (Döbereiner et al. 1974). It was concluded that the bulging of the maxillae was not due to an overall increase in bone thickness, as would be the case in osteodystrophia fibrosa.

The results of these studies on the collagen of Cara inchada diseased cattle do not provide support for the hypothesis that primary collagen changes play a pathogenetic role in Cara inchada.

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