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Kenhardt revisited – a study in a high fluoride area

D H Retief*, E L Bradley**, F H Barbakow, M Friedman, J I Bischoff and E H M van der Merwe

Medical Research Council / University of the Witwatersrand Dental Research Institute, Johannesburg, South Africa and **Department of Biostatistics, School of Medicine, University of Alabama in Birmingham, Birmingham, U.S.A.

Keywords: Endemic fluorosis; fluoridation; fluorosis

SUMMARY

The caries inhibitory effect of optimally fluoridated water is well documented. Communities are often exposed to naturally fluoridated water with F content exceeding the optimal level of 1 ppm. The purpose of this study was to determine the relationship between enamel fluoride concentration, and mass fluoride expressed in picograms (pg) and mass in an endemic fluoride area.

Sixty-one 14 to 17-year-old White children (27 males and 34 females) who were born and had been living continually in Kenhardt were selected for this study. The DMFT and DEGF were determined clinically. Acid etch biopsies were done on both the maxillary central incisors of each pupil. F and Ca analyses were carried out on the biopsy solutions and mass fluoride expressed in picograms (pg) and mass enamel in micrograms (µg). Data transformations to $\log_e$ mass F (lnF), $\log_e$ mass En (lnEn) and $\sqrt{DMFT}$ were made. The lnF values were adjusted to a standardized depth of 10 µm (enamel equivalent mass of 208.6 µg) by covariance analysis.

In order to determine the association between $\sqrt{DMFT}$, lnF and DEGF, correlations between the various pairs of variables were calculated for all the pupils, for pupils with DMFT ≤2 and DMFT >2, and for pupils with DEGF ≤1 and DEGF >1.

The following significant associations were established: A negative correlation significant at the 10 per cent level ($p=0,0902$) between $\sqrt{DMFT}$ and lnF for DEGF≤1; A highly significant ($p=0,0004$) positive correlation between lnF and DEGF for all the pupils and for pupils with DMFT >2.

OPSOMMING

Die kariesbeperkende effek van optimale gefluoreerde water is reeds deeglik beskryf. Gemeenskappe word dikwels blootgestel aan natuurlike gefluoreerde water met 'n F inhoud wat die optimale vlak van 1 dpm oorskry. Die doel van hierdie ondersoek was om die verhouding te bepaal tussen glasuur fluoried konsentrasie, graad van fluoridasie (DEGF) en tandkaries insidensie (DMFT) in 'n endemiese fluoried gebied. Een-en-sestig 14 tot 17-jarige wit kinders (27 manlike en 34 vroulik) wat gebore is en opgegroei het in Kenhardt, is vir hierdie ondersoek gekies. Die DMFT en DEGF is klinies bepaal. Suurets biopsies is gedoen op beide die maksillêre eerste snytande van elke skolier. F en Ca analises is uitgevoer op biopsieoplossings en massa fluoried is uitgedruk in pikogramme (pg) en massa glasuur in mikrogramme (µg). Data transformasies na $\log_e$ massa F (lnF), $\log_e$ massa En (ln En) en $\sqrt{DMFT}$ is gedoen. Die lnF waardes is aangepas tot 'n gestandardiseerde diepte van 10 µm (glasuur ekwivalente massa van 208,6 µg) deur kovariante analise. Ten einde die assosiasie tussen $\sqrt{DMFT}$, lnF en DEGF te bepaal, is korrelasies tussen die verskillende pare variante vir al die skoliere bereken, vir skoliere met DMFT≤2 en DMFT>2, en vir skoliere met DEGF ≤1 en DEGF >1.

Die volgende betekenisvolle assosiasies is gevind: 'n negatiewe korrelasie betekenisvol op die 10 persent vlak ($P=0,0902$) tussen $\sqrt{DMFT}$ en lnF vir DEGF≤1; 'n hoogs betekenisvolle ($P=0,004$) positiewe korrelasie tussen lnF en DEGF vir al die skoliere en vir skoliere met DMFT >2.

*Present Address: Dr D H Retief
Institute of Dental Research School of Dentistry University of Alabama in Birmingham
Birmingham, AL 35294 U.S.A.

INTRODUCTION

Endemic dental fluorosis or mottled enamel is widely distributed throughout the world. In the Republic of South Africa it has been observed in the North Western, Western and Karoo regions of the Cape Province, Western and Central Free State and Northern, Eastern and Western areas of the Transvaal (Ockerse, 1946).

Ockerse (1941) examined 6 to 16-year-old White children in the Kenhardt School. Thirty-eight of these children were born and grew up in the village of Kenhardt, and 24 per cent had dental caries while all showed signs of mottled enamel. The fluoride content of the drinking water of Kenhardt at that time was 6,8 ppm F. Of the 142 children born in the Kenhardt district, 9 had questionable mottling and the degree of mottling in the remainder ranged from mild to severe. The fluoride content of four well waters in the Kenhardt district averaged 7,7 ppm.

In 1960 Dodd, Levy and Jackson reported that an unknown bone disorder occurred in Coloured children resident in the Rooiblok residential area of Kenhardt. They postulated that the high fluoride content of the drinking water obtained from ghorras (shallow wells sunk to a depth of 3 to 6 meters) was probably the most important factor responsible for the bone deformities observed in children born and reared in this township. They also reported that caries, which occurred at the gingival margins of the teeth of children with severe fluorosis, was common throughout the district.

Steyn, Jordaan and Theron (1962) observed that 33 of the 150 Coloured children of school-going age from the Rooiblok area showed clinical signs of bone deformities. The drinking water consumed by the afflicted children contained from 3,6 to 13,0 ppm F. The teeth of 30 of the children with skeletal deformities were examined. All the children had dental fluorosis but caries only occurred in those children with advanced hypoplastic enamel.

Later in 1962 Kenhardt was again visited by Jackson and, amongst others, he determined the extent of dental fluorosis in 7 to 10-year-old White children resident in Kenhardt and Coloured children from "Die Woonbuurt" and Rooiblok residential areas respectively. He reported that 23 of the 25 White children and 29 of the 32 Coloured children from "Die Woonbuurt" had dental fluorosis while none of the 37 children from Rooiblok was fluorosis-free. The drinking water of Kenhardt and "Die Woonbuurt" was from an alternative source and the fluoride content ranged from 2,6 to 3,2 ppm.

In 1975 we revisited Kenhardt to determine the interrelationship between fluoride concentration of surface enamel, degree of fluorosis (DEGF) and caries incidence (DMFT) in 14 to 17-year-old White school children who were born and had been living continually in Kenhardt.

MATERIALS AND METHODS

Kenhardt is a village located in the Northwestern part of the Cape Province in the Republic of South Africa. The average annual rainfall is between 12,5 and 25 cm and the general geologic formation of the district is old granite and gneiss (Ockerse, 1941). The White population of the town is 850 and of the Kenhardt district 2,500. The central water supply of the village is obtained from boreholes situated 8 km from the town and is piped to the village. The drinking water was analyzed by the Department of Health on 23 February, 1978 and part of the analysis is given in Table I. The fluoride content of the drinking water is 3,2 ppm. Analysis of the drinking water collected from several points in the town was done in our laboratory and the fluoride content ranged from 3,16 to 3,22 ppm. Drinking water, mostly obtained from boreholes, was collected from 13 farms in the district and the fluoride content determined (Table II). Several of the children reported that their drinking water was obtained from fountains or "syferwater" but unfortunately none of this water was available for analysis.

Pupils from the Kenhardt High School were selected for this study. The procedures involved in the clinical examination and biopsy procedure were carefully explained to them and their approval to be included in this investigation obtained. Only 14 to 17-year-old children who were born and had been living continually in the village or district of Kenhardt were selected. The 61 pupils consisted of 27 males and 34 females.

Table I. Composition of the drinking water of Kenhardt*

pH	8,1	
Nitrate	2,4	
Chloride	320	
Sulphate	100	
Fluoride	3,2	mg/ℓ
Calcium	98	
Magnesium	42	
Potassium	3	
Sodium	160	
Boron	440	
Copper	21	g/ℓ
Iron	70	
Manganese	6	

* Department of Health, Republic of South Africa, February 23, 1978.

Table II. Fluoride content of the drinking water obtained from farms in the Kenhardt district

Farm	Source	ppmF
Sandkolk	Borehole	2,44
Witkoppies	Borehole	2,74
Crieff	Borehole	0,98
Lushof	Borehole	0,49
Nooitgedacht	Cistern	0,06
Arbeidsvreug	Borehole	2,58
Perdevlei	Borehole	2,26
Reniersrus	Borehole	2,94
Sonderhuis	Borehole	3,02
Kalkputs	Borehole	2,44
Geduld	Borehole	1,00
Afspring	Borehole	2,38
Voorspoed	Borehole	1,08

Table III. DMFT distribution in male and female pupils

DMFT	Frequency		Cumulative frequency		Per cent		Cumulative per cent	
	M	F	M	F	M	F	M	F
0	7	10	7	10	25,9	29,4	25,9	29,4
1	4	3	11	13	14,8	8,8	40,7	38,2
2	7	4	18	17	25,9	11,8	66,7	50,0
3	-	1	18	18	-	3,0	66,7	52,9
4	5	4	23	22	18,5	11,8	85,2	64,7
5	1	2	24	24	3,7	5,9	88,9	70,6
6	2	4	26	28	7,4	11,8	96,3	82,4
7	1	-	27	28	3,7	-	100,0	82,4
8	-	4	-	32	-	11,8	-	94,1
9	-	1	-	33	-	3,0	-	97,0
12	-	1	-	34	-	3,0	-	100,0

Table IV. DEGF distribution in male and female pupils

DEGF	Frequency		Cumulative frequency		Per cent		Cumulative per cent	
	M	F	M	F	M	F	M	F
0	1	3	1	3	3,7	8,8	3,7	8,8
1	11	10	12	13	40,7	29,4	44,4	38,2
2	6	12	18	25	22,2	35,3	66,7	73,5
3	9	9	27	34	33,3	26,5	100,0	100,0

Table V. Various parameters for the male, female and combined pupils

Parameters	Male	Female	T	P	Combined pupils
n	27	34			61
Unadjusted InF	13,08 ± 0,08*	12,94 ± 0,06	-1,4330	0,1571	13,00 ± 0,05
Enamel F (ppm)	2410 ± 220	2011 ± 129			
Adjusted InF	13,08 ± 0,07	12,94 ± 0,07	-1,43	0,1557	
Unadjusted InEn	5,39 ± 0,03	5,41 ± 0,03	0,4464	0,6569	5,40 ± 0,02
Biopsy depth (µm)	10,46 ± 0,34	10,66 ± 0,30			
DMFT	2,30 ± 0,41	3,44 ± 0,57			2,93 ± 0,37
√DMFT	1,25 ± 0,17	1,48 ± 0,19	0,8920	0,3760	1,38 ± 0,13
DEGF	1,85 ± 0,18	1,79 ± 0,16	-0,2364	0,8140	1,82 ± 0,12
Age	15,22 ± 0,22	15,35 ± 0,21			

* Mean ± SE

The caries incidence (DMFT) of each participant was determined in good natural light with the use of mouth mirrors and sharp dental probes by a dental surgeon (DHR). Teeth lost as a result of trauma or those congenitally missing were not included in determining the DMFT index. The DMFT distribution in the male and female pupils is given in Table III and the mean DMFT ± S.E. in Table V. The degree of fluorosis was assessed by a dental surgeon (FHB) using Dean's (1934) classification as a guide.

Class I	Normal teeth with no evidence of fluorosis. Score 0.
Class II	Mild fluorosis characterized by localized white streaks or areas. Score 1.
Class III	Moderate fluororis characterized by extensive white opaque mottling and isolated pitting of enamel. Score 2.
Class IV	Severe fluorosis characterized by marked pitting and often accompanied by brown staining. Score 3.

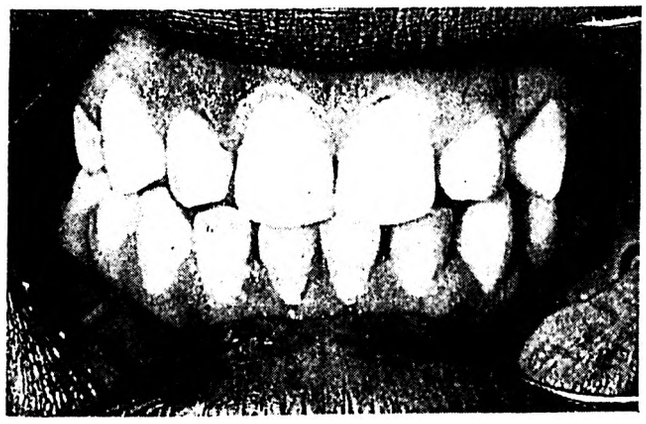


Fig. 1. No fluorosis.

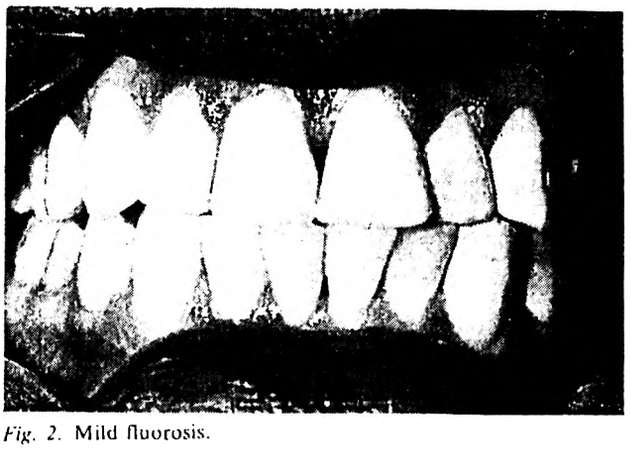


Fig. 2. Mild fluorosis.

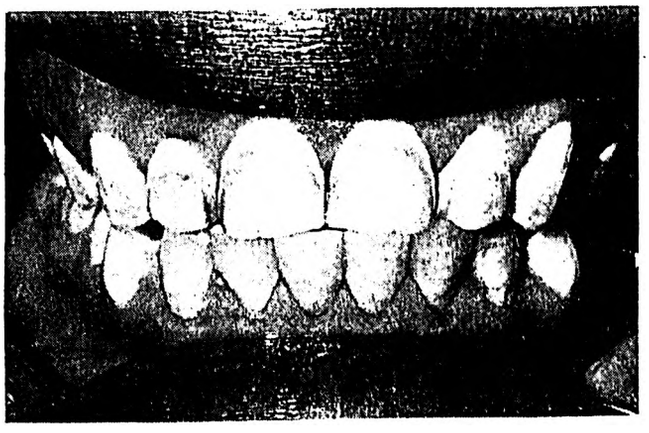


Fig. 3. Moderate fluorosis.

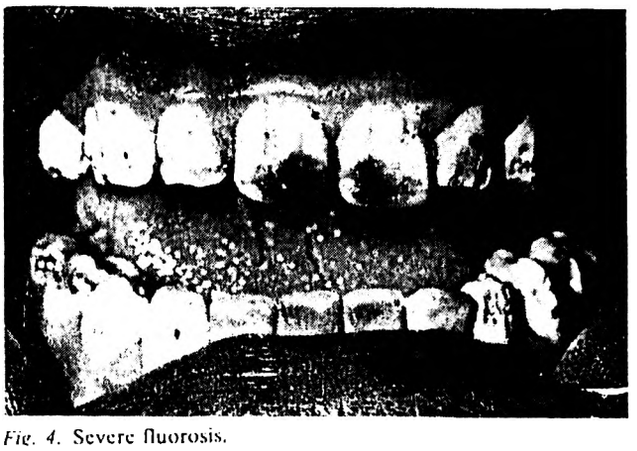


Fig. 4. Severe fluorosis.

The DEGF distribution in male and female pupils is given in Table IV and the mean DEGF ± S.E. in Table V.

The facial surfaces of the maxillary central incisors of each pupil were cleaned with a water slurry of flour of pumice and a slowly rotating rubber cup. The teeth were washed and dried well. Areas, 3 mm in diameter, were demarcated on the middle third of the crowns of the incisors by means of annular adhesive discs (No. 471; 3 M Co., St. Paul, MI). The discs were burnished well to ensure good marginal adaptation to the underlying enamel. Enamel specimens were obtained from the demarcated surface areas by means of an acid etch biopsy procedure (Van der Merwe *et al*, 1974). The etching solutions were transferred to plastic tubes containing 1 ml of Total Ionic Strength Adjustment Buffer [1] diluted with an equal amount of deionized water.

The test solutions were analyzed for fluoride and calcium. The fluoride content was determined with a Model 96-09 Orion combination fluoride electrode coupled to an Orion Model 801A pH/mv meter. The mass of fluoride in the etching solutions was expressed in picograms (pg). The calcium content was determined in aliquots of etching solutions diluted 25 times with 1 per cent lanthanum chloride to prevent interference from phosphorus and aluminium (Willis, 1961). Duplicate calcium determinations were carried out with a Zeiss Model PMQII with flame attachment FA2 atomic absorption spectrophotometer [2]. The mass of enamel in each etching solution was calculated by assuming that human enamel contains 37 per cent calcium (Retief *et al*, 1971) and expressed in micrograms (μg). The fluoride concentrations in the enamel of the central incisors were calculated as follows:

$$\text{Unadjusted fluoride concentration (ppm)} = \frac{\text{Mass F (pg)}}{\text{Mass Enamel } (\mu g)}$$

The mean and S.E. of the average values for left and right maxillary central incisors of each individual were determined for the male and female pupils (Table V). The biopsy depth for each tooth was calculated from the following formula:

$$\text{Biopsy depth } (\mu m) = \frac{\text{Mass enamel } (\mu g)}{\text{Density of enamel} \times \text{Biopsy area } (mm^2)} = \frac{\text{Mass enamel } (\mu g)}{20{,}86}$$

[1] (TISAB, Orion Research Inc., Cambridge, MA)

[2] (Carl Zeiss, Oberkochen/Württ, West Germany)

Density of enamel is 2,95 (Manly and Hodge, 1939). The mean and S.E. of the average biopsy depths for left and right maxillary central incisors were determined for the male and female children (Table V).

Because of the steep fluoride gradient in the outermost enamel (Brudevold *et al*, 1956), the mass of fluoride in a given biopsy obtained from a standardized area depends on the biopsy depth or equivalently, on the mass of enamel in the biopsy solution. The biopsy depth is an uncontrollable variable and ranged from 6,8 µm to 14,8 µm. In order to make valid comparisons between the fluoride content of the central incisor teeth, the fluoride concentrations were corrected to a uniform depth of 10 µm (equivalent enamel mass of 208,6 µg).

Statistical analysis

The following data transformations were made:

Mass F in picograms to $\log_e$ mass F (lnF)
Mass enamel in micrograms to $\log_e$ mass En (lnEn)
DMFT to $\sqrt{DMFT}$

The data were transformed in order to stabilize variation and to make the data more nearly follow a Gaussian distribution. Preliminary t-tests were carried out to determine the significance level of the differences between lnF and lnEn respectively for the left and right incisors of the pupils involved in this study. The differences between lnF (t=1,40, p=0,167) and lnEn (t=1,70, p=0,0947) were not significant. The means of lnF (unadjusted) and lnEn (unadjusted) for the sexes were determined from the average values of lnF (left) and lnF (right) and lnEn (left) and lnEn (right) for each individual. Two sample t-tests were used to determine the significance level of the differences for lnF (unadjusted), lnEn (unadjusted), $\sqrt{DMFT}$ and DEGF between the sexes.

A covariance analysis (Steel and Torrie, 1960) was run to test the differences between sexes for lnF (adjusted) using lnEn as the covariate. The adjusted lnF values were obtained by correcting the unadjusted lnF values to a standardized depth of 10 µm (equivalent enamel mass of 208,6 µg).

In order to determine the association between $\sqrt{DMFT}$, lnF and DEGF, correlations (Steel and Torrie, 1960) between the various pairs of variables were calculated for all the pupils, for pupils with DMFT≤2 and DMFT>2, and for pupils with DEGF≤1 and DEGF>1.

All computations were performed utilizing the Statistical Analysis System (Barr *et al*, 1976).

RESULTS

Of the male pupils 25,9 per cent were caries-free and 3,7 per cent showed no signs of fluorosis. The corresponding percentages for the females were 29,4 and 8,8. The mean (± S.E.) values for DMFT, DEGF, enamel fluoride concentration (ppm) and biopsy depth for the male and female pupils are given in Table V. Two sample t-tests of the transformed data $\sqrt{DMFT}$, lnF (unadjusted), lnEn (unadjusted) and DEGF showed that the differences between sexes for these parameters were not significant (Table V). The covariance analysis revealed that the covariate (lnEn) was not significant (t=0,21, p=0,83) and that the difference in lnF (adjusted) between sexes was also not significant (t=1,43, p=0,1577). The correlations between the parameters $\sqrt{DMFT}$, lnF and DEGF are given in Table VI. The following significant associatons were established:
A negative correlation significant at the 10 per cent level (p = 0,0902) between $\sqrt{DMFT}$ and lnF for DEGF≤1; A highly significant (p =0,0004) positive correlation between lnF and DEGF for all the pupils and for pupils with DMFT>2 (Table VI).

DISCUSSION

Sixty-one of the 70 odd children in the Kenhardt High School who were born and had been living continually in the Kenhardt village and district were selected for this study. Sixteen of these children were resident in the village and the remainder were from the district. Approximately one-half of the children from the district were boarding in the school hostels during the school term. They were approximately 7 years old when first admitted to the hostels. The children examined were representative of the area as only 10 per cent of the Kenhardt children attended other schools.

The drinking water of the district was mainly obtained from boreholes with a fluoride content ranging from 0,49 to 3,02 ppm F. Rainwater collected from the roofs of dwellings in cisterns is used on some of the farms, but this supply of drinking water is sporadic as it is dependent on the rainfall in a semi-arid region. The school hostels and the high school are supplied with the same drinking water as the village which contains 3,2 ppm F.

The adjustment of lnF to a standardized depth of 10 µm was made because the biopsy depths ranged from

Table VI. Correlations between DMFT, lnF and DEGF

	All pupils		DMFT ≤2		DMFT >2		DEGF ≤1		DEGF >1	
	r	p	r	p	r	p	r	p	r	p
$\sqrt{DMFT}$ and lnF	-0,0532	0,6838	0,0549	0,7539	-0,0204	0,9214	-0,3460	0,0902*	0,1268	0,4612
$\sqrt{DMFT}$ and DEGF	-0,0061	0,9626	0,2597	0,1319	-0,1271	0,5360	-0,2805	0,1745	0,1723	0,3151
lnF and DEGF	0,4368	0,0004*	0,1941	0,2640	0,6418	0,0004*	0,2219	0,2864	0,2450	0,1498
n	61		35		26		25		36	

*Significant correlations

6,8 µm to 14,8 µm. This could have resulted in dissimilar biopsy depths between the male and female pupils. In this investigation, however, the difference in InEn (or equivalent biopsy depth) between the male pupils (5,39 ± 0,03) and the female students (5,41 ± 0,03) was not significant (P = 0,6569, Table V). In addition, the biopsy procedure used resulted in mean biopsy depths of approximately 10 µm (the adjusted depth) for both the male and female groups (Table V). Consequently similar values for InF (unadjusted) and InF (adjusted) were obtained for the male and female pupils respectively.

No significant differences between InF (t = 1,40, p = 0,167) and InEn or equivalent biopsy depth (t = 1,70 p = 0,0947) respectively for the left and right maxillary incisors of the pupils involved in this study were observed. This is in accordance with previous findings by Keene *et al,* (1973) and Bischoff *et al,* (1976). Aasenden (1974) reported that the mean fluoride concentration of biopsies taken to comparable depths was generally about 10 per cent higher in males than in females. He suggested that this observed difference in F concentration could be related to the longer pre-eruptive maturation time of the enamel in boys than in girls. In the present study the difference in the InF values adjusted to a standardized depth of 10 µm between sexes was not significant (t = 1,43, p = 0,1577).

The present investigation is based on the premise that the average F concentration of the biopsied left and right maxillary central incisors of an individual is an index of the fluoride status of the entire dentition of that individual. This premise is supported by the experimental work of Aasenden *et al,* (1973) and Richards *et al,* (1977). Aasenden *et al,* (1973) determined the fluoride concentration in surface enamel of maxillary central and lateral incisors and canines of children from three communities living in areas supplied with different F levels in the drinking water. Their study showed that there were differences between the F concentrations in the surface enamel of various tooth types but that the F concentrations of the three tooth types within a given community differed from those of their homologous teeth in another community by a relatively constant factor. They concluded that in comparative studies on the effect of water fluoridation, conclusions based on the F levels of only one tooth type were applicable to the general F status of a community. This was confirmed by Richards *et al,* (1977) who reported that the fluoride content of the maxillary central incisors was closely correlated to the grand mean fluoride content of all the other maxillary teeth from the same individual.

Several attempts have been made to determine the relationship between parameters such as dental fluorosis, caries incidence and fluoride content of enamel. As early as 1936, Bowes and Murray reported that the fluoride content of pooled bulk enamel from mildly mottled teeth was higher than that of non-mottled teeth. Armstrong and Brekhus (1938) found that the mean fluoride concentration in bulk enamel of sound teeth was higher than that of carious teeth. McClure (1948), however, reported that he could not relate differences in the fluoride content of sound and carious teeth from the same individuals to their caries status. McClure and Likins (1951) found that an increase in the fluoride content of the drinking water resulted in an increased fluoride uptake by bulk enamel and that this was associated with a marked reduction in dental caries experience.

A study was undertaken by Forrest (1956) to correlate the incidence of caries and mottling with different levels of fluoride in the drinking water. She reported that the caries incidence was markedly lower in the fluoride than in the non-fluoride areas and that the incidence and severity of fluoride mottling was directly related to the amount of fluoride in the water. Examination of her results showed that the caries incidence in children resident in an area containing 5,8 ppm F was twice as high as the caries incidence in children living in an area containing 3,5 ppm F (2,8 vs 1,4). The caries experience of both groups of children was, however, considerably lower than that of children living in low fluoride (0,1 - 0,2 ppm F) areas.

Binder (1973) carried out a survey to determine the effects of natural fluoride in the drinking water on dental caries and fluorosis. He found that the DMFT values of children resident in high fluoride areas decreased while the incidence of fluorosis increased. The interrelationship between fluoride exposure, dental fluorosis, concentrations of fluoride in surface enamel obtained by an abrasive biopsy procedure and caries experience was examined in young Naval recruits (Keene *et al,* 1973). Enamel from the caries-free group contained significantly higher levels of fluoride than the enamel from the groups with caries experience. Dental fluorosis and years of exposure to fluoride in drinking water were significantly higher in the caries-free group. To obtain further information on the interrelationships of the same parameters, the study was extended to individuals with obvious manifestations of dental fluorosis (Keene *et al,* 1975). They found that the mean enamel fluoride concentrations were significantly higher in the fluorosis group than in the control group but that the difference in the mean DMFT scores was not statistically significant.

DePaola *et al,* (1975) determined the caries experience (DMFS index) and surface enamel fluoride concentrations in a large number of 12-16-year-old pupils living in selected fluoridated and non-fluoridated areas. Fluoride was expressed as $\log_e$ mass F corrected to a standardized depth and a square root transformation of DMFS was carried out. An analysis of the data revealed a highly significant inverse relationship between $\log_e$ mass F and $\sqrt{DMFS}$. Bischoff *et al,* (1976), however, could find no correlation between caries experience (DMFT) and fluoride concentration in surface enamel in a population group living in an endemic fluorosis area.

In the present study the following significant correlations between the parameters DMFT, InF and DEGF were established: A weak negative correlation (p = 0,0902) between $\sqrt{DMFT}$ and InF for pupils with DEGF <1.

A strong positive correlation (p = 0,0004) between InF and DEGF for all the pupils involved and for children with DMFT>2. There was a tendency for the caries incidence to decrease with increased enamel fluoride

concentration in pupils with no or mild fluorosis. This could not be demonstrated for pupils with DEGF>1. Retief *et al*, (1979) had previously reported that there was a significant positive ($p<0,02$) correlation between $\sqrt{DMFT}$ and DEGF in Coloured children resident in Kenhardt. The DEGF of the Coloured pupils was much more severe than in the White children studied in the present investigation, but it may explain why no significant correlation could be established between $\sqrt{DMFT}$ and lnF in pupils with moderate and severe fluorosis.

The highly significant positive correlation ($p = 0,0004$) between lnF and DEGF for all the pupils was also observed for Coloured children resident in Kenhardt (Retief *et al*, 1979)

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