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X-RAY DIFFRACTION INVESTIGATION ON THE
EFFECT OF EXPERIMENTAL AND IN SITU RADIATION
ON MATURE HUMAN TEETH
A PRELIMINARY REPORT

by

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INTRODUCTION

The use of radiotherapy in the treatment of certain carcinomas of the oral cavity, pharynx and larynx can cause caries-like destruction in the hard dental tissues, enamel and dentine (*Leist*, 1925; *del Regato*, 1939; *Cernea & Bataille*, 1947; *Wildermuth & Cantril*, 1953; *Chochia & Shimanovskaya*, 1958; *NG et al.*, 1959; *Gowgiel*, 1960; *Osswald*, 1962; *Kimeldorf et al.*, 1963; *Frank et al.*, 1965; *Poyton*, 1968). In all cases in which the radiation has involved dental defects, the damage has been done by therapeutic radiation and not by X-ray examinations of teeth and jaws.

Radiation damage to the hard dental tissues can be considered from two main points of view: Damage to developing and not fully mineralized teeth and damage to fully formed and mineralized teeth.

The present investigation deals only with radiation-caused defects on the components of fully mineralized enamel and dentine of mature human teeth.

There are two opinions concerning the pathogenesis of radiation-caused dental defects in man: One is based on the assumption that denaturation of the organic components of the tooth substance is caused primarily by radiation and that this is followed by dissolution of the calcified components (*Leist*, 1926; *Lüdin & Müller*, 1936; *Bianchi*, 1943; *Poppe*, 1963). The

other suggests that the radiation-caused dental defects are secondary in relation to the radiation-caused hyposalivation with consequent changes in the micro-physiological and micro-biological oral milieu (*del Regato*, 1939; *Cernea & Bataille*, 1947; *Frank et al.*, 1965).

The apparent lack of research on radiation-caused changes of the inorganic components of hard dental tissues gave rise to the present investigation.

The purpose of this investigation is to demonstrate that both experimental and in situ radiation on mature human teeth causes changes of the crystalline structure of enamel and dentine.

MATERIAL AND METHODS

Two cariesfree, extracted and non-irradiated mature human teeth, a premolar and a molar, were used in this investigation. The teeth were divided by means of a diamond disk with water cooling. The dried teeth were pulverized in an agate mortar with repeated sifting and controlled by binocular microscope until the whole quantity had a particle size $\leq 5 \mu$ (*Trautz*, 1967). The enamel and dentine were separated by means of the flotation method (*Manly & Hodge*, 1939). One half of the enamel and dentine fractions of the tooth was experimentally irradiated. The other half of the fractions was used as control material. Furthermore, an in situ irradiated mature human molar was used. As the enamel on this molar had almost disappeared, the dentine only was studied.

The radiation was carried out with ^{60}Co .*) The enamel and dentine samples from the premolar were irradiated with 1 Mega-rad (M-rad) in a single dose. The enamel and dentine samples from the molar were irradiated with 10×100 Kilo-rad (k-rad) equal to a total dose of 1 M-rad. The in situ irradiated molar received a total dose of 12,000 R cumulatively from a 250 kV source over a period of $2\frac{1}{2}$ years and the tooth was extracted 2 years later.**)

The mineral components of enamel and dentine consist of calcium phosphate. X-ray diffraction investigations have shown that the mineral components of enamel and dentine are analogous with the mineral apatite (*Gross*, 1926; *De Jong*, 1926; *Bale*, 1940; *Jensen & Møller*, 1944; *Carlström*, 1955; *Trautz*, 1955).

*) The experimental material was kindly irradiated at AEC, Research Establishment Risø, Denmark.

**) The in situ irradiated molar was kindly provided by Dr. S. Björnsson, LDS, Dental Clinic, University Hospital, Copenhagen.

A natural hydroxyapatite from Zillerthal, Austria, was used as reference material in the X-ray diffraction investigation, untreated as well as irradiated with 1 M-rad ^{60}Co . *) An X-ray diffractometer with filtered Cu $K\alpha$ -radiation was used. The range $24^\circ, 2\theta$ to $35^\circ, 2\theta$ was mainly investigated because it includes the most important reflections of hydroxyapatite.

To express the changes in the crystal structure of enamel and dentine caused by radiation the numerical values for crystallinity: \bar{X} for hydroxyapatite, enamel and dentine respectively were calculated. The calculation of crystallinity was carried out according to the formula:

$$\bar{X} = \frac{P}{B},$$

where P was the area of the diffraction peaks, and B the area of the background. The background-line which is interrupted by the peaks was drawn to one uninterrupted line. The diffractogram within the given range from $25^\circ, 2\theta$ to $35^\circ, 2\theta$ was transferred to special paper with homogeneous thickness. The background area (B), i.e. the area from the zero-line to the background-line, and the peak-area (P), i.e. the area from the background-line to the curve, were cut out and weighed. The weight is an expression for the area. \bar{X} for the natural hydroxyapatite was arbitrarily assigned a value of 100. \bar{X} for enamel and dentine is expressed in terms of percentages of this value (*Le Geros et al.*, 1963). Fig. 1 shows the diffractograms of hydroxyapatite, non-irradiated, and experimentally irradiated enamel samples from premolar. Fig. 2 shows the diffractograms of non-irradiated dentine sample from the premolar, of the dentine sample from the premolar experimentally irradiated with 1 M-rad in single dose, and for molar dentine sample irradiated with 12.000 R cumulatively in situ. \bar{X} for all samples were calculated as shown in Table I.

The diffractograms in Fig. 1 illustrate that the value for crystallinity of the enamel samples experimentally irradiated with 1 M-rad, both in single dose and cumulatively becomes greater than that of non-irradiated samples, but it does not reach the degree of crystallinity of natural hydroxyapatite (see Table I). The diffractograms in Fig. 2 illustrate, that the value for crystallinity of the dentine samples experimentally irradiated, both in single dose and cumulatively with 1 M-rad, and the in situ irradiated molar dentine

*) The hydroxyapatite was kindly provided by dr.phil. Harry Micheelsen, Mineralogical Museum of the University of Copenhagen.

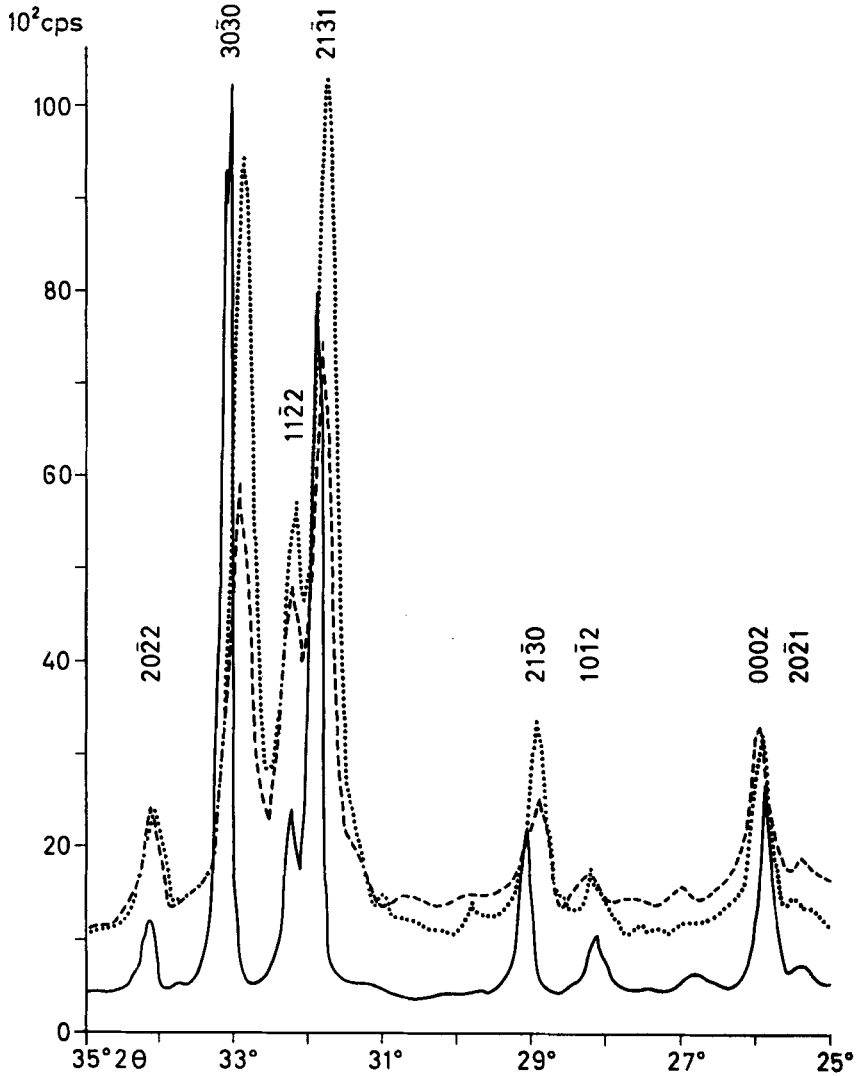


Fig. 1. X-ray diffraction patterns obtained from powdered hydroxyapatite (—), premolar enamel non-irradiated (---), and premolar enamel experimentally irradiated with 1 M-rad (.....). Cu $K\alpha$ -radiation.

decreased when compared to the values of the non-irradiated samples (see Table I).

The radiation-induced linebroadening caused the reflections $21\bar{3}1$, $11\bar{2}2$ and $30\bar{3}0$ almost to disappear for both the experimentally and the *in situ*

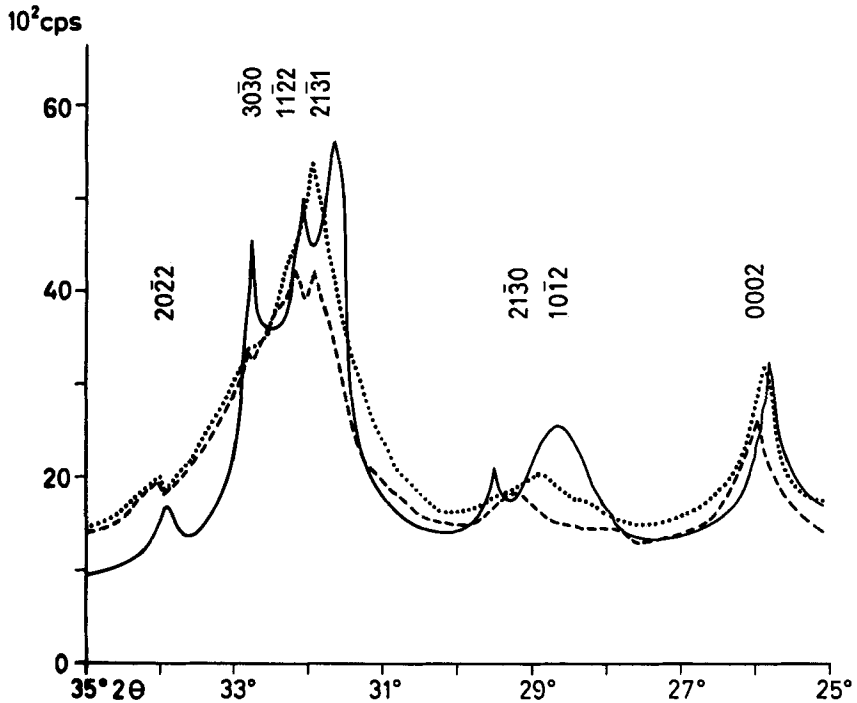


Fig. 2. X-ray diffraction patterns obtained from powdered non-irradiated molar dentine (—), molar dentine experimentally irradiated with 1 M-rad in single dose (---), and molar dentine in situ irradiated with 12,000 R cumulatively (.....). Cu $K\alpha$ -radiation.

irradiated samples. The disappearance of the reflections was most distinct for the in situ irradiated sample.

Table I.

Crystallinity values of non-irradiated and irradiated hydroxyapatite, enamel, and dentine samples

Sample	Crystallinity degree		Radiation
	non-irradiated	irradiated	
Enamel, premolar	76	89	1 M-rad
Enamel, molar	71	91	10×100 k-rad
Dentine, premolar	59	42	1 M-rad
Dentine, molar	57	43	10×100 k-rad
Dentine, molar	—	45	12,000 R, in situ cumulative
Hydroxyapatite	100	100	1 M-rad

In this material it was not possible to definitely demonstrate differences in the diffractograms from experimentally irradiated enamel or dentine samples, indicating whether the radiation had been given in single dose or cumulatively. Neither was it possible to demonstrate any radiation-caused crystalline changes in the hydroxyapatite samples.

From the present material it is not possible fully to explain the radiation-caused differences in the diffractograms, compare Figs. 1 and 2. The *in situ* irradiated molar received 12.000 R which is a 100 times smaller dose than was received by the experimentally irradiated molar. (1 R = 0.97 rad). Yet the same crystallographic changes can be observed in the two diffractograms. The reason might be due to an ion transport taking place in the dentine *in vivo*, but not *in vitro*. This transport might be activated by radiation.

It is peculiar that \bar{X} for enamel irradiated with ^{60}Co increased. In contrast, \bar{X} for dentine diminished after both experimentally and *in situ* radiation.

This is not to say that the effect of radiation on mature human teeth is exclusively a radiation-induced defect in the crystal structure. It is also possible that the defect in the crystal is the result of a chemical reaction caused by radiolysis.

Further investigations regarding the causal relations of this form of tooth defects are in progress.

DISCUSSION

Crystallographic investigation of irradiated mature human teeth shows that the two opinions, mentioned in the introduction, concerning the pathogenesis of the radiation-caused dental defects, neither alone nor in combination provide the complete explanation of the emergence of the dental defects.

For the experimentally irradiated enamel, whether irradiated in a single dose or cumulatively, a change of the particle size occur, so that \bar{X} increases.

For the experimentally irradiated dentine, both in a single dose and cumulatively, and for the dentine of the *in situ* cumulatively irradiated molar the particle size in the crystal structure diminishes. This increases the absolute surface of the crystal.

This radiation-caused change in the crystalline structure might, together with the hyposalivation-induced changes in the micro-physiological and micro-biological oral milieu, further the development of the carious lesions.

Acknowledgment. This investigation was supported in part by a grant from the Danish Dental Society Foundation: Fonden til støtte for videnskabelige og praktiske undersøgelser inden for tandlaegekunsten.

SUMMARY

The X-ray diffraction investigation demonstrates that experimental radiation with 1 M-rad, in both single dose as cumulatively, of mature human teeth as well as in situ radiation with 12,000 R induces changes in the crystalline structure of human enamel and dentine. The experimental radiation of dentine requires a 100 times greater dose to obtain the same crystalline change as the in situ irradiated molar dentine.

The radiation-induced crystallographic changes must be considered in addition to the hyposalivation-caused changes in the micro-physiological and micro-biological oral milieu.

RÉSUMÉ

ÉTUDE PAR DIFFRACTION DES RAYONS X SUR L'ACTION D'UNE IRRADIATION EXPÉRIMENTALE OU IN SITU SUR LES DENTS HUMAINES MATURES

Une étude par diffraction des rayons X a montré qu'une irradiation expérimentale de 1 M-rad, en dose unique ou cumulative, de même qu'une irradiation in situ de 12.000 R, provoquaient des modifications de la structure cristalline de la dentine et de l'émail humains.

L'irradiation expérimentale de la dentine exigeait une dose 100 fois plus grande pour obtenir la même modification cristalline que celle que provoquait dans la dentine des molaires l'irradiation in situ.

Il convient de faire le rapprochement entre les modifications cristallographiques provoquées par l'irradiation et les modifications du milieu micro-physiologique et micro-biologique buccal provoquées par l'hyposialie.

ZUSAMMENFASSUNG

RÖNTGENDIFFRAKTIONSUNTERSUCHUNG ÜBER DIE WIRKUNG VON EXPERIMENTELLER UND IN SITU BESTRAHLUNG VOLLENTWICKELTER HUMANER ZÄHNE

Die Röntgendiffraktionsuntersuchung zeigte, dass sowohl die experimentelle Bestrahlung vollentwickelter humaner Zähne mit 1 M-rad, in einfacher Dosis wie kumulativ, als die in situ Bestrahlung mit 12.000 R in der kristallinen Struktur humanen Zahnschmelzs und Dentins Änderungen hervorrief.

Die experimentelle Bestrahlung von Dentin erforderte eine hundertfach grössere Dosis, um die gleiche kristalline Änderung wie in dem in situ bestrahlten molaren Dentin hervorzurufen.

Die durch Bestrahlung hervorgerufenen kristallographischen Änderungen sind zusätzlich zu den durch die Hyposalivation bewirkten Änderungen im mikrophysiologischen und mikrobiologischen oralen Milieu zu sehen.

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