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CELLULAR AND HISTOCHEMICAL CHARACTERISTICS OF OSTEOID FORMED IN EXPERIMENTAL FLUORIDE POISONING

(Fluoride intoxication; neobone; glycosaminoglycans)

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SUMMARY

The present study on the cellular and histochemical characteristics of osteoid formed in iliac crest bone during fluoride poisoning in rabbits was carried out as there is no information available to date either on its structural or biochemical characteristics. Osteoid formation in bone is prevalent both in fluorosis and in sodium fluoride therapy for osteoporosis. The report provides evidence which indicates that fluoride induces osteophytic activity leading to the formation of cartilagenous loci (fibrocartilage) in bone, the matrix of which is loaded with glycosaminoglycans (GAG) and proteoglycans. The occurrence of dermatan sulphate is demonstrated histochemically. Accumulation of dermatan sulphate in the cartilagenous matrix possibly prevents the normal process of mineralization.

INTRODUCTION

Osteoid formation is an intermediary phase in normal developmental events of bone tissue when the pre-existing connective tissue cells or the chondrocytes begin to develop a thin layer of matrix around them which, under favourable conditions, calcify. However, the term osteoid is also used to refer those loci in well developed bone tissue which undergo osteophytic changes during fluoride poisoning and in fluoride therapy [1]. Teotia et al. [2] have reported that in patients with skeletal fluorosis, histopathological studies on bone revealed irregular islands of osteoid tissue in the deeper layers of the calcified trabeculae. Franke [3] has reported osteoid

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Abbreviations: CPC, cetyl pyridinium chloride; GAG, glycosaminoglycans.

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formation in bone, as a result of sodium fluoride therapy for osteoporosis. Jowsey et al. [4, 5] have also provided evidence which suggests that fluoride as a therapeutic agent in the treatment of osteoporosis produces osteoid formation in bone. The osteoid thus formed has been described as an abnormal bone which is irregular and contained incompletely mineralized bone with larger osteocytes compared to the surrounding normal bone tissue.

Osteoid formation in fluoride toxicity is also referred to as 'neobone' formation, possibly due to the poorly mineralized nature of the osteoid.

It was evident that there is paucity of information on the structural and biochemical characteristics of the osteoid formed in fluoride toxicity and in fluoride therapy. The present communication reports on the cellular and histochemical characteristics of osteoid formed due to excessive ingestion of fluoride in rabbits.

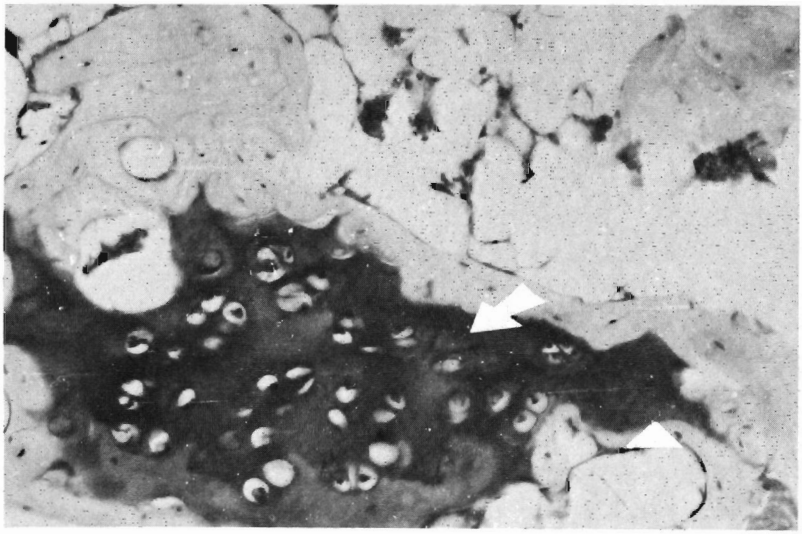
MATERIALS AND METHODS

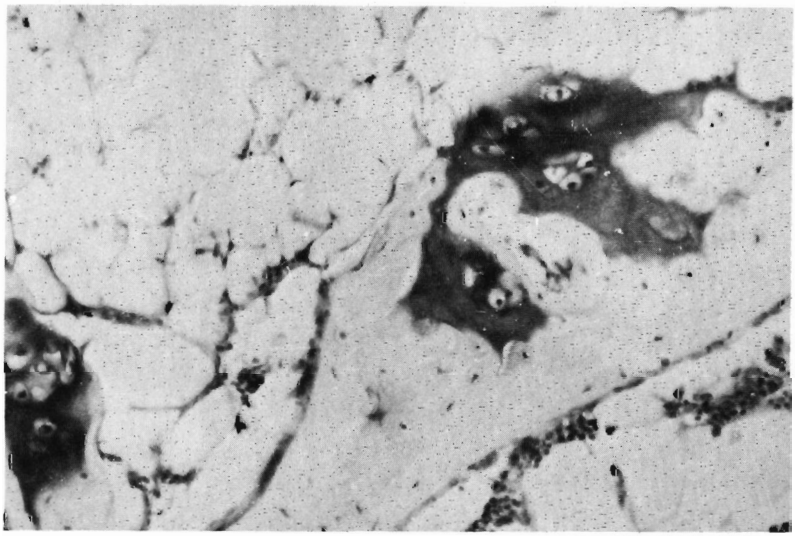
Rabbits aged 2 months were fed 10 mg of sodium fluoride/kg body wt. daily intragastrically up to a period of 10 months. Rabbits were killed at 8 and 10 months of fluoride treatment. The iliac crest region of the pelvic girdle were dissected out and fixed in 10% neutral formalin containing 1% CPC to preserve the GAG. The fixed tissue was decalcified in a mixture of 10% formic acid and 20% sodium citrate mixed in ratio of 1:1. Decalcification was terminated when the decalcifying fluid showed negative calcium reaction to oxalate. The decalcified tissue was blocked in paraffin and 5 μm thick sections were prepared. These sections were deparaffinized, stained with alcian blue (acidified with acetic acid to pH 2.6) and freshly prepared aqueous solution of ruthenium red to localize GAG and proteoglycans (protein complex of GAG), respectively.

Sections were also incubated in a solution containing 3000–4000 I.U. of hyaluronidase/ml of 0.15 M NaCl (pH 6) for 10 to 60 min at 37°C, prior to alcian blue staining, to digest those GAG which are susceptible to enzymic digestion. Control sections were incubated for the same length of time under the same conditions in 0.15 M NaCl prior to alcian blue staining. Bone sections obtained from age-matched control animals which were not exposed to NaF were also treated in a similar way.

RESULTS

Bone samples from the iliac crest region of the pelvic girdle obtained from those animals which were on fluoride for 8 and 10 months revealed osteoid formation. Osteoid formation was found in various regions of the trabecular bone (Figs. 1 and 2). Morphologically, the cells confined to the osteoid region resemble chondrocytes. It was also possible to observe stacking arrangement of chondrocytes (Fig. 3). The intercellular matrix of the osteoid revealed alcian blue and ruthenium red positive





Figs. 1 and 2. The iliac crest bone of the rabbit showing osteoid formation (arrow) in the trabeculae. Fig. 1. Ruthenium red staining, magnification 750 ×. Fig. 2. Alcian blue staining, magnification 300 ×. The intercellular matrix of the osteoid is positive for ruthenium red and alcian blue.

material indicating the presence of GAG and proteoglycans. Isolated patches of alcian blue and ruthenium red positive areas were also observed in the trabeculae which revealed the presence of cells which were not well differentiated into chondrocytes. Osteoids at various stages of formation were thus seen in the bone sections obtained from rabbits exposed to sodium fluoride. Even after

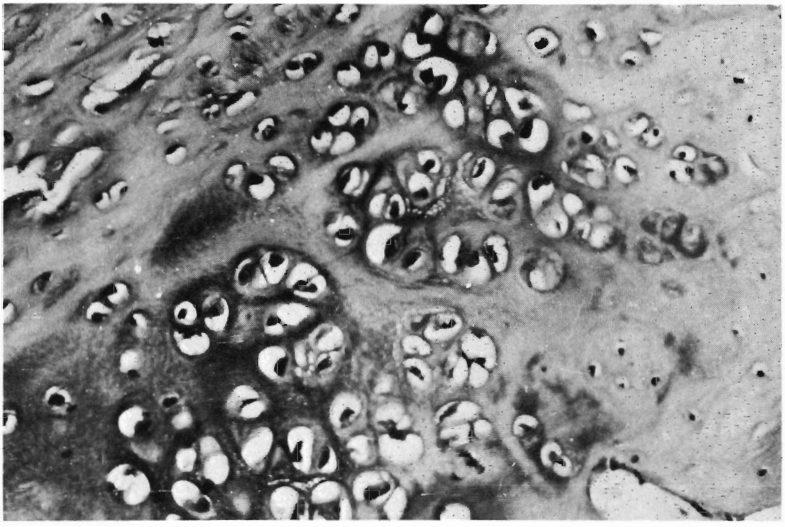


Fig. 3. The osteoid formed revealing stacking arrangement of chondrocytes. Alcian blue staining, magnification 750 ×.

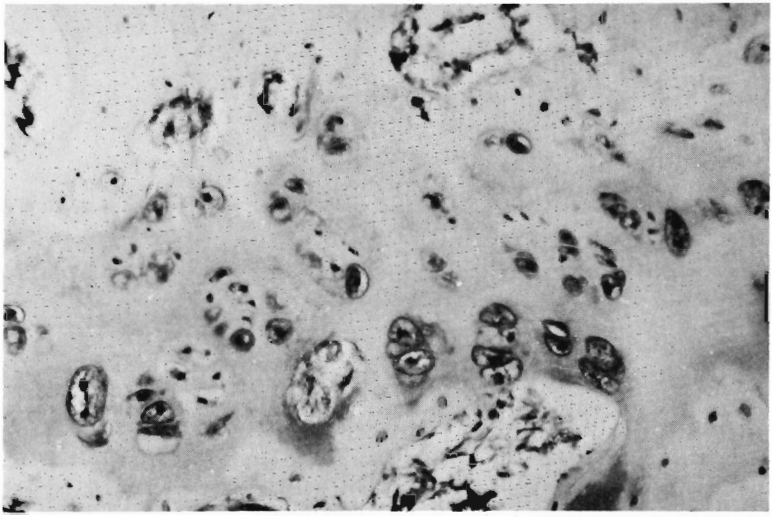


Fig. 4. The intercellular matrix of the osteoid revealing alcian blue-positive material after hyaluronidase digestion for 60 min. Magnification 750 ×.

hyaluronidase digestion, alcian blue positive intercellular material was observed in the osteoids indicating the presence of hyaluronidase-resistant GAG in the matrix (Fig. 4).

DISCUSSION

From the nature of the cells present in the osteoid it appears that they resemble cartilage cells. It was also evident that a kind of stacking arrangement of chondrocytes was seen in certain regions of the osteoid formed and therefore from the morphological observations it appears that osteoids formed during fluoride intoxication resemble fibrocartilage. The appearance of chondrocytes in the trabeculae may possibly be due to activated differentiation of totipotent mesenchymal cells into chondrocytes under the influence of fluoride. Alternatively, previously existing osteocytes and osteoblasts get dedifferentiated into totipotent cells which further undergo redifferentiation into chondrocytes. This may possibly be true as evidence exists suggesting that osteoblasts get activated during fluoride intoxication [6].

The intercellular matrix of the osteoid has revealed high contents of GAG and proteoglycans. An earlier communication from this laboratory has reported significantly high GAG content in iliac crest bone after fluoride ingestion for periods ranging from 3 to 10 months. It has also been found that among the various constituents of GAG investigated, the sulphated GAG were enhanced [7]. The presence of hyaluronidase-resistant alcian blue-positive material detected in the intercellular matrix of osteoids is due to the presence of dermatan sulphate and/or keratan sulphate [8]. However, the quantitative analysis of purified GAG and the isomeric components of chondroitin sulphate have confirmed the occurrence of dermatan sulphate in iliac crest bone [9]. Habuchi et al. [10] reported on the occurrence of dermatan sulphate–chondroitin sulphate copolymers in the meniscus of fibrocartilage which was never replaced by bone as mineralization failed to occur. Therefore, the formation of fibrocartilage and presence of dermatan sulphate in the osteoids may possibly be one of the reasons for poor mineralization occurring in osteoid formed during fluoride ingestion/therapy.

This communication, however, has distinctly shown that the osteoid formed due to fluoride ingestion, is an aggregation of chondrocytes resembling fibrocartilage, the matrix of which has high concentrations of GAG as well as proteoglycans. It is also evident that among the sulphated GAG, dermatan sulphate is laid down in the osteoid which prevents the normal process of mineralization.

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