BLOODTYPING IN HUMAN DENTAL PULP BY IMMUNOENZYME TECHNIQUES

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Introduction

Genetically determined markers increase the chances of individual identification of human cadavers, especially in cases in which tests cannot be made by direct methods because of detoriation, in putrefied and skeletonized cadavers.

The ABH-system is important, because it appears to be more stable concerning autolytic processes than other antigens and is usually documented of most persons. ABH-bloodtyping in human teeth and dental pulps by absorption-elution test is possible but can easily lead to false results (von Crainic et al 1981).

The extensive vascularity of the human dental pulp and its protection by dentine offers excellent possibilities for bloodtyping by application of immunoenzyme techniques.

Material and Method

Studies were made of 24 extracted molar teeth after 10 to 18 month storage at room temperature. After cutting the tooth longitudinally the pulp was separated from the dentine. Tissue sections were made using a cryostat at -20°C and from formalin fixed and paraffin embedded material. Immunoenzyme techniques as peroxidase-anti-peroxidase (PAP) method and alkaline-anti-alkaline phophatase (APAAP) method were applied for ABH-bloodgrouping as described by Bourne 1983.

Isoelectric focusing was performed essentially by the method of Pflug (1986) using LKB-Immobiline Dry Plates ^{TM}pH 4,5-5,4 followed by alkaline phosphatase-linked secondary antibody immunoenzyme technique staining system.

Materials: DaKo-Universal-Mäuse-PAP$_{Kit}$(K 550), Universal-DaKo-APAAP-Kit$_{TM}$-Maus (K 670), Monoclonal Anti A (A 581), Monoclonal Anti B (A 582), Mäuseserum (X 910), Schweineserum (X 901), Glycergel Einschlußmedium (C 563) DAKOPATTS, Hamburg, FRG. LKB Immobiline Dry Plates ^{TM}pH 4,5-5,4 (Pharmacia LKB, Freiburg, FRG). Goat-anti Human Gcglobulin-IgG fraction (ATAB, Scarborough, USA). Anti-Ziege IgG alkalische Phosphatase markiert, 5-Brom-4-chlor-3-Indolylphosphat (Sigma Chemie, München, FRG).

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Results

Groupspecific ABH-antigen could be determined in the lumen of capillaries filled with erythrocytes. In a few cases positive staining of endothel cells could also be observed. The groupspecific component (Gc) system was detected in dental pulp tissues in all cases tested and its activity was fairly well maintained.

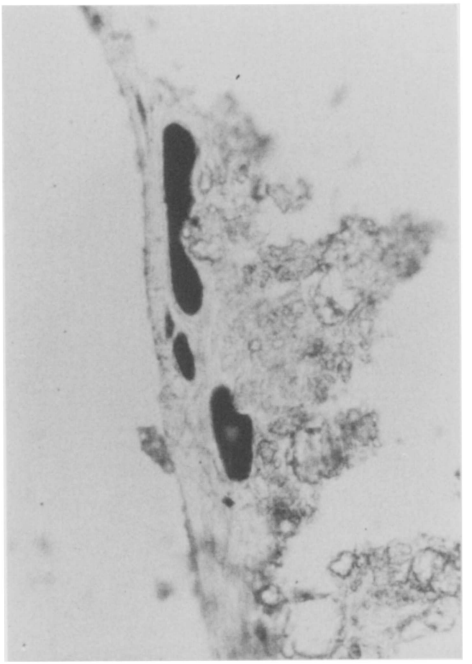

Fig. 1: Cryosection of human dental pulp. ABH-group-specific staining by APAAP procedure.

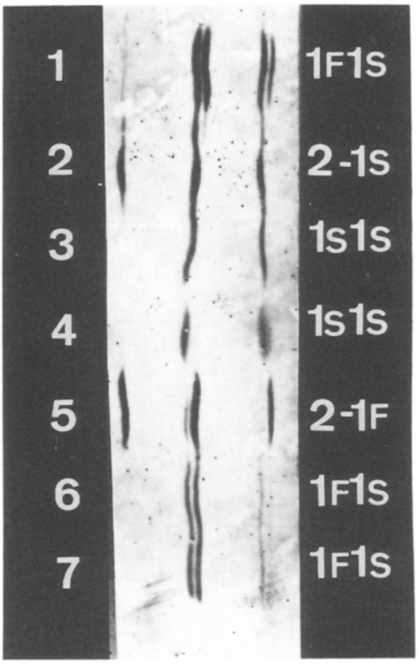

Fig. 2: Gc-subtyping.
Isoelectric focusing on LKB-Immobiline Dry plates™ ph 4,5-5,4 and detection with alkaline-phosphatase-linked secondary antibody system.
References: 1,2,5,6,7
Bloodstain: 4
Dental pulp: 3

Discussion

The determination of ABH-bloodgroup in the dental pulp by immunoenzyme technique establishes an essential control of the results obtained by absorption-elution test for confirmation of the ABH-phenotype.

There have been several polymorphic enzymes occuring in the dental pulp reported earlier; these are PGM_1, PGD, ADA, AK, EsD and Fu polymorphism (Henke et al 1982; Kido et al 1987; Petersen et al 1974; Turowska et al 1977).

In the present study we have demonstrated the presence of groupspecific component (Gc) activity in the dental pulp and added it to the list of enzymes named above. The results show that Gc-subtyping is applicable to bloodstain typing in crime laboratory casework as well as to individual identification. Therefore and due to its high discrimination power (DP = 0,74) Gc-marker is of value concerning forensic investigations.

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