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Histochemical Study Of The Effects Of Ethanol On Alkaline Phosphatase In The Female Femur Of Wistar Rat Foetuses

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ABSTRACT

The teratogenic effects of ethanol include the assaults of the various developmental processes of tissues exposed in utero, and particularly the mineralisation bones. An experimental investigation of the mechanisms of action of this toxic agent was conducted in the femoral bones of the foetal Wistar rat by the histochemical assessment of the activity of alkaline phsophatase, an enzyme present within the osteoblasts and actively involved in the mineral deposition in bones during development.

Enzyme was relatively sparse and less intense activity in the growing end of ethanol treated bone compared to the control. This appears to be a possible indication of the mechanism by which ethanol suppresses osteoblasts functions, hence, the ability to potentate its toxic and teratogenic effects.

Keywords: Ethanol; Alkaline phasphatase; Wistar rat fetuses; Bone mineralisation.

Enzymes and hormones play vital roles in skeletal growth by regulating calcium mobilization from the skeleton to maintain the osmotic balance of the body. In particular, during the skeletal tissue development, the association of alkaline phosphatase with the process of calcification in bone, cartilage and tooth had been confirmed by numerous workers (Sampson et al., 1996; 1999; Hogan et al., 1997); and since Gomori (1939) published his well-known histochemical methods, a number of investigators have studied alkaline phosphatase activities in the skeletal tissues at the cytological level and it is now well known that osteoblasts, odontoblasats and chondroblast cells exhibit a high degree of the enzyme activity. Friday and Howard (1991) and Klein et al., (1996) associated the ethanol induced skeletal malformation with the inhibitory effect of ethanol on the proliferation and functions of the osteogenic cells (osteoblasts), which are involved both in calcification and bone matrix synthesis. Alkaline phosphatases had been reported to be available in the osteoblast nuclei and are liberated to the cytosol during active osteogenic activity where they hydrolyse phosphate esters to produce an excess of free inoroganic phosphate ions. This elevates the Ca^{2+} and $PO4^{2-}$ ions products at specific calcification enters to a degree necessary to produce precipitation of apatites.

The enzyme is thus considered as useful marker for studying bone development, Pritchard (1952) and Jeffrey (1976).

MATERIALS AND METHODS

Sixty, (60) adult healthy Wistar rats comprising of twenty, (20) males and forty, (40) females were procured for the experiment, they were housed in the animal holdings of the Department of Anatomy; the room was kept tidy and well ventilated. The animals were fed on rat pellets (Agro Feeds Ltd., Ibadan). The animals were grouped into two: A, control and B, experimental groups. The experimental group orally received 0.79g/kg of 30%v/v ethanol using the oesophageal tube, on gestational days 9,10 and 11, which coincide with the osteogenic period in this animal (Murphy (1964). Ethanol dosage was calculated from its g/ml equivalent weight, and 30%v/v was found tolerable to the animals following a pilot test. The control group received normal saline in lieu of ethanol. In each cage were 2 females and 1 male for the purpose of mating. Confirmation of pregnancy and commencement of gestational dating were done according to the methods of Asling (1960). On the 12^{th} day the animals were sacrificed by chloroform inhalation and the fetuses retrieved. The femoral bones were carefully excised from the

feotuses and quickly frozen in the cryostat (Lriyz rotary cryotome); 8um serial sections were cut at-20^{0}C, mounted on untreated cover slips and post-fixed in 10% neutral – buffered formalin. The modified coupling azo dye method of Kaplow (1955) was employed to demonstrate alkaline phosphatase activity. This technique employed sodium @-naphthyl phosphate as substrate and the diazonium salt Fast Black B as coupler, incubation were run for 45 minutes at pH 8.3 and room temperature, yielding black granular reaction product. Mountin of tissues was done in glycerine jelly. Control reaction for false positive enzyme activity was prepared under similar conditions with the substrate omitted from the incubating medium.

RESULTS

Intense black granular precipitates or formazan indicating sites of alkaline phosphatase activity in the growing end of the femoral bones were characteristics of the control rats. Variable and less intense granules characterized these sites in the experimental or ethanol treated rats. The differences in enzyme activity observed in both the control and ethanol treated rats are evidence of the reactions of the enzymes following the treatments. Optimal enzyme activity was seen at about 45 minutes of incubation. Increasing incubation time beyond this point did not reveal any amore intense enzyme activity than was observed at the end of the optimal time period. The control and experimental tissues incubated in the medium from which substrates had been omitted showed complete absence of reaction product.

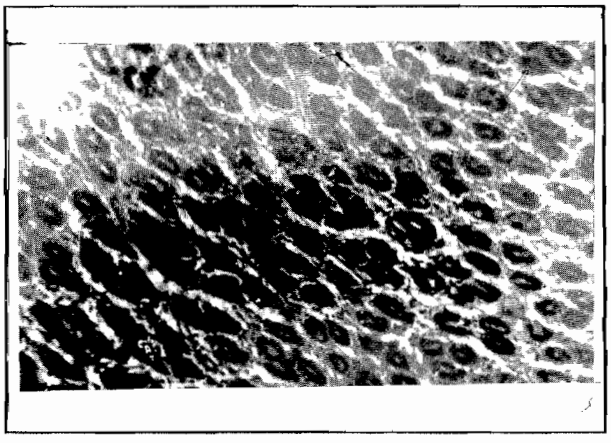

Fig. 1: **Alkaline phosphatase activity in the growing end of Femur of Wister rats (Control Group).**

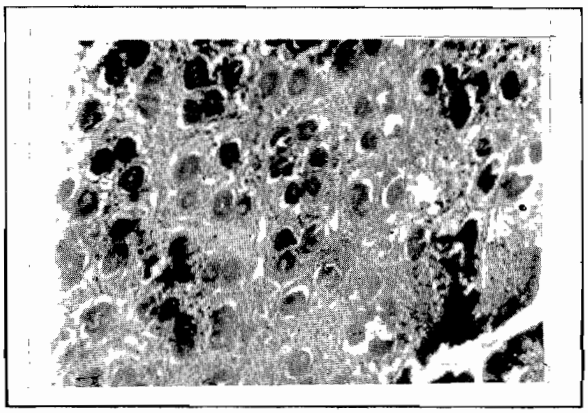

Fig. 2:**Alkaline phosphatase activity in the growing end of femur of Wister rats (Ethanol Treated Group)**

DISCUSSION

Alkaline phosphatase activity was localized at the endosteal and growing ends of the femoral bones in both the control and experimental rats, thus indicative of the active osteogenic sites. Enzyme activity in the ethanol treated femur was rather weak but quite intense in the control bone at the same site. This could possibly serve as useful diagnostic test in the pathological conditions of the bones.

It had been demonstrated that virtually any level of alcohol consumed produce a toxic effects on the individuals including bone tissues (Peng et al., 1982; Sampson et al., 1999). Acute alcohol intoxication causes transitory hypocalcaemia and hypercalciuria. Prolonged moderate drinking elevates serum parathtyroid hormone levels, while chronic alcoholics are characterized by low serum levels of vitamin D, with resultant malabsorption of calcium, thereby leading to hypocalcaemia and hypoclaciuria (Laitinen and Valimaki, 1991; Miralles-Flores and Delgado-Baeza 1992; Lazareseu et al., 1995; Habbick et al., 1998). Moreover, Peng et al (1982) and Kusy et al., (1989) showed that a relationship exists between bone strength and required to break the fermur and the dose of ethanol administered.

It is suggested that the enzyme, alkaline phosphatase play a key role in bone mineralisation, probably by making inorganic phosphatase available at sites of calcification. A peak in alkaline phosphatase activity is found to precede an increase in the rate of mineralisation as determined by Ca^{45} incorporation, Reddi (1982), and its activity correlates with the pattern of calcium uptake.

Earlier investigators have indicated such factors that affect bone development as poor nutrition

with low calorie and protein levels leading to retarded growth and suppressed alkaline phosphatase activity, Gudehithlu and Ramkrishnam (1990a,b).

The mitochondria in the osteoblasts had been reported to act as a storage site for the Ca^{2+} and PO^{4-} oins, Borle (1973), which are then gradually incorporated into newly laid down bone matrix (osteoid) during calcification. Poor bone mineralisation is common in alcoholics who have been found to be vitamine D deficient, Laitinen and Valimaki (1991), and low vitamine D was reported to reduce the absorption of calcium, Lucille (1977).

Moreover, Israel (1990) suggested that ethanol ingestion induces agglutination or rosettes formation by the red blood cells which consequently lowers the circulating oxygen level resulting in the impairment of normal cell function, particularly that of osteoblasts. Depressed osteocalcin level in the serum during acute alcohol intoxication was reported to indicate that alcohol acutely retards osteoblasts activity (De Verjoul et al., 1983). Ostecalcin is a vitamin K dependent protein synthesized by osteoblasts and released into the circulation. Its concentration in the serum is elevated in states of high osteoblast activity and reduced in sites of diminished bone synthesis (Laitinen and Valimaki, 1991).

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