**THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH**

<table>
<thead>
<tr>
<th>Position</th>
<th>Name</th>
<th>Institution and Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>President</td>
<td>Prof H Tsunoda</td>
<td>Iwate Medical University, Morioka, Japan</td>
</tr>
<tr>
<td>Vice President</td>
<td>Prof Ming-Ho Yu</td>
<td>Western Washington University, Bellingham, WA, USA</td>
</tr>
<tr>
<td>Second Vice President</td>
<td>Dr Miklos Bely</td>
<td>National Inst of Rheumatology, Budapest, Hungary</td>
</tr>
<tr>
<td>Secretary</td>
<td>Prof Gene W Miller</td>
<td>Biology Dept, Utah State University, Logan, Utah, USA</td>
</tr>
<tr>
<td>Treasurer</td>
<td>Dr John Colquhoun</td>
<td>216 Atkinson Road, Titirangi, Auckland, NZ</td>
</tr>
</tbody>
</table>

**ADVISORY BOARD**

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution and Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr Miklos Bely</td>
<td>Nat Inst of Rheumatology, Budapest, Hungary</td>
</tr>
<tr>
<td>Prof A W Burgstahaler</td>
<td>University of Kansas, Lawrence, Kansas, USA</td>
</tr>
<tr>
<td>Dr G Embry</td>
<td>University of Wales Med College, Cardiff, Wales UK</td>
</tr>
<tr>
<td>Prof J Franke</td>
<td>Medical Academy, Erfut, Germany</td>
</tr>
<tr>
<td>Prof G W Miller</td>
<td>Utah State University, Logan, Utah, USA</td>
</tr>
<tr>
<td>Dr B P Rajan</td>
<td>Madras Dental College, Madras, India</td>
</tr>
<tr>
<td>Prof Shouren Cao</td>
<td>Chinese Academy of Preventive Medicine, Beijing, China</td>
</tr>
<tr>
<td>Dr Edward Czerwinski</td>
<td>Krakow Academy of Medicine, Krakow, Poland</td>
</tr>
<tr>
<td>Prof A K Susheela</td>
<td>All India Inst of Med. Sciences, New Delhi, India</td>
</tr>
<tr>
<td>Prof Zan-Dao Wei</td>
<td>Guiyang Medical College, Guizhou, China</td>
</tr>
<tr>
<td>Prof K Yoshitaka</td>
<td>Shiga University of Med Science, Shiga-Ken, Japan</td>
</tr>
<tr>
<td>Prof Y Yoshida</td>
<td>Osaka Medical College, Osaka, Japan</td>
</tr>
</tbody>
</table>

**EDITORIAL BOARD**

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution and Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr D J Ballentyne</td>
<td>University of Victoria, Victoria BC, Canada</td>
</tr>
<tr>
<td>Prof Shouren Cao</td>
<td>Chinese Academy of Preventive Medicine, Beijing, China</td>
</tr>
<tr>
<td>Dr Edward Czerwinski</td>
<td>Krakow Academy of Medicine, Krakow, Poland</td>
</tr>
<tr>
<td>Dr K A V R Krishnamachari</td>
<td>Director, Regional Medical Research Centre, Bhubaneswar, India</td>
</tr>
<tr>
<td>Dr K Kono</td>
<td>Osaka Medical College, Osaka, Japan</td>
</tr>
<tr>
<td>Prof Lennart Krook</td>
<td>College of Veterinary Medicine, Cornell University, Ithaca</td>
</tr>
<tr>
<td>Dr John R Lee</td>
<td>9620 Bodega Hwy, Sebastopol, California USA</td>
</tr>
<tr>
<td>Dr K A V R Krishnamachari</td>
<td>Director, Regional Medical Research Centre, Bhubaneswar, India</td>
</tr>
<tr>
<td>Dr F Murray</td>
<td>Murdoch University, Murdoch WA, Australia</td>
</tr>
<tr>
<td>Dr S P S Teotia</td>
<td>LLRM Medical College, Meerut, India</td>
</tr>
<tr>
<td>Dr James C Pushnik</td>
<td>California State University, Chico, California USA</td>
</tr>
<tr>
<td>Dr Sally W Wheeler</td>
<td>Hawkesbury Agric Research Unit, Richmond, NSW, Australia</td>
</tr>
</tbody>
</table>

---

**Note:** The above text is a sample of the content and is not intended to be comprehensive or exhaustive. It represents a snapshot of the organization and its leadership and advisory structure as of the publication date.
EDITORIAL
WE WILL MEET IN CHINA, IN 1994 ................................................................. 165

ANNOUNCEMENTS
XXth ISFR CONFERENCE, BEIJING, CHINA 10-13 October 1994 ...................... 166
Vth FLUORIDE SYMPOSIUM, POLAND, 14-16 September 1994 ...................... 166

RESEARCH REPORTS
SPIN LABEL ESR STUDY OF THE INFLUENCE OF FLUORIDE ON ERYTHROCYTE MEMBRANE FLUIDITY
  Y Wang, X Li and W Xin, China ................................................................. 167-176
ENDEMIC FLUOROSIS IN THE VILLAGE RALLA ANANTAPURAM IN ANDHRA PRADESH: AN EPIDEMIOLOGICAL STUDY
  D Saralakumani and P Ramakrishna Rao, India ........................................ 177-180
MONITORING OF NEIGHBOURHOOD FLUOROSIS THROUGH A DENTAL FLUOROSIS SURVEY IN SCHOOLS
  V K Desai, D M Solanki, S L Kantharia and B S Bhavsar, India ................. 181-186
EPIDEMIOLOGICAL STUDY OF GOITRE IN ENDEMIC FLUOROSIS DISTRICT OF GUJARAT
  V K Desai, D M Solanki and R K Bansal, India ........................................ 187-190
NUCLEIC ACID LEVELS IN THYROID GLAND IN ACUTE AND CHRONIC FLUORIDE INTOXICATION
  A Shashi, India ......................................................................................... 191-196

RESEARCH REVIEW
DEVELOPMENTS IN THE ANALYSIS OF FLUORIDE 1980-1990
  C Y Wang and J Xu, China ......................................................................... 197-202

CONFERENCE ABSTRACTS
On Analytical Methods for Fluoride:
DETERMINATION OF TOTAL FLUORIDE IN CORN: COMBUSTION-HYDROLYSIS/
FLUORIDE-ION ELECTRODE METHOD  R Ji, X Quan, S Cao and Y Li, China ........ 203
PREPARATION AND CERTIFICATION OF FLUORIDE COMPOSITION IN CORN
AND FLY ASH REFERENCE MATERIALS  R Ji, R Yang, S Cao and Y Li, China ...... 203
IN VIVO LOCALISATION OF STRONTIUM AND FLUORIDE IN BONE TISSUE
  S Bang, Switzerland and J S Lee, Korea ................................................... 204
EMISSION SPECTROCHEMICAL ANALYSIS OF FLUORINE USING
SHOCK WAVE PLASMA INDUCED BY A TEA CO₂ LASER
  K Kagawa, Y Deguchi, M Tani and Y Takagi, Japan .................................... 204-205
FLUORIDE MICRODETERMINATION OF BIOLOGICAL MATERIALS BY FAU-1100 AND FAU-2100
  K Itai and H Tsunoda, Japan ................................................................. 205
FLOW INJECTION ANALYSIS OF FLUORIDE IONS USING ION-SELECTIVE ELECTRODE AND ELIMINATION OF INTERFERENCES FROM IRON (III) AND ALUMINUM (III) M Chikuma, A Miki, Y Okabayashi and H Tanaka, Japan ...... 206

On Environmental Fluoride Pollution and Biological Effects on Humans:
THE EVALUATION OF INDOOR AIR QUALITY IN FLUOROSIS PREVALENCE AREAS CAUSED BY COAL BURNING S Cao, R Ji and Y Li, China ......................... 207
RESEARCH PROGRESS ON ENDEMIC FLUOROSIS IN CHINA Z D Wei, China .... 207
RESULTS OF BONE DENSITOMETRY USING DPX IN PATIENTS WITH IATROGENIC FLUOROSIS J Franke and S Hauch, Germany ..................... 208
PHYSICOCHEMICAL PROPERTIES OF HETEROGENEOUS FLUORIDATED APATITES M Okazaki, J Takahashi and H Kimura, Japan ........ 208-209

On Biological Effects of Fluoride:
THE ORIENTATION OF CAPSULAR AND INTERCAPSULAR LINEARLY ORIENTED POLYSACCHARIDES OF BONE AND ARTICULAR CARTILAGE IN EXPERIMENTAL OSTEOPATROUS M Bely, Hungary .......... 209
THE MECHANISM OF CHANGES IN METABOLISM AND TRANSPORT OF GLUCOSE CAUSED BY FLUORIDE ADMINISTRATION TO RATS T Sakurai, K Suzuki, T Taki and Y Suketa, Japan .................................................... 210
RESTORATION OF CALCIUM DISTURBANCE IN KIDNEY OF FLUORIDE-TREATED RATS: ROLE OF PROTEIN KINASE C AND CALCIUM PROTEASE (CALPAIN) Y Suketa, M Yamamoto, T Ibuki and T Imagawa, Japan ........................................ 210-211
CHRONIC FLUORIDE TOXICITY: A BIOCHEMICAL AND SCANNING ELECTRON MICROSCOPIC STUDY OF ENAMEL SURFACE OF RABBIT TEETH M Bhoomarag and A K Susheela, India ................ 211
THE EFFECT OF ORGANIC AND INORGANIC FLUORINE COMPOUND ADMINISTRATION ON FLUORIDE LEVELS IN THE PLASMA AND IN THE SOFT AND HARD TISSUES OF RATS BREEDING ON A LOW-FLUORIDE DIET M Tomita, T Sugimura, M Koyama and Y Kaneko, Japan ........ 212
EFFECTS OF FLUORIDE ON SHEEP MOLARS G E Milhaud, France .......... 213

Poster Presentations:
STUDY OF REGIONAL FLUORIDE STANDARDS FOR AMBIENT AIR QUALITY IN BAOTOU X Wu, China ........................................ 214
CORRELATION BETWEEN THE FLUORIDE CONCENTRATION IN AIR AND CANADA POPULAR LEAVES X Wu, China .................. 214
FLUORIDE CONCENTRATION IN WATER FROM LAKES MAGADI, BOCORIA, AND ELEMENTAITA, KENYA J K Gikunju and T E Maithe, Kenya and P Lokken, Norway .................... 215
EXPERIMENTAL STUDIES ON FLUORIDE-INDUCED DIABETIC HYPERGLYCEMIA A Shashi, India ........................................ 215
AN OVERVIEW OF FLUORIDE RESEARCH: ANALYTICAL LEVEL AND MEDICOBIOLOGICAL APPROACH K Yoshitake, G Yamamoto, T Kimura and T Ando, Japan ........................................ 216
AIR-POLLUTION FLUOROSIS IN THE REGION OF PINGXIANG, JIANGXI, PEOPLE'S REPUBLIC OF CHINA Y Chen, M Lin, Z He and X Xie, China ........ 216
STUDY OF THE EFFECT OF THE MICROELEMENT MOLYBDENUM ON ENDEMIC FLUOROSIS Z M Yao and L H Chang, China ........ 217
FURTHER IDENTIFICATION OF A DFP-HYDROLYZING ENZYME IN PLANTS TISSUES S Sakurai, Japan and M-H Yu, USA ........ 217
HIGH-RESOLUTION ELECTRON MICROSCOPY OF ENAMEL AND DENTIN CRYSTALS IN FLUOROTIC TEETH Y Mieake, T Yanigisawa, H Tohda, Japan and O Fejerskov, Denmark ........ 218
CONFERENCE ABSTRACTS continued:

ON ENDEMIC FLUOROSIS IN NONG-AN COUNTY, JILIN PROVINCE, CHINA D Zhang, K Liao, A Gu et al, China ......................... 218-219

RELATIONSHIP BETWEEN FLUORIDE AND IONIC FLUORINE CONTENT IN DRINKING GROUNDWATER AND FLUOROSIS ON THE SONGNEN PLAIN, CHINA S L Xiong, China ........................................... 219

INVESTIGATION OF MAJOR TECHNOLOGIES AND FACILITIES FOR DRINKING WATER DEFLUORIDATION IN CHINA. C Huang and J Liu, China ...... 220

EVALUATION OF THE EFFECT OF LOWERING FLUORIDE LEVELS IN DRINKING WATER AFTER A TEN-YEAR PERIOD IN SHUNYI COUNTY, CHINA L Hao and F Chen, China ..................... 220

MOTION MODEL OF FLUORINE AND OPTIMIZED FLUORIDE CONTENT CONTROL IN GROUNDWATER OF CHANGCHUN CITY AND SUBURBS, CHINA Y Q Zhao, China ........................................... 221

EFFECTS OF VARIOUS FEEDS ON RATS WITH REDUCED BONE MINERAL DENSITY J Senda, M Shimahara, N Hashiguchi et al, Japan ......................... 221

PROBLEM OF EXCESS FLUORINE IN WATER IN INDIA AND ITS REMOVAL BY THE NALGONDA TECHNIQUE D R Bulusu and W G Nawlakhe, India ......................... 222

EFFECTS OF FLUORIDE ON EXPERIMENTAL POSTMENOPAUSAL OSTEOPOROSIS WITH KIDNEY DYSFUNCTION T Dote, Y Yoshida, K Kono et al, Japan ......................... 222

RESEARCH ON CHRONIC EFFECTS OF OCCUPATIONAL EXPOSURE TO ORGANIC FLUORINE G Z Zhang, Z Y Zhang, B C Xu et al, China ......................... 223

EFFECTS OF ORGANIC FLUORINE EXPOSURE ON THE REPRODUCTIVE FUNCTION OF FEMALE WORKERS AND THE DEVELOPMENT OF THEIR OFFSPRING Z Y Zhang, G Z Zhang, X J Liu et al, China ......................... 223-224

STUDIES ON THE TREATMENT OF HYDROFLUORIC ACID SKIN BURNS K Kono, Y Yoshida, M Watanabe et al, Japan ......................... 224

EFFECTS OF FOOD INTAKE ON SERUM AND URINARY FLUORIDE CONCENTRATIONS AS AN INDICATOR OF OCCUPATIONAL FLUORIDE EXPOSURE H Nagaie, Y Yoshida, K Kono et al, Japan ......................... 224-225

EFFECTS OF ANTACID (CIMETIDINE) ON FLUORIDE ABSORPTION FROM THE GASTRO-INTESTINAL TRACT Y Besso, Y Yoshida, K Kono et al, Japan ......................... 225

FLUORIDE CONCENTRATION IN URINE AND SERUM AMONG INHABITANTS OF A FISHING VILLAGE Y Tanioka, Y Yoshida, K Kono et al, Japan ......................... 225-226

URINARY FLUORIDE EXCRETION AFTER DIETARY INTAKE IN HEALTHY JAPANESE ADULTS M Watanabe, Y Yoshida, K Kono et al, Japan ......................... 226

EFFECTS OF LONG-TERM LOW-FLUORIDE INTAKE ON BONE IN GROWING, SENESCENCE-ACCELERATED MICE (SAM-P/6) T Imai, Y Takeki and M Niwa, Japan ......................... 226-227

DEPENDENCE OF CELL FLUORIDE SENSITIVITY TO THE PERIOD OF THE CELL CYCLE T Sato and M Niwa, Japan ......................... 227

EPIDEMIOLOGICAL STUDY ON THE PREVALENCE OF DENTAL FLUOROSIS IN CHILDREN AFTER DRINKING WATER IMPROVEMENT AND DEFLUORIDATION S Weibin and W Xuesong, China ......................... 228

STUDY ON THE COMBINED EFFECT OF FLUORIDE AND SULPHUR DIOXIDE FROM INDOOR COAL BURNING ON EXPERIMENTAL ANIMALS THROUGH NATURAL INHALATION C K Liang et al, China ......................... 228
ABSTRACTS

PREVALENCE OF RESPIRATORY DISORDERS AMONG ALUMINIUM POTROOM WORKERS IN RELATION TO EXPOSURE TO FLUORIDE

Vidar Soyseth and Johny Kongerud, Norway ......................................................... 229

LOW CONCENTRATIONS OF SODIUM FLUORIDE INHIBIT Ca2+ INFLUX INDUCED BY RECEPTOR-MEDIATED PLATELET ACTIVATION

Y Ozaki, K Sato, Y Yatomi and S Kume, Japan .................................................. 229

THROMBIN AND NaF, BUT NOT EPINEPHRINE, RAISE CYTOSOLIC FREE Na⁺ IN HUMAN PLATELETS

V Stamouli, C Vakirtzilemonias and W Siffert, Germany ................................. 230

THE INFLUENCE OF FLUORIDE ON PROTEOGLYCAN STRUCTURE USING A RAT ODONTOBLAST INVITRO SYSTEM

R J Waddington, G Embery and RC Hall, Wales ........................................ 230-231

DENTAL FLUOROSIS, DENTAL CARIES AND FLUORIDE EXPOSURE AMONG 7-YEAR-OLDS

P J Riordan, Australia .......................................................................................... 231-232

MEASUREMENT OF INTRINSIC BONE QUALITY INVIVO BY REFLECTION ULTRASONOC: CORRECTION OF IMPAIRED QUALITY WITH SLOW-RELEASE SODIUM FLUORIDE AND CALCIUM CITRATE

P P Antich, C Y C Pak, J Gonzales et al, USA ................................................... 232

DISTRIBUTION OF FLUORIDE IN CORTICAL BONE OF HUMAN RIB

K Ishiguro, H Nakagaki, S Tsuboi et al, Japan .................................................. 233

EFFECT OF FLUORIDE ON THE ATP REQUIREMENT FOR GLYCEROLIPID BIOSYNTHESIS IN ADIPOSE TISSUE OF 4 MAMMALIAN SPECIES

D C Rule, USA ........................................................................................................ 233

CORRELATION AND CAUSE: AN ISSUE IN THE EPIDEMIOLOGY OF FLUOROSIS

John Colquhoun, New Zealand ........................................................................... 234

INHIBITORY EFFECT OF FLUORIDE ON INSULIN RECEPTOR AUTOPHOSPHORYLATION AND TYROSINE KINASE ACTIVITY

F Vinals, X Testar, M Palacin and A Zorzano, Spain ......................................... 234-235

LETTERS TO THE EDITOR

NON-SKELETAL FLUOROSIS: COMMENT ON SURVEY

Mark Diesendorf .................................................................................................... 235

WATER FLUORIDE AND GOITRE

Richard C Foulkes .................................................................................................. 236
WE WILL MEET IN CHINA, IN 1994

During his Presidential opening address to the XIXth ISFR Conference in Kyoto, Professor Humio Tsunoda observed:

"there are many participants from China who are taking part in this conference . . . China has the world's severest fluoride-related environmental problems, but the country is making a nationwide effort to resolve these problems, and high-level fluoride research covering a wide range of fields is being conducted. I believe that scientific interchange on fluoride research between the Chinese scientists and other participants will be extremely meaningful and most welcome at this conference."

Professor Tsunoda's belief was confirmed, and it was most appropriate that, at the Conference conclusion, it was unanimously resolved to accept the invitation of the Chinese delegates to hold the next ISFR Conference in Beijing, China. The following information about the Conference (see also next page) has now been received from Professor Cao Shouren, Chairman of the Organizing Committee.

President
Professor Humio Tsunoda

XXth ISFR Conference Organizing Committee
Dr Gao Shufen (Honorary Chairman)
Prof Cao Shouren (Chairman)
Prof Wei Zandao (Vice-Chairman)
Dr Wang Huanzeng (Vice-Chairman)
Dr Gan Dekun (Secretary General)
Dr Shen Erli (Secretary General)
Prof Wang Zhicheng
Prof Wang Yunzhao
Dr Sun Weibin
ISFR 94
BEIJING, CHINA
October 10-13, 1994

THE XXth CONFERENCE OF THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH
will be held in Beijing, People's Republic of China, on October 10-13, 1994.
The official language of the Conference will be English.
The registration fee is US$200.

Main Topics:
1. Environmental fluoride pollution.
2. Biological effects of fluoride.
3. Influence of fluorides on health.
4. Analytical methods and monitoring of fluorides.
5. Dechlorination methods.

Submission of Abstracts:
Abstracts should reach the Secretariat, if possible, by end of October 1993.
Forms for abstract submission are available from the Secretariat.

Secretariat
ISFR '94 Scientific Secretariat, Dr Liang Chaoke
Institute of Environmental Health and Engineering
Chinese Academy of Preventive Medicine
29 Nan wei Road
Beijing 100050
People's Republic of China
FAX 0086-01-3011875
Phone 0086-01-3038761-2611

MESSAGE FROM POLAND
Professor Zygmunt Machoy has informed us that the sixth Fluorine Symposium will take place in Szczecin, Poland, on September 14-16, 1994. Further information will appear in *Fluoride.*
SPIN LABEL ESR STUDY OF THE INFLUENCE OF FLUORIDE ON ERYTHROCYTE MEMBRANE FLUIDITY

Yingyan Wang,* Xiaojie Li and Wenjuan Xin
Beijing, China

SUMMARY: The fluidity of intact human erythrocyte membrane and the effect of fluoride on fluidity were studied by using two kinds of fatty acid spin labels and measuring the time and temperature dependence of order parameter S and rotational correlation time $\tau_c$. The time dependance of S or $\tau_c$ indicated that there is an effect of time in the course of the F$^-$ action on the cell membrane. The plot of S vs. temperature for I [12, 3] showed an abrupt discontinuity at 29.0°C in untreated and 36.0°C in F$^-$-treated but for I [1, 14] showed no discontinuity. Plots of $\tau_c$ vs. temperature for I [12, 3] and I [1, 14] showed a point of phase transition at 38.0°C and 40.0°C in untreated and at 36.6°C and 44.6°C in F$^-$-treated, which indicated that a difference in fluidity exists between the surface and the interior of the lipid bilayer, and that the above fluidity differences is changed by F$^-$ exposure. It is concluded that F$^-$ has little effect on membrane fluidity but, when combining with a factor of time or temperature, has a marked effect on it.

Key words: Fluoride; Human erythrocyte membrane; Spin label ESR.

Introduction

In recent years, the influence of fluoride (F$^-$) on the cell membrane has been studied (1). Such studies were concerned mainly with biochemical changes of the membrane under the action of F$^-$, particularly on the biophysical function, e.g., permeability (2) and temperature adaptation (3). Among the several techniques is the spin label ESR spectrum method (4). We applied the method of spin labeling with various chain-lengths of stearic acid, in order to study the influence of F$^-$ on cell membrane fluidity. The erythrocyte membrane was chosen as the model because it has been shown to be particularly appropriate for studying the biological properties of the cell membrane (5). In this paper, we report the changes of membrane fluidity of the different layers (surface and depth) of the healthy human erythrocyte membrane treated with F$^-$, and the effect of different times and temperatures.

Materials and Methods

Reagents: The spin labels, 2-[3-carboxypropyl]-2-tridecyl-4,4-dimethyl- and 2-[14-carboxytetradecyl]-2-ethyl-4,4-dimethyl-3-oxazolidinylxyloxy - abbreviated as I [12, 3] and I [1, 14] - were purchased from Sigma and Aldich Chemical Co., respectively. Other reagents obtained from China are AR grade.

Erythrocyte: Healthy human erythrocyte suspensions (ACD-B), containing $1 \times 10^9$ cells/mL, were provided by the Blood Centre of the Red Cross Society in Beijing. The original erythrocyte suspensions were diluted to $1 \times 10^7$ cells/mL with Tris-HCl-glucose isotonic solutions (pH 7.3).

* Beijing Municipal Research Institute of Environmental Protection, Beijing, Peoples Republic of China.
Spin labeling of erythrocytes: The spin label was dissolved in the small volume of ethanol and then prepared to 0.01 M I [12, 3] or I [1, 14] solutions with Tris buffer. 10 μL of label solutions were added to 5 mL of the dilutions of erythrocyte suspensions and, after a thorough shaking, the mixture was incubated for 25 min at 37°C. The spin-labeled erythrocytes were immediately centrifuged for 8 min at 3000 rev/min and the supernatants were removed. The labeled membranes were then washed with isotonic solutions (5 mL at a time) until no ESR signal was detected in the supernatants - that is, the signals finally recorded were merely contributed from the membranes into which the probe I [12, 3] or I [1, 14] was inserted on the surface and into the deeper part below the surface.

Treatment with NaF: This experiment was divided into two groups. In the F- treated group, 10 μL of 0.05 M sodium fluoride were added to labeled cells, and in the untreated group, no drug was added. After a thorough shaking the two groups were incubated for 10 min at 37°C. Finally, the labeled enthrrocytes, F- treated and untreated, were collected and spectra were recorded.

Electron spin resonance (ESR) measurements: ESR spectra were recorded using a Varian E-109 ESR spectrometer equipped with a variable temperature accessory. The measuring conditions were: microwave power 5 mW, X-band, 100 KHz field modulation, modulation amplitude 1.0 G, central magnetic field 3324, scan width 200, scan speed 25 G/min, time constant 0.18 s, temperatures generally 37°C, except when recording temperature alterations, ranging from 0°C to 55°C.

Results and Discussion

Figure 1 shows the representative ESR spectra obtained from erythrocyte membranes, labeled respectively I [12, 3] and I [1, 14], with or without fluoride treatment. The spectra of both F- treated and untreated membranes indicate that there is an anisotropic motion as observed with spin probes incorporated into the lipid bilayers, exhibiting hyperfine splittings, peak-to-peak width and peak heights. The corresponding parameters calculated from the ESR spectra are presented in Table 1, including maximum peak height $A_{\text{max}}$, minimum peak height $A_{\text{min}}$, order parameter $S$, mean fluctuation angle $\theta$, rotational correlation time $\tau_c$ and microviscosity $\eta$, the measurement conditions being for 90 min at 37°C. As shown from Table 1, S values of F- treated membrane labeled with probe I [12, 3] and I [1, 14] are slightly greater than those of the untreated, but $\tau_c$ values of the former are less than the latter. The results suggest that fluoride has an effect on the original fluid state of the human erythrocyte membrane lipid bilayer.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>$A_{\text{max}}$</th>
<th>$A_{\text{min}}$</th>
<th>$S$</th>
<th>$\theta$</th>
<th>$\tau_c$</th>
<th>$\eta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>I [12, 3]</td>
<td>Control</td>
<td>54.54</td>
<td>18.34</td>
<td>0.627</td>
<td>29.94</td>
<td>23.67</td>
</tr>
<tr>
<td></td>
<td>NaF</td>
<td>54.81</td>
<td>18.17</td>
<td>0.635</td>
<td>29.54</td>
<td>18.71</td>
</tr>
<tr>
<td>I [1, 14]</td>
<td>Control</td>
<td>37.53</td>
<td>25.16</td>
<td>0.177</td>
<td>47.84</td>
<td>7.28</td>
</tr>
<tr>
<td></td>
<td>NaF</td>
<td>35.82</td>
<td>23.20</td>
<td>0.199</td>
<td>46.98</td>
<td>6.54</td>
</tr>
</tbody>
</table>
S, θ, τc and η of the membrane treated with F⁻ at various times from 10 to 240 min are presented in Table 2. The plots of four parameters vs. time indicated that a peak is reached in the course of F⁻ action on the cell membrane and the time of its appearance differs from that of the control. For instance, S or τc on the surface of the bilayer appears at 10 or 90 min in the untreated but at 120 or 10 min in the F⁻-treated; and the peak of S or τc in the deeper part appears at 30 or 180 min in the untreated but at 90 or 30 min in the F⁻-treated. On the surface, the appearance time of the S peak is delayed about 110 min, and that of τc is made earlier by about 80 min; in the deeper part, the appearance time of the S peak is delayed about 60 min, and that of τc is made earlier by about 150 min. As a result, F⁻ delays the appearance time of the S peak on the surface and in the interior, but shortens the appearance time of the τc peak in both. Compared with the untreated group, F⁻ increased the order parameter S - that is, fluidity is decreased. And the effect which F⁻ produces occurs earlier in the bilayer interior than on the surface, and thus the time which is needed to effect a change along the hydrocarbon chain on the membrane fluidity is shorter as a function of depth than on the surface. That is to say: there is an effect of time in the course of F⁻ acting on human erythrocyte membranes.
<table>
<thead>
<tr>
<th>Time (min)</th>
<th>S mean ± SE</th>
<th>( \frac{1}{S} ) mean ± SE</th>
<th>( \frac{1}{S} ) mean ± SE</th>
<th>NaF Control</th>
<th>NaF</th>
<th>Unit of ( \tau_c ) is 10^{-10} s; unit of ( \eta ) is Pa s</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.30 ± 0.02</td>
<td>0.25 ± 0.02</td>
<td>0.23 ± 0.02</td>
<td>0.30 ± 0.02</td>
<td>0.25 ± 0.02</td>
<td>10^{-10} s, Pa s</td>
</tr>
<tr>
<td>30</td>
<td>0.40 ± 0.03</td>
<td>0.33 ± 0.03</td>
<td>0.31 ± 0.03</td>
<td>0.40 ± 0.03</td>
<td>0.33 ± 0.03</td>
<td>10^{-10} s, Pa s</td>
</tr>
<tr>
<td>60</td>
<td>0.50 ± 0.04</td>
<td>0.43 ± 0.04</td>
<td>0.41 ± 0.04</td>
<td>0.50 ± 0.04</td>
<td>0.43 ± 0.04</td>
<td>10^{-10} s, Pa s</td>
</tr>
<tr>
<td>90</td>
<td>0.60 ± 0.05</td>
<td>0.53 ± 0.05</td>
<td>0.51 ± 0.05</td>
<td>0.60 ± 0.05</td>
<td>0.53 ± 0.05</td>
<td>10^{-10} s, Pa s</td>
</tr>
<tr>
<td>120</td>
<td>0.70 ± 0.06</td>
<td>0.63 ± 0.06</td>
<td>0.61 ± 0.06</td>
<td>0.70 ± 0.06</td>
<td>0.63 ± 0.06</td>
<td>10^{-10} s, Pa s</td>
</tr>
<tr>
<td>180</td>
<td>0.80 ± 0.07</td>
<td>0.73 ± 0.07</td>
<td>0.71 ± 0.07</td>
<td>0.80 ± 0.07</td>
<td>0.73 ± 0.07</td>
<td>10^{-10} s, Pa s</td>
</tr>
<tr>
<td>240</td>
<td>0.90 ± 0.08</td>
<td>0.83 ± 0.08</td>
<td>0.81 ± 0.08</td>
<td>0.90 ± 0.08</td>
<td>0.83 ± 0.08</td>
<td>10^{-10} s, Pa s</td>
</tr>
</tbody>
</table>

Note: Unit of \( \tau_c \) is 10^{-10} s; unit of \( \eta \) is Pa s.
The spectral parameters at the various temperatures are given in Tables 3 and 4, including activation energies (Eₐ) and point of phase transition (Ppt) to be calculated from Arrhenius plots on the surface and in the interior besides S, θ, τₑ and η. Meanwhile, Figures 2 and 3 also show the plots of order parameter S vs. temperature and the Arrhenius plots of rotational correlation time τₑ vs. temperature. The temperature alteration curves of order parameter S show that a break point of S appears only on the surface and is a straight line without a break point below the surface. So the membrane of the whole erythrocyte has a transition temperature at one position of I [12, 3] in the hydrocarbon region. In the untreated membrane the break point appears at 29.0°C where the straight line inflects below, i.e., the line at lower temperature is steeper than the one at higher temperatures which exhibits a more gentle slope. In the F⁻-treated membrane, however, the break point appears at 36.5°C where the straight line inflects above, i.e., the line at lower temperatures is more gentle and the one at higher temperatures has a steeper slope. As a result, the temperature dependance of S reflects that F⁻ has an influence on lipid fluidity on the surface of the membrane bilayer, making the temperature of the break point rise 7.5°C. Secondly, the phase transition temperatures of τₑ are featured both on the surface and in the interior. So the membranes of whole cells have two transitions at I [12, 3] and I [1, 14] in the hydrocarbon region. On the surface, Ppt of F⁻-treated and untreated, respectively, are at 36°C and 38°C, making the temperature of Ppt decrease 1.7°C; in the interior they are respectively at 44.6°C and 40.0°C, making the temperature of Ppt increase 4.6°C. Thus, F⁻ decreases the phase separation temperature on the surface but increases it with depth. Besides, the Arrhenius plot of the τₑ value from F⁻-treated membrane with I [1, 14] differs from those of the other three plots. Its Ppt, being inflected abruptly at 44.6°C, is featured above the whole curve, i.e., the straight line at the lower temperature is steep and the one at the higher temperature is gently sloped, but Ppt of the rest are featured below the curve, i.e., the straight lines at the lower temperature are gently sloped and the ones at the higher temperature are steeply sloped. The temperature dependences of τₑ support the view that F⁻ influences the lipid fluidity of the human erythrocyte membrane, particularly in the interior. Hence, the temperature dependence of both S and τₑ indicate that F⁻ interferes in a process of phase transition or separation in lipid bilayer during temperature alteration. Finally, Eₐ in the interior of F⁻-treated membranes, as with τₑ, differs from the other three Eₐs. The former Eₐ, which is above the upper break temperature, is larger than than that below the temperature, but the Eₐs of the latter three are inverse. For example, Eₐ decreases with depth from 8.13°C to 5°C; one on the surface increases from 3.136 to 6.872 kcal/mol with decreasing of the temperature from 55°C to 0°C. So, all Eₐs except Eₐ in the interior of F⁻-treated membrane are decreased by F⁻ exposure. In a word, activation energies (Eₐs) of phase separation from human erythrocyte membrane phase separation are influenced by F⁻.

At the normal body temperature of 37°C, when F⁻ is incorporated into the membrane, unsaturated bonds of unsaturated fatty acid chains in lipid molecules may be altered to form saturated acids (1). So, it is possible to induce an increase of linearly aligned saturated fatty acids, and the reduction of effective volumes of molecules, thereby raising the membrane medium viscosities and the increase of hydrophilicity, etc. Thus, it is observed that S and θ are maximized and τₑ and η are minimized. Further, it is noted that the alterations mentioned above are responsive to time and temperature. The time dependences on S or τₑ show, both on the surface and in the interior, that the appearance of the peak of S or τₑ on the surface,
TABLE 3. Spectral parameters in the various temperatures of F^-treated and untreated erythrocyte membrane labeled by probe I [12, 3]

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>50</th>
<th>45</th>
<th>40</th>
<th>35</th>
<th>30</th>
<th>25</th>
<th>20</th>
<th>15</th>
<th>10</th>
<th>5</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0.537</td>
<td>0.577</td>
<td>0.592</td>
<td>0.604</td>
<td>0.635</td>
<td>0.678</td>
<td>0.702</td>
<td>0.735</td>
<td>0.937</td>
<td>0.929</td>
<td>0.909</td>
</tr>
<tr>
<td>θ</td>
<td>32.06</td>
<td>32.09</td>
<td>31.42</td>
<td>30.92</td>
<td>29.57</td>
<td>26.67</td>
<td>26.45</td>
<td>26.34</td>
<td>11.74</td>
<td>12.51</td>
<td>14.20</td>
</tr>
<tr>
<td>τc</td>
<td>9.45</td>
<td>11.04</td>
<td>14.83</td>
<td>22.20</td>
<td>38.52</td>
<td>60.98</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>η</td>
<td>2.44</td>
<td>2.85</td>
<td>3.83</td>
<td>5.73</td>
<td>9.94</td>
<td>15.73</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E_a</td>
<td>3.841 Kcal/mol</td>
<td>8.061 Kcal/mol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PPt</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>NaF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0.472</td>
<td>0.539</td>
<td>0.567</td>
<td>0.635</td>
<td>0.665</td>
<td>0.675</td>
<td>0.700</td>
<td>0.749</td>
<td>0.940</td>
<td>0.919</td>
<td>0.917</td>
</tr>
<tr>
<td>θ</td>
<td>36.38</td>
<td>36.58</td>
<td>32.51</td>
<td>29.56</td>
<td>28.20</td>
<td>27.74</td>
<td>26.53</td>
<td>24.60</td>
<td>11.51</td>
<td>13.38</td>
<td>13.60</td>
</tr>
<tr>
<td>τc</td>
<td>8.33</td>
<td>9.88</td>
<td>12.56</td>
<td>17.18</td>
<td>25.47</td>
<td>45.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>η</td>
<td>2.15</td>
<td>2.55</td>
<td>3.24</td>
<td>4.43</td>
<td>6.61</td>
<td>11.63</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E_a</td>
<td>3.136 Kcal/mol</td>
<td>6.872 Kcal/mol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PPt</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Unit of τc is 10^{-10} s; unit of η is Pa; PPt is point of phase transition; all values are averages from three measurements
<table>
<thead>
<tr>
<th>T (°C)</th>
<th>S</th>
<th>η</th>
<th>$\tau_\nu$</th>
<th>$E_\nu$</th>
<th>Control</th>
<th>NaF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5S</td>
<td></td>
<td></td>
<td>1564 Kcal/mol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>0.093</td>
<td>1.013</td>
<td>2.32</td>
<td>6.94</td>
<td>5.98</td>
<td>3.82</td>
</tr>
<tr>
<td>40</td>
<td>0.109</td>
<td>1.012</td>
<td>2.51</td>
<td>6.94</td>
<td>5.98</td>
<td>3.82</td>
</tr>
<tr>
<td>35</td>
<td>0.121</td>
<td>1.011</td>
<td>2.67</td>
<td>6.94</td>
<td>5.98</td>
<td>3.82</td>
</tr>
<tr>
<td>30</td>
<td>0.132</td>
<td>1.010</td>
<td>3.05</td>
<td>6.94</td>
<td>5.98</td>
<td>3.82</td>
</tr>
<tr>
<td>25</td>
<td>0.162</td>
<td>1.009</td>
<td>3.47</td>
<td>6.94</td>
<td>5.98</td>
<td>3.82</td>
</tr>
<tr>
<td>20</td>
<td>0.190</td>
<td>1.008</td>
<td>4.36</td>
<td>6.94</td>
<td>5.98</td>
<td>3.82</td>
</tr>
<tr>
<td>15</td>
<td>0.203</td>
<td>1.007</td>
<td>4.85</td>
<td>6.94</td>
<td>5.98</td>
<td>3.82</td>
</tr>
<tr>
<td>10</td>
<td>0.228</td>
<td>1.006</td>
<td>5.51</td>
<td>6.94</td>
<td>5.98</td>
<td>3.82</td>
</tr>
<tr>
<td>5</td>
<td>0.258</td>
<td>1.005</td>
<td>7.18</td>
<td>6.94</td>
<td>5.98</td>
<td>3.82</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PPL</th>
<th>$E_\nu$</th>
<th>Kcal/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>40.0°C</td>
<td>2.483</td>
<td></td>
</tr>
<tr>
<td>44.6°C</td>
<td>1.547</td>
<td></td>
</tr>
</tbody>
</table>

Note: Unit of $\tau_\nu$ is 10^{-10} s, unit of $E_\nu$ is Pa. PPL is point of phase transition; all values are averages from three measurements.
30 min later than in the interior, is probably due to the changes at the surface being greater than those in the interior. Accordingly, an abrupt inflection of S on the surface but not shown in the interior may be relevant to the fluidity difference between two layers and not related to the effects of fluoride. In the F−-treated surface membrane, however, the break temperature of S raised 7.5°C from untreated may be involved with the action of fluoride on unsaturated bonds in lipid molecules. On the basis of the Fluid Mosaic Theory (6), the greater the concentrations of saturated fatty acid, the larger the S value and the more it is temperature dependent, the greater the influence on fluidity. Thus, the increase of fluidity needs to compensate the raising of temperature for the increase of S or the decrease in fluidity. In general, order parameter S represents the degree of long-range alignment of hydrocarbon chains along the membrane normal (z-direction) but is not directly related to the rotational correlation time τc (7). Therefore, the break point

**FIGURE 2. Temperature dependence of S from erythrocyte membrane labeled with I [12, 3] and I [1, 14]**

![Graphs showing temperature dependence of S](image-url)
temperatures of $\tau_c$ in the Arrhenius plot are different from those of S and are functions of depth with both F⁻-treatments. The fact that abrupt inflections or phase transition result from combined actions of fluoride and temperature may be accounted for by the changes among chains and chain-chain interactions. The reason the activation energies of $\tau_c$ in the interior are higher than those prior to reaching phase transition points is the requirement of more energy to complete transition from gel to liquid-crystalline state, when fluidity is decreased with fluoride effects in the course of temperature alteration from low to high.

**FIGURE 3.** Temperature dependence of $\tau_c$ from erythrocyte membrane labeled with I [12, 3] and I [1, 14]

A untreated  B F⁻-treated
Conclusions

Fluoride alone has little influence on fluidity in the intact human erythrocyte membrane but, when combining with a factor of time or temperature, does have a marked influence on fluidity. These observations lead us to postulate that the changes of fluidity in the healthy human erythrocyte membrane may be closely related to fluoride-induced changes from unsaturated fatty acid to saturated acid in the lipid molecule. Obviously, our work provides a new clue for studying fluoride membrane toxicology.

References

3 Cossins AR, Prosser CL. Evolutionary adaptation of membranes to tem perature. Proceedings of the National Academy of Science (USA) 75 2040-2043 1978.
ENDEMIC FLUOROSIS IN THE VILLAGE
RALLA ANANTAPURAM IN ANDHRA PRADESH:
AN EPIDEMIOLOGICAL STUDY

D Saralakumari and P Ramakrishna Rao
Anantapur, India

SUMMARY: Epidemiological data are presented from an endemic fluorotic village, Ralla Anantapuram, in Anantapur District of Andhra Pradesh. Duration of residence in the village, nutritional and socio-economic status, and clinical symptomology were correlated with the onset and severity of the disease and fluoride content of the potable water. The survey revealed higher incidence and severity of skeletal manifestations in males. With age the severity became more evident, restricting physical movements.

Key words: Endemic fluorosis; India; Ralla Anantapuram.

Introduction

Endemic fluorosis is a well defined clinical entity, characterized by dental mottling as well as skeletal and non-skeletal manifestations. Fluorosis is a major public health problem in 15 States of the 30 States and Union Territories of the country. The fluoride content, chiefly of water, and also of air and food, determine the human intake of fluoride. The population in areas of high fluoride content in drinking water are exposed to the risk of endemic fluorosis. Anantapur in Andhra Pradesh is one of the districts in the State afflicted by endemic fluorosis. Many villages in this area are identified as fluorotic (1). The present study was conducted in the village Ralla Anantapuram, Mudigubba mandal, Anantapur District, Andhra Pradesh State, India.

Material and Methods

Epidemiological data were collected using an individual sampling technique with an emphasis on economic, social and nutritional status. Detailed information was recorded on the family history, duration of residence and the source of drinking water. Drinking water samples were analysed using a fluoride specific ion electrode by the method of Neil and Mary (2).

Examinations were carried out to determine the incidence of dental and skeletal fluorosis. Careful attention was paid to discoloration of the teeth, joint pains, stiffness and pain in the back, difficulty in rising from squatting position, knock knees and crippling (3).

The degree of dental fluorosis was recorded using the following classification (4). Grade I: White opacities, faint yellow lines; Grade II: Same as Grade I plus brown stains; Grade III: Brown lines, pitting and chipped edges; Grade IV: Brown and black plus loss of teeth.

Department of Biochemistry, Sri Krishnadevaraya University, Anantapur - 515 003, Andhra Pradesh, India.
Results and Discussion

The sources of water were three bore wells, two open dug wells and one pond. Of these, only the first five were used for drinking and cooking purposes, the fluoride content ranging from 7.20 to 10.70 ppm with an average of 9.02 ppm (Table 1).

The population of the village are manual labourers working either in the fields or in lime stone kilns. Owing to the hot climatic conditions and their occupation they consume 4 to 6 litres of water, equal to 36 to 54 mg F, per day, and loss of water is more through sweat. Sweat fluoride concentration is lower than that of plasma and is considered to be of minor importance as a route of fluoride excretion (5).

Out of the total population, 160 are affected by either dental of skeletal fluorosis, or both - an incidence of 94.7%. The non-skeletal manifestations were not taken into consideration during this survey. All the male and 38 female members have resided in the village since birth. The remaining 40 female members migrated to the village after their marriages. The disease incidence was slightly more in males (95.6%) than in females (93.6%). Dental fluorosis afflicted 95.6% of males and 88.5% of females. Dental fluorosis severity reached a peak at the age of 20 years affecting 92.3% of the total population (Table 2).

Table 2 shows the village prevalence of various degrees of dental fluorosis. A higher percentage were affected with Grade III.

The earliest evidence of dental fluorosis was observed among children around 6-10 years of age, and of skeletal fluorosis around 11-20 years of age (Table 3). As the age advanced, the manifestations of skeletal fluorosis became more evident, restricting physical movements and causing difficulty in walking. More than one symptom of skeletal fluorosis were found in each patient (Table 4 and Figure). The incidence of the mild form of skeletal fluorosis, like joint pains and back pain, was similar in both sexes. The severe form predominated in males (Table 4).

Earlier studies have indicated that the incidence and severity of chronic fluoride intoxication are greatly influenced by socio-economic, climatic and nutritional status, being higher in poorer segments of the population with signs of nutritional deficiency (6-12). The majority of the families in this survey belonged to the poorer section. The dietary survey revealed that the major staple food is ragi (millet), and then jowar (millet), and rarely rice. Tamarind, chillies, onions and groundnuts are used for the preparation of side dishes. Red chillies are consumed with almost every meal. Leafy vegetables are consumed once, twice or thrice a week in the seasons (2 to 3 months in a year) when they are available. The villagers are occasional non-vegetarians.

Conclusion

These results reported from Ralla Anantapuram village in Andhra Pradesh, one of the Southern States of India, have the characteristic pattern of endemic fluorosis observed in numerous other regions of India. As the age advanced, the manifestations of skeletal fluorosis became more evident, restricting physical movements and impeding walking. As reported in previous studies (12,13) the symptoms of skeletal fluorosis are more severe in males. It is also evident that drinking water is the main source of fluoride ingestion. The results suggest that non-skeletal manifestations may be severe, although this report does not focus upon that aspect.
TABLE 1. Fluoride content of Ralla Anantapuram drinking water from different sources

<table>
<thead>
<tr>
<th>Source of water</th>
<th>Fluoride content in ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bore well I</td>
<td>9.2</td>
</tr>
<tr>
<td>Bore well II</td>
<td>7.2</td>
</tr>
<tr>
<td>Bore well III</td>
<td>10.7</td>
</tr>
<tr>
<td>Dug well I</td>
<td>8.4</td>
</tr>
<tr>
<td>Dug well II</td>
<td>9.6</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>9.02</strong></td>
</tr>
</tbody>
</table>

TABLE 2. Gender distribution of fluorosis

<table>
<thead>
<tr>
<th>Group</th>
<th>Total number surveyed</th>
<th>Affected with fluorosis</th>
<th>Affected with Dental Flu' sis</th>
<th>Skeletal Flu' sis</th>
<th>Distribution of degrees of dental fluorosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grade I</td>
</tr>
<tr>
<td>Male</td>
<td>91</td>
<td>87 (95.6%)</td>
<td>87 (95.6%)</td>
<td>74</td>
<td>9</td>
</tr>
<tr>
<td>Female</td>
<td>78</td>
<td>73 (93.6%)</td>
<td>69 (88.5%)</td>
<td>61</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>169</td>
<td>160 (94.7%)</td>
<td>156 (92.3%)</td>
<td>129</td>
<td>13</td>
</tr>
</tbody>
</table>

TABLE 3. Distribution of dental and skeletal fluorosis by duration of residence

<table>
<thead>
<tr>
<th>Group</th>
<th>Dental fluorosis</th>
<th>Skeletal fluorosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Duration of residence in years</td>
<td>0-5</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. affected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. affected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. affected</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Percent distributions are in parentheses

TABLE 4. Gender incidence of skeletal fluorosis in patients with >10 years residence

<table>
<thead>
<tr>
<th>Group</th>
<th>Affected with skeletal fluorosis</th>
<th>Joint pains</th>
<th>Back pain</th>
<th>Stiffness of back</th>
<th>Difficulty in rising</th>
<th>Crippled</th>
<th>Knock knees</th>
<th>Loss of sense and perception</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>68</td>
<td>65 (95.6)</td>
<td>49 (72.1)</td>
<td>36 (52.9)</td>
<td>25 (36.8)</td>
<td>19 (27.9)</td>
<td>10 (14.5)</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Female</td>
<td>60</td>
<td>58 (96.7)</td>
<td>42 (70.0)</td>
<td>12 (20.0)</td>
<td>18 (30.0)</td>
<td>11 (18.3)</td>
<td>3 (5.0)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Total</td>
<td>128</td>
<td>123 (96.1)</td>
<td>91 (71.1)</td>
<td>48 (37.5)</td>
<td>43 (33.6)</td>
<td>30 (23.4)</td>
<td>13 (10.2)</td>
<td>2 (1.6)</td>
</tr>
</tbody>
</table>

Percent incidences are in parentheses
FIGURE. Patients with endemic fluorosis from Ralla Anantapuram

References


Published by the International Society for Fluoride Research
Editorial Office: 216 Atkinson Road, Titirangi, Auckland 7, New Zealand.
MONITORING OF NEIGHBOURHOOD FLUOROSIS THROUGH A DENTAL FLUOROSIS SURVEY IN SCHOOLS

V K Desai, D M Solanki, S L Kantharia and B S Bhavsar
Gujarat, India.

SUMMARY: A baseline survey of neighbourhood fluorosis was conducted in 1976-77 in villages around a fluorspar processing plant located on the west coast of India, north of Bombay. A resurvey in a sample of villages was conducted after twelve years. The industry had reduced fluoride air pollution by 90 - 96% during the interim period. Dental fluorosis was selected as an indicator of health for monitoring. The results indicate a significant decline in the prevalence of dental fluorosis between baseline and resurvey, confirming the effectiveness of control of fluoride air pollution. It is concluded that dental fluorosis prevalence is a sensitive and easily identifiable indicator of high fluoride exposure.

Key words: Dental fluorosis; Neighbourhood fluorosis.

Introduction

Neighbourhood fluorosis is a problem resulting from emissions of fluoride-containing gas and discharge of fluoride-containing effluent by industrial plants. The polluted air or water can cause fluoride toxicity in crops, cattle and human beings around the industry.

A baseline study of neighbourhood fluorosis (1) was conducted in 1976-77 in the vicinity of an industry on the west coast of India north of Bombay, which processes fluorspar as raw material and manufactures fluoride-based compounds. The baseline study was conducted after eleven years operation of the industry.

With increasing realisation of the need for safety measures in industrial processes and development of technologies to prevent pollution, the industry under study adopted, from the year 1977-78, several engineering preventive measures to control emission of fluoride-containing gas and particles. Following regular improvements and modifications, the company claims a 96% reduction in gaseous and over 90% reduction in particulate fluoride emission from 1975-1986.

Dental fluorosis is one of the early manifestations of high fluoride intake during childhood. Dental fluorosis surveys in schools can be conducted with minimum resources. Hunter (2) has suggested that dental fluorosis should be regarded as a sign of neighbourhood fluorosis.

This report on available environmental, health baseline and follow-up survey data initiates the first planned monitoring of the impact of the control of fluoride air pollution.

Department of Preventive and Social Medicine, Government Medical College, Surat, Gujarat, India.
Materials and Methods

A total of 15 villages and 3 urban colonies located at different directions and distances from the fluorspar processing industry were surveyed in the 1976-77 baseline survey. These villages were located between 500 and 3500 metres from the industry. Because of wind patterns the villages to the north-east and south-west were more affected by industrial air pollution (1). Eight of the villages, representing all the directions and distances, were selected by stratified random sampling for the 1988 resurvey (see Figure).

![Diagram showing direction and distance of villages from factory]

A survey of dental fluorosis in schoolchildren was carried out in the selected villages and the results compared with baseline survey data for the same villages. All registered schoolchildren were examined during the baseline and the resurveys.

Drinking water samples were collected from the villages and fluoride estimations done with an ion specific electrode.

Results and Discussion

During the baseline survey water, soil and fodder fluoride levels were studied as environmental indicators, while dental and skeletal fluorosis prevalences, supported by radiological investigations and urinary fluoride levels, were studied as human health indicators. The observations revealed that manifestations of fluoride toxicity, and the magnitude of the health problem, were closely associated with the pattern of fluoride air pollution.

During the resurvey the aim was to evaluate the sensitivity of dental fluorosis to environmental changes in the neighbourhood fluorosis problems.

From the eight villages selected for resurvey 1317 schoolchildren had been examined during the baseline survey while 3789 schoolchildren were examined during the resurvey. Prevalences of dental fluorosis were 29.7% and 18.1% at baseline and resurvey respectively. This decline of 11.6% was statistically significant.
The decline in prevalence was higher in the younger age group (6 - 10 years) than in the older age group (11 - 16 years) - 14.2% and 8.9% respectively - and also statistically significant (Table 1).

Dental fluorosis is an irreversible condition, so age-group prevalences are useful for study of the time factor in the epidemiology of fluorosis. The higher decline in the younger age group indicates a lower occurrence of new cases of dental fluorosis. The cases recorded during the baseline survey had shifted to higher age groups.

Water fluoride is an additional source of fluoride for the children under study. If the water fluoride level does not change between baseline and resurvey, the decline in dental fluorosis prevalence is totally due to fluoride air pollution control.

Drinking water sources were ground water in all villages during baseline, and no new source was added during the time before resurvey. As seen in Table 2, during the baseline survey all the villages had drinking water sources with less than 1 ppm fluoride, while during resurvey the drinking water fluoride level was raised in six villages. In the village with 1.6 ppm water fluoride, there was no significant change in the prevalence of dental fluorosis, while in villages with more than 2 ppm water fluoride there was a significant increase in prevalence compared to baseline survey.

Villages with raised fluoride level during resurvey were not located on the course of effluent discharge of the industry and therefore these rises can be due to natural contamination. A study in Mehsana district of Gujarat State revealed that 40% of drinking water sources showed a rise in their fluoride level each year (3). So the rise in water fluoride levels in the present resurvey may be the result of similar phenomenon.

Comparison of dental fluorosis prevalences according to drinking water fluoride levels at the time of resurvey is shown in Table 3. There was no significant decline in the prevalence of dental fluorosis in villages with more than 1.5 ppm drinking water fluoride. In other villages, due to the control of fluoride air pollution and water fluoride levels within permissible limits, a significant decline in the prevalence of dental fluorosis during the interim period was observed.
<table>
<thead>
<tr>
<th>Z Value</th>
<th>Reservoir</th>
<th>Baseline</th>
<th>Dental Fluorosis Prevalence</th>
<th>Water Fluoride Level ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.40</td>
<td>0.60</td>
<td>95 (10.1)</td>
<td>68 (7.5)</td>
</tr>
<tr>
<td>1.18</td>
<td>0.80</td>
<td>0.80</td>
<td>95 (22.3)</td>
<td>29 (3.1)</td>
</tr>
<tr>
<td>4.48</td>
<td>0.90</td>
<td>1.00</td>
<td>10 (24.6)</td>
<td>29 (3.1)</td>
</tr>
<tr>
<td>6.00</td>
<td>1.10</td>
<td>1.10</td>
<td>10 (24.6)</td>
<td>29 (3.1)</td>
</tr>
<tr>
<td>1.16</td>
<td>0.80</td>
<td>0.80</td>
<td>95 (10.1)</td>
<td>68 (7.5)</td>
</tr>
<tr>
<td>1.40</td>
<td>1.00</td>
<td>1.00</td>
<td>10 (24.6)</td>
<td>29 (3.1)</td>
</tr>
<tr>
<td>2.00</td>
<td>0.90</td>
<td>0.90</td>
<td>95 (10.1)</td>
<td>68 (7.5)</td>
</tr>
</tbody>
</table>
TABLE 4  
Comparison of dental fluorosis prevalences in relation to direction from industry

<table>
<thead>
<tr>
<th>Direction</th>
<th>Dental fluorosis prevalence</th>
<th>Z value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline  Resurvey</td>
<td></td>
</tr>
<tr>
<td>NE + SW</td>
<td>241/794 (30.4) 326/2260 (14.4)</td>
<td>8.99</td>
</tr>
<tr>
<td></td>
<td>P &gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>NW + SE</td>
<td>151/523 (28.9) 359/1529 (23.4)</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>P &gt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 5  
Comparison of dental fluorosis prevalences in relation to distance from industry

<table>
<thead>
<tr>
<th>Distance</th>
<th>Dental fluorosis prevalence</th>
<th>Z value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline  Resurvey</td>
<td></td>
</tr>
<tr>
<td>II + III</td>
<td>206/675 (30.5) 520/2611 (19.9)</td>
<td>5.57</td>
</tr>
<tr>
<td></td>
<td>P &gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>185/642 (28.8) 165/1108 (14.9)</td>
<td>6.76</td>
</tr>
<tr>
<td></td>
<td>P &gt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>

Baseline survey analysis indicated that villages in NE and SW direction, and in distant zones, were at higher risk of air fluoride pollution than villages in other directions and in nearby zones (1). Tables 4 and 5, comparing the resurvey results with baseline, show that the decline in dental fluorosis prevalence was significantly higher in villages in high risk zones according to direction (NE and SW 16%, NW and SE 5.5%) and in villages in high risk zones according to distance (IV 13.9%, II and III 10.6%).

The significantly higher decline in dental fluorosis prevalence in high risk zones for air fluoride pollution indicate that the control of air fluoride pollution during the interim period was effective.

Desai et al (4), in an epidemiological study of dental fluorosis in relation to water pollution from effluent discharge of a fluor spar processing plant, concluded that prevalence of dental fluorosis, without grading, was related to water fluoride level.
The present study strongly indicates that simple prevalences of dental fluorosis without grading are positively related to air fluoride levels and changes in level over a period of time.

Conclusion

We conclude that dental fluorosis is an easily identifiable and sensitive health indicator of high fluoride exposure, and can be used for regular monitoring of health problems related to environmental fluoride pollution.

Acknowledgement

The authors acknowledge the financial support of the Indian Council for Medical Research. We are also grateful to the principal investigator of the baseline survey, Dr N R Mehta, for his guidance.

References

1 Bhavsar BS, Desai VK, Mehta NR et al. Neighbourhood Fluorosis in Western India. Parts I and II. Fluoride 18 (2) 80-92 1985.
3 Desai VK, Buch JU, Shah SC, Gupta SC. Health and social cost benefit evaluation of a drinking water supply project aimed at fluorosis control in Gujarat (India). Project Report, Training Course, Rural Watersupply and Sanitation DPPC Bradford University, Bradford, 1990
EPIDEMIOLOGICAL STUDY OF GOITRE IN ENDEMIC FLUOROSIS DISTRICT OF GUJARAT

V K Desai, D M Solanki and R K Bansal
Gujarat, India

SUMMARY: Soft tissue fluoride toxicity is well established. Several animal and human studies of the effect of fluoride on the thyroid gland have shown conflicting results. Endemic fluorosis and goitre are widespread in India with considerable overlapping in different geographical zones. We examined 22,276 individuals for presence of goitre and dental fluorosis and estimated the fluoride and iodine content of their drinking water. Overall goitre and dental fluorosis prevalences were 14.0% and 12.2%, respectively, and were significantly and positively correlated. No significant relationship was observed between water iodine level and goitre. In the study area only 0.3% of cases were visible goitre (Grade-II and above) and all goitre cases were euthyroid. This suggests that fluoride-induced goitres are brought about by anatomical or structural changes rather than functional changes.

Key words: Dental fluorosis; Goitre.

Introduction

Soft tissue involvement in fluoride toxicity is now well established. In the past, the toxic effect of fluoride on the thyroid gland was suspected, based on clinical experience in the treatment of Grave's disease (1). A high prevalence of goitre has been observed in countries where skeletal fluorosis is endemic (2). Controversy still exists because several animal experiments and human studies have shown conflicting results (1).

In India endemic fluorosis exists in 15 States and the problem of endemic goitre occurs in 12 States, with significant overlapping of the problem in some regions. Such widespread co-existence provides an opportunity to conduct epidemiological studies, in order to better understand the interactions of the trace elements involved, the effects on human health, and methods of control and prevention.

Materials and Methods

The Amreli District in the Saurashtra region of the State of Gujarat, on the west coast of India, has ten administrative blocks, two with hilly terrain. The major source of drinking water is ground water.

Our clinical examination of 10,029 residents by a house to house survey and 12,247 children by a school survey, covering 1% of the total population and 5% of school children of randomly selected villages, conformed to WHO recommendations for a goitre survey (3). Water samples were collected from all drinking water sources. Fluoride and iodine estimations were carried out, with an ion-specific electrode and titration method, respectively.

Preventive and Social Medicine Department, Government Medical College, Surat, Gujarat, India.
Results and Discussion

Amreli District is one of the first identified endemic fluorosis districts in Gujarat State. The overall district prevalence of dental fluorosis was 12.2% and of goitre 14.0%. Age specific prevalences of dental fluorosis and goitre are shown in Table 1. Both problems were predominant in school children and young adults.

Dental fluorosis prevalence ranged from 6.0 to 59.0%, and goitre from 9.5 to 37.5%, in some blocks of the district. Distribution of blocks according to dental fluorosis and goitre prevalence is shown in Table 2. Only one block had goitre prevalence < 10%. In two blocks with more than 20% goitre prevalence dental fluorosis prevalence was more than 50%. There was a significant positive correlation between prevalence of goitre and dental fluorosis in the study area ($r = +0.4926$, $p<0.001$).

Iodine deficiency is the major cause of endemic goitre (more than 10% prevalence) in India. In the study area all but one of the blocks had goitre prevalence conforming to WHO criteria for endemic goitre (3). An earlier study of goitre and associated health consequences in Surat District of Gujarat State (4) revealed endemic goitre in regions with less than 20 μg/litre water iodine level. In the present study endemic goitre was present even in six District blocks with water iodine levels greater than 20 μg/litre. Moreover, there was no significant correlation between water iodine level and goitre prevalence ($r = 0.1443$, $p>0.05$).

The significant correlation between prevalences of goitre and dental fluorosis, and the absence of significant negative correlation between water iodine level and goitre prevalence, indicate a probable role of fluoride toxicity in the development of goitre cases. This conclusion is further supported by the fact that dietary patterns were uniform in the study area and the use of goitrogen containing foods was not predominant in any block.

In the study area there was no village with a water fluoride level less than 1 ppm (the "safe" limit for India) and a water iodine level less than 10 μg/litre. In regions with a normal iodine environment (water iodine level more than 20 μg/litre), goitre prevalence was significantly higher in regions with more than 2 ppm water fluoride level (27.8%) than in regions with less than 2 ppm water fluoride level (17.1%). The present study thus indicates that fluoride toxicity in a normal iodine environment can cause a goitre problem. A study in Andhra Pradesh has also revealed direct correlation between water fluoride level and goitre in the 14-17 years age group (5), while a study in 17 Himalayan villages showed a positive correlation between goitre prevalence and water fluoride level in the presence of uniformly low water iodine levels (6).

According to Stanbury's classification of goitre (7) the prevalence of visible goitre (Grade-II and higher) was only 0.3% in the study area. In districts of Gujarat State with endemic goitre due to iodine deficiency visible goitre prevalence was 5.8%. Thus there were more early goitre cases in the study area than is usually seen in goitre endemic areas.

Goitre cases were clinically euthyroid in the present study, and cases with clinical manifestations suggestive of cretinism were significantly lower (0.1%) than in the endemic goitre zone (0.9%) in Gujarat State (8). These observations indicate
that goitre due to fluoride toxicity does not have functional changes and does not affect the hormonal profile in the community under study. Large scale demographic surveys in U.S.A. and Great Britain have also indicated that drinking water fluoride does not impair thyroid function (1).

Attempts have been made by several workers to study the mode of action of fluoride on the thyroid gland. Experimental studies in animals have suggested that high fluoride intake causes hypertrophy of parafollicular cells and high glycerophosphate dehydrogenase activity (9), increased lipid component, total lipids and triglyceride (10). Whether or not a similar mechanism operates in humans in endemic fluorosis areas is not known. Whether these changes are responsible for observed small enlargements of the thyroid gland is also not known.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Percent prevalence of Dental fluorosis</th>
<th>Goitre</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5</td>
<td>1.5</td>
<td>2.6</td>
</tr>
<tr>
<td>5 - 12</td>
<td>16.3</td>
<td>16.8</td>
</tr>
<tr>
<td>13 - 19</td>
<td>15.4</td>
<td>21.4</td>
</tr>
<tr>
<td>20 - 30</td>
<td>15.3</td>
<td>21.2</td>
</tr>
<tr>
<td>31 - 40</td>
<td>15.1</td>
<td>16.4</td>
</tr>
<tr>
<td>&lt; 40</td>
<td>9.6</td>
<td>6.4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>12.2</strong></td>
<td><strong>14.0</strong></td>
</tr>
</tbody>
</table>

**TABLE 2. Distribution of blocks according to prevalence of dental fluorosis and goitre**

<table>
<thead>
<tr>
<th>Percent goitre prevalence</th>
<th>Percent prevalence of dental fluorosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 10</td>
</tr>
<tr>
<td>&lt; 10</td>
<td>1</td>
</tr>
<tr>
<td>10 - 20</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 20</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2</td>
</tr>
</tbody>
</table>

(Figures in parentheses show % of administrative blocks with given goitre prevalence)
Controversies about fluoride toxicity on the thyroid gland still remain. But this study confirms the widespread co-existence of fluorosis and goitre, and indicates the need for uniform epidemiological field studies in different parts of the country. Such studies should be supported by laboratory investigations into water chemistry, dietary iodine and fluoride estimations, thyroid function and fluoride metabolism, to explore the mode of action of fluoride toxicity on the thyroid gland and its consequences on human health.

Acknowledgement
The authors are grateful to the State Health Department for providing the opportunity to study the above problem in Amreli District of Gujarat State.

References
9 Poonam Khan. Studies on the effect of fluoride ingestion in excess on parafollicular cells of rabbit thyroid gland. MSc dissertation. All India Institute of Medical Sciences, New Delhi, 1985.
NUCLEIC ACID LEVELS IN THYROID GLAND IN ACUTE AND CHRONIC FLUORIDE INTOXICATION

A Shashi
Patiala, India

SUMMARY: To assess the effect of fluoride on nucleic acid content of the thyroid gland during experimental fluorosis, sodium fluoride as 5, 10, 20 and 50 mg/kg body weight/day was injected subcutaneously for 100 days into rabbits of both sexes. Controls were given 1 cc double distilled water/kg body weight/day for the same period. In both sexes, when compared with the controls, there was a significant decline (P < 0.001) in the level of RNA in the thyroid glands of experimental animals of the 10, 20 and 50 mg groups and a significant increase (P < 0.001) in females of the 5 mg group. The DNA content declined significantly (P < 0.001) in the thyroid glands of both sexes, except in the 5 mg group.

Key words: Fluoride; DNA; Rabbit; RNA; Thyroid gland.

Introduction

The relationship between fluoride intoxication and thyroid function is highly debatable. A number of investigators reported that fluoride induces changes of one sort or another in thyroid function. Goitre has been produced in experimental animals by feeding salts (1,2), and fluoride exerts an antithyroid effect in humans when used in treatment of thyrotoxicosis (3). Association of dental fluorosis and endemic goitre has also been established (4).

Alterations in various physiological and biochemical parameters in the thyroid gland in experimental fluorosis have been reported (5). The current experimental work was undertaken to elucidate the effect of fluoride on nucleic acid levels in the thyroid gland in experimental animals.

Materials and Methods

Albino rabbits of both sexes (400-650 g) were maintained on standard laboratory chow, and water was supplied ad libitum. The first group of twelve animals were administered 1 cc distilled water/kg body weight/day for 100 days, and served as the control. The second, third, fourth and fifth groups of animals were administered subcutaneous injections of fluoride (NaF) at doses of 5, 10, 20 and 50 mg/kg/body weight/day respectively for the same period. The control and the treated groups of animals were sacrificed under ether anaesthesia. The thyroid gland was carefully excised and utilised for the analysis of nucleic acids. The extraction of nucleic acids from the thyroid gland was carried out according to the method of Webb and Levy (6). The determination of RNA was made according to the method of Markham (7). DNA was estimated by the method of Burton (8). The data were subjected to statistical analysis.

Department of Zoology, Punjabi University, Patiala 147002, India.
Results

The nucleic acid levels in the thyroid glands of control and fluoridated animals are given in Tables 1 and 2. The RNA content in thyroid glands of both sexes (Figures 1 and 2) showed a significant decline (P < 0.001) in animals treated with 10, 20 and 50 mg fluoride/kg body weight as compared to the control. The highest percent decrease was in the 50 mg fluoride group (60.92% in males, 65.27% in females). In males, the RNA level did not show any significant change in the 5 mg fluoride group. However, in females a significant elevation (P < 0.001) in RNA content of the thyroid gland was noticed. In both sexes, RNA content fell significantly (P < 0.001) in 5 mg vs 10 mg fluoride groups, 10 mg vs 20 mg fluoride groups and 20 mg vs 50 mg fluoride groups. In both sexes, the DNA level in the thyroid gland was not significantly affected by NaF treatment in the 5 mg fluoride group. In the remaining groups, it was significantly (P < 0.001) reduced (Figures 3 and 4).

<table>
<thead>
<tr>
<th>TABLE 1. Effect of fluoride on RNA in the thyroid gland of rabbit (values are Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Level of significance between the groups was calculated by Student's t-test.
Significance values in treated groups cf. control: a P<0.001, b P<0.01
Significance values in 5 mg vs. 10 mg F^- group, 10 mg vs. 20 mg F^- group and 20 mg vs. 50 mg F^- group: c P<0.001, d P<0.02

<table>
<thead>
<tr>
<th>TABLE 2. Effect of fluoride on DNA in the thyroid gland of rabbit (values are Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Level of significance between the groups was calculated by Student's t-test.
Significance values in treated groups cf. control: a P<0.001, b P<0.01
Significance values in 5 mg vs. 10 mg F^- group, 10 mg vs. 20 mg F^- group and 20 mg vs. 50 mg F^- group: c P<0.001, d P<0.02
FIGURE 1. RNA in thyroid gland of male rabbit

FIGURE 2. RNA in thyroid gland of female rabbit
Discussion

It is well documented that fluoride inhibits nucleic acid synthesis (9, 10). Fluoride alters DNA synthesis in bone organ culture (11) and cultured mouse fibroblasts (12). At the subcellular levels, protein synthesis is altered (13). Treatment of He La cell monolayers with toxic concentrations of fluoride inhibited DNA, RNA and protein synthesis (14). During the present experimental studies, fluoride intoxication resulted in rapid decline of DNA and RNA in thyroid glands of rabbits of both sexes. The decrease in nucleic acid synthesis may be due to reduced protein synthesis. Fluoride interferes with the normal functioning of follicular cells by inhibiting the proteinases responsible for splitting thyroglobulin into thyroxine and triiodothyroxine (15). Sodium fluoride depresses the endocytosis of colloid and thyroid secretion by inhibiting aerobic glycolysis in the follicular cell (16). Juvenal et al (17) suggest that fluoride ions affect the polymerization of nucleotide precursors of RNA in beef thyroid gland. Intracellular i-mRNA is decreased. Polyribosomes are partially disintegrated and RNA is reduced (18, 19).

The disturbance in nucleic acid synthesis in fluorosis is also attributed to decreased activity of certain enzymes, e.g. deoxyribonuclease (20) and 5'-nucleotidase, adenosidases catalysing certain stages of biosynthesis of nucleotides and nucleic acids. Thymine uptake by DNA and uridine uptake by RNA are decreased (21). Fluoride inhibits DNA polymerase activity (22). Hence, DNA synthesis might decrease as a result of reduced protein synthesis (12).

Sodium fluoride treatment could result in DNA damage which is involved in the initiation of transformation (23). Fluoride is supposed to play a disruptive role towards DNA through a N-H-F-H-N hydrogen bond in the base pair of thymidine adenine base pairing (24) or may be mediated by a toxicological complex of amido-F-H, reducing protein synthesis (25). The inhibition of protein synthesis may retard the progression of DNA repair following DNA damage.

Acknowledgement

The author is grateful to the Council of Scientific and Industrial Research, New Delhi, for providing a Research Fellowship and grant to support this study.

References


DEVELOPMENTS IN THE ANALYSIS OF FLUORIDE 1980-1990

C Y Wang and J Xu
Kunming, China

Within the last ten years two excellent reviews of analytical methods for the determination of fluoride in biological and related materials have been presented by Venkateswarlu (1,2). Here we review the literature and focus on five trends in this field for the period 1980-1990.

1. Trace and Ultratrace-Analysis

Molecular absorption spectrometry has shown that the best sensitivity for the determination of F\(^-\) is 0.38 ng of F\(^-\) in urine (3), blood serum (4), milk (5), and potable water (6).

After liquid-liquid extraction of F\(^-\) with triphenylantimony(V) dihydroxide, the detection limit is 0.3 ng of F\(^-\) (7). Determining F\(^-\) in drinking water at 620 nm as the lanthanum-alizarin complexan, with use of a total-reflection 4 cm-long capillary cell, has a detection limit of 10 ng/l of F\(^-\) (8).

Gas chromatography-flame ionization detection (GC-FID) is based on the reaction:

\[
\begin{array}{c}
\text{CH}_3 \quad \text{Cl} \quad \text{Si} \quad \text{CH}_3 \\
\text{CH}_3
\end{array} + F^- \xrightarrow{\text{CCl}_4} \begin{array}{c}
\text{CH}_3 \\
\text{CH}_3
\end{array} + \begin{array}{c}
\text{F} \quad \text{Si} \quad \text{CH}_3 \\
\text{Cl}
\end{array}
\]

The fluorotrimethylsilane is determined by GC with FID. The detection limit is 0.3 to 10 ppb of F\(^-\) (9) in the aqueous solution.

Determination of trace levels of fluoride in water, urine and coal fly ash by microwave-induced plasma (MIP) emission-spectrometric and spark-source mass spectrometry has also been studied (10,11).

Wang et al (12) described a method for trace analysis of fluorine in vegetables and foods, in which the sample is decomposed in an oxygen flask followed by the multi-standard application of Gran's method with a fluoride ion-selective electrode and use of a minicomputer to process the data. This method is sensitive, rapid and simple.

Singer et al (13) conducted a study on several food groups from market basket collections. Greek investigators (14) presented a new TISAB containing aluminoxon (10 g/l), Na citrate dihydrate (29.41 g/l), NaCl (58 g/l), and acetic acid (57 ml/l). Its pH is adjusted to 5.0 with 6 M NaOH. Calibration graphs for the aluminoxon TISAB were rectilinear down to 5 μM F\(^-\), and the detection limit (0.3 μM) was lower than with standard TISAB.

Determination of nanogram amounts of fluorine in breast milk by ashing diffusion-fluoride electrode analysis has been reported (15). For pre-concentration of trace F\(^-\) and HF from aqueous samples, 0.05 M acetate buffer was added to the sample, and F\(^-\) was absorbed on hydroxyapatite containing 15% of polyacrylamide. A 100-fold excess of anions or Al did not interfere (16).
2. Micro and Ultramicro-Analysis

The measurement of fluoride in microliter and nanoliter volumes of samples has been discussed by Vogel et al (17). They presented a microanalytical technique with an inverted F\textsuperscript{-} electrode (18-22). The Figure shows an inverted F\textsuperscript{-} electrode adapted for nanoliter volumes. Concentration of over 5 \textmu M F\textsuperscript{-} could be measured in samples as small as 0.1 nl.

**FIGURE.** Inverted F electrode adapted for nanoliter volumes

Chiba et al (23) described an F\textsuperscript{-} electrode-plate shaped Ag-AgX reference electrode for determination of F\textsuperscript{-} in microliter volumes. Ag-AgX reference electrodes were prepared by anodic oxidation of silver plates (2 cm x 2 cm x 0.5 mm) in 0.1 M KCl, KBr or KI at +0.5 V vs. a saturated calomel electrode (SCE) for 5 min. A few \mu l of the F\textsuperscript{-}-containing sample were placed on the plate, a F\textsuperscript{-} electrode was inserted into the liquid, and after 5 min the potential was measured.

Wang (24) recommends use of a quartz oxygen flask to decompose mg quantities of sample, with auxiliary combustible adhesive paper around the filter paper wrapping the sample before ignition, in combination with a new measuring technique of the F\textsuperscript{-} electrode. The polymers of high F content, fluorocarbons, and compounds containing the -CF\textsubscript{3} group can be quantitatively decomposed. The presence of N, Cl, Br, I, S, P, As, B, Si, K, Na, Hg, Mg, Al, Fe, Pb, Ag, Cu, and Zn does not interfere with the quantitative microdetermination of organic fluorine. The absolute error is \pm 0.3\%. 
3. Simultaneous Multi-Element Determination

For multi-element trace analysis, it is efficient to use neutron activation analysis (NAA), ion chromatography (IC) and microwave induced plasma (MIP) or inductively coupled plasma (ICP).

Ward (25) determined 25 elements: F, Cl, Na, Al, Se, Br, Ca, Cu, I, Mg, Mn, Mo, Rb, V, K, Ag, As, Au, Co, Cr, Cs, Fe, Hg, Sb, and Zn in biological material by NAA.

Chinese and Japanese investigators have described the IC with a conductivity detector for micro-determination of $F^-$, $SCN^-$, $Cl^-$, $Br^-$, $PO_4^{3-}$, $SO_4^{2-}$, and $I^-$ (26), and for determination of $F^-$, $Cl^-$, $PO_4^{3-}$, $Br^-$, $NO_3^-$, and $SO_4^{2-}$ in seawater (27).

Fry et al (28) determined fluorine by atomic emission spectroscopy (AES) in the argon ICP.

Simultaneous determination of F, C, H, N, O, Cl, Br, and S by GC with microwave-induced plasma-fourier-transform (MIP-FP) in the near IR (infrared) and by AES has also been reported (29).

Simultaneous determination of $F^-$, Mo, and W by differential spectroscopy has been applied to the determination of $F^-$ based on a ternary complex formation with Ce and alizarin complexan (30).

4. Rapid and Automatic Analysis

For rapid and automatic analysis of $F^-$, use of a microprocessor coupled to a $F^-$ electrode in an ion-selective electrode-flow injection analysis (ISE-FIA) has been developed (see Table).

<table>
<thead>
<tr>
<th>METHOD</th>
<th>APPLICATION</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>F electrode-microprocessor</td>
<td>in coffee and tea</td>
<td>(31)</td>
</tr>
<tr>
<td>ISE-FIA</td>
<td>potable water, 360 samples/hr</td>
<td>(32)</td>
</tr>
<tr>
<td>Computer controlled ISE-FIA</td>
<td>in the concentration range $F^-$ 20-200 ppb 60 samples/hr</td>
<td>(33)</td>
</tr>
<tr>
<td>ISE-FIA</td>
<td>research of buffer systems</td>
<td>(34)</td>
</tr>
<tr>
<td>ISE-FIA</td>
<td>agricultural, pharmaceutical, clinical and environmental samples</td>
<td>(35)</td>
</tr>
</tbody>
</table>

5. Biopsy Technique

Tyler et al (36) presented a novel multi-electrode system for the direct and simultaneous determination of $F^-$ and Ca in acid solution. The system has been successfully applied as a biopsy technique for the determination of fluoride concentration profiles within the outer 100 μm of human tooth enamel. Two independent electrode systems were described: a new ultra-sensitive fluoride-glass pH electrode differential cell, and a calcium electrode monitor for the direct and simultaneous measurement of both fluoride and calcium in unbuffered acid solution. $F^-$ Nernstian response may be extended at least tenfold towards the lower limit of detection, with appreciable reduction in electrode response time at low concentrations.
Considerable research has been directed toward the evaluation of fluoride in dental enamel. One current technique used for the determination of fluoride to calcium ratios in mineralized tissues depends upon the serial acid etching of small isolated areas, for example on a tooth enamel surface, followed by the analysis of individual etch solution for fluoride and calcium using F⁻ ISE and atomic-absorption spectrometry.

Smith et al (37) studied a new procedure for separation of the enamel and dentin of human teeth. The cementum is coated with denture rebase mixture and the enamel is dissolved in 0.6 M HNO₃ at 90°C. After heating the remains at 140°C in order to peel off the rebase, residual enamel and the cementum are removed by digestion with 3 M HNO₃ at 90°C. The dentin remnants are then dissolved in 1.2 M HNO₃ at 90°C during 75 min. All dissolutions are carried out in a Parr digestion bomb. The separated dentin and enamel solutions are analysed for 13 elements (12 metals plus B) by ICP-AES, and for F⁻ by ISE.

Canadian investigators (38) studied the measurement of F in bone biopsies by NAA for the clinical study of osteoporosis. The F content is expressed relative to that of Ca and therefore no sample weighing is required. The method has been applied to clinical studies in the treatment of osteoporosis with F⁻.

Since the NAA procedure is non-destructive for the bone biopsies, it can be used for histological evaluations. Also, human demineralized dental samples were analysed for F by either secondary ion mass spectroscopy (SIMS) or a microdrill method. The results by the two methods were similar (39).

Taves et al (40) have reported a precise diffusion colorimetric method for determination of F in unashed soft tissues.

Paez et al (41) distinguished the fluorine in blood as the free (or inorganic) form and in combined (or organic) form. For blood organic fluorine analysis, a method based on reductive cleavage of the C-F bond with the sodium biphenyl reagent, has been developed by Venkateswarlu (42). Free F⁻ can be measured with a F⁻ electrode without prior separation. To determine total F, prior calcination in the presence of an alkaline stabiliser is recommended (43). The molecular absorption method has been found possible for total or organic fluorine in human serum samples after all covalent fluorine in the sample has been converted into inorganic fluoride by use of the sodium biphenyl reagent (44).

Finally, German investigators (45) have presented a hybrid biosensor based on plant potato tissue and immobilized glucose oxidase with a Clark oxygen electrode for the measurement of fluoride and phosphate. The oxygen consumption corresponding to the formation of glucose is sensed electrochemically with a Clark oxygen electrode. Since potato acid phosphatase is inhibited by fluoride and phosphate, both substances can be determined with the biosensor by their ability to diminish glucose formation.

References


10 Chiba K, Yoshida M, Tanabe K et al. Determination of ultratrace levels of fluorine in water and urine samples by a gas chromatographic-atmospheric pressure helium microwave induced plasma emission spectrometric system. *Analytical Chemistry* 54 (4) 761-764 1982.


31 Moody GJ, Ong B, Quinlan K et al. Determination of fluorine in coffee and tea using a microprocessor coupled with a fluoride ISE. *Journal of Food Technology* 15 (3) 335-343 1980.
The following are abstracts of papers presented at the XIXth Conference of the International Society for Fluoride Research at Kyoto, Japan, September 1992, in the session on Analytical Methods for Fluoride.

DETERMINATION OF TOTAL FLUORIDE IN CORN BY THE COMBUSTION-HYDROLYSIS/FLUORIDE-ION ELECTRODE METHOD

R Ji, X Quan, S Cao and Y Li

Several methods for the determination of fluoride were compared. It was found that the combustion-hydrolysis/fluoride-ion selective electrode method is ideal for the determination of total fluoride in corn. The combustion-hydrolysis method is a specific decomposition procedure in an oxygen stream. The different forms of fluoride in the sample are converted into hydrogen fluoride and other volatile fluorides. Reference cabbage samples were analysed by this method. The average value was 5.77 µg/g compared to 5.87 ± 0.97 µg/g for the standard sample. The recovery of added NaF and CaF₂ was 90.4-102.5% and 96.4-105.4%, respectively. The method is thus accurate and reliable.

Institute of Environmental Health and Engineering, Chinese Academy of Preventive Medicine, CHINA.

PREPARATION AND CERTIFICATION OF FLUORIDE COMPOSITION IN CORN AND FLY ASH REFERENCE MATERIALS

R Ji,¹ R Yang,² S Cao¹ and Y Li¹

Corn and fly ash reference materials were prepared and certified for fluoride content according to the need of analytical quality control in monitoring domestic soot fluoride pollution. These two kinds of reference materials were homogeneous and stable for more than a year. On the basis of standard metrological technology for reference materials, the certified reference materials were analyzed by accurate and reliable methods according to three kinds of principles and units. The guarantee and uncertainty of corn (two concentrations) and fly ash reference materials containing fluoride are $33.7 \pm 2.2$, $1.91 \pm 0.81$, and $113.6 \pm 13.0$ µg/g, respectively. These corn and fly ash reference materials filled the vacancy of similar domestic reference material containing fluoride. User response to the reference materials has been good. The reference materials have now been approved by the State Bureau of Technical Supervision and are numbered GBW 08506-GBW 08507.

¹ Institute of Environmental Health and Engineering, Chinese Academy of Preventive Medicine.
² National Research Center for Certified Reference Materials, CHINA.
IN VIVO LOCALISATION OF STRONTIUM AND FLUORIDE IN BONE TISSUE

S Bang¹ and J S Lee²

It has been known that systemically ingested strontium (Sr) and fluorine (F) deposit in the calcified tissues, and that these elements readily substitute for calcium and hydroxyl ions, respectively, in the crystalline lattice of the apatitic phase of bone mineral substance.

To study the effects of these elements on bone tissue, weanling mice were maintained either on a high Sr diet or on F-containing drinking water for up to 18 months in separate animal experiments.

Electron-probe X-ray microanalysis of the topographic distribution pattern of Sr or F in the femoral bone sections showed that these elements were predominantly deposited in the periosteal and endosteal layers, the areas of bone tissue formed during the experiment, whereas bone tissue existing before the beginning of the experiment showed only a small amount of these elements.

The X-ray diffraction analysis of powdered whole bone samples of both Sr- or F-treated animals revealed the modification of the α parameter of the bone mineral substance, indicating the incorporation of Sr or F into the bone apatite crystal lattice.

¹ University Medical Center, Geneva, SWITZERLAND.
² Asan Medical Center, Seoul, KOREA.

EMISSION SPECTROCHEMICAL ANALYSIS OF FLUORINE USING SHOCK WAVE PLASMA INDUCED BY A TEA CO₂ LASER

K Kagawa,¹ Y Deguchi,² M Tani¹ and Y Takagi³

Detection of fluorine atoms by an emission or absorption spectroscopic technique is usually very difficult due to the high-lying electronic-energy levels. Nowadays, inductively coupled helium plasma is employed for detection of fluorine atoms with F I 685.6 nm emission line. On the other hand, we have developed a new method of emission spectrochemical analysis using the shock wave plasma induced by bombarding with a high-power short-pulse laser when the pressure of the surrounding gas is reduced to around 1 Torr. The plasma has some characteristics favorable to spectrochemical analysis. In a previous study (Japanese Journal of Applied Physiology 30 1899 1991) we reported that Ca in food material, such as milk powder, can be analyzed with a good linear relationship between the content of the element and the emission intensity. The laser-induced shock wave plasma generates rather high temperature of more than several thousand degrees in centigrade, and therefore, it can be expected that fluorine can also be detected.

In this study, a TEA CO₂ laser (500 mJ, 100 ns) was used under the operation at 5 Hz, and the laser light was focused on the surface of samples placed in a vacuum-tight metal chamber. The plasma light was imaged on the entrance slit of a monochromator. The electrical signal from a photomultiplier was fed to a digital
boxcar signal system. In order to identify the fluorine lines, a KF pellet, a CaF$_2$ pellet, and a teflon block were used as samples.

Helium as surrounding gas gives the highest emission intensity for fluorine lines compared to argon and xenon, whereas air gives no emission. Comparison is made between the neutral emission line (F I 685.6 nm) and the ionic lines (F II 402.5, 384.7, 350.6, 350.3 nm) on the pressure dependence and the spatial distribution. These ionic lines have some advantages for spectrochemical analysis over the neutral one. The minimum determinable concentration of fluorine is estimated to be around 10 ppm in glass samples. Practical application to the quantitative analysis of food and biological materials will also be presented.

1 Department of Physics, Faculty of Education, Fukui University, Fukui 910,
2 Department of Environmental Health, Fukui Medical School, Matsuoka-cho, Fukui 910-11,
3 Department of Welfare and Health, Fukui Prefecture Government, Fukui 910, JAPAN.

FLUORIDE MICRODETERMINATION OF BIOLOGICAL MATERIALS BY FAU-1100 AND FAU-2100

K Itai and H Tsunoda

Fluoride concentrations in human and animal tissues are usually at levels below several mg/kg except in bone tissues. Fluoride concentrations in serum have extremely low levels; i.e., less than 20 μg/L (1.05 μM). Consequently, a method which can determine trace levels of fluoride is needed for studies of the fluoride behaviour in biological systems. For determination of fluoride in biological samples, there are two requirements to be met. The first requirement is a method which can isolate fluoride from various samples rapidly, with a high recovery rate and without external contamination. The second requirement is a method to determine trace levels of fluoride in sample solutions. We have developed an apparatus for the isolation of fluoride by pyrohydrolysis; FAU-1100. With this apparatus, it is possible to determine both organic and inorganic fluorocompounds with a recovery close to 100% and precision within 5%. A high recovery rate can be expected even for highly heat-resistant compounds such as CaF$_2$, without using WO$_3$ as a catalyst.

For determination of the isolated fluoride, we developed a separate apparatus in which flow injection analysis was used in conjunction with a fluoride selective electrode as a detector; FAU-2100. With this apparatus, concentrations of fluoride as low as 0.5 μg/L (0.026 μM), in a sample solution volume as small as 0.2 mL can be determined within 3 minutes with a precision of 2-3%.

Combined use of these methods makes it possible to determine fluoride in different biological materials within 10-15 minutes with a precision of several percent, free from external contamination. By selecting suitable conditions for analysis and using a 1 g sample, it is possible to determine fluoride at a concentration as low as 5 ng/g.

Department of Hygiene and Public Health, School of Medicine, Iwate Medical University, Morioka 020, JAPAN.
FLOW INJECTION ANALYSIS OF FLUORIDE IONS USING
ION-SELECTIVE ELECTRODE AND ELIMINATION OF
INTERFERENCES FROM IRON(III) AND ALUMINUM(III)

M Chikuma,¹ A Miki,¹ Y Okabayashi² and H Tanaka³

The ion-selective electrode method has been widely used for determining fluoride ions owing to its precision and stability. However, determinations at near detection limit often suffer from poor reproducibility in batch operations.

We have devised a flow system with a fluoride-selective electrode and a wall-jet type cell for the analysis of trace amounts of fluoride ions. The system is shown in the figure. Interferences from iron(III) and aluminum(III) were serious for the determination of fluoride ions using the system, because the metals form stable complexes with fluoride. In order to eliminate the interferences from these metals, separation of fluoride ions using fluoride-selective adsorbents and masking of the metals using proper reagents were investigated in the present work.

The fluoride-selective adsorbents were prepared by the modification of an anion-exchange resin with lanthanide metal complexes of alizarin fluorine blue and zirconium(IV) complexes of some chelating agents. Fluoride ions were collected and eluted completely with dilute sodium hydroxide solution. However, the interference from aluminum(III) was not eliminated completely by the separation method.

CyDTA and deferoxamine as masking agents eliminated the interferences based on their strong chelate-forming ability with iron(III) and aluminum(III).

Application of the flow system to the analysis of fluoride ions in biological and chemical samples is now underway.

FIA System
A: Carrier solution containing fluoride  B: TISAB

¹ Osaka University of Pharmaceutical Sciences, 2-10-65 Kawai, Matsubara, Osaka 580,
² Shionogi Research Laboratories, Shionogi & Co., Fukushima-ku, Osaka 553,
³ Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607, JAPAN.
The following are abstracts of papers presented at the XIXth Conference of the International Society for Fluoride Research at Kyoto, Japan, September 1992, in the sessions on Environmental Fluoride Pollution and Biological Effects on Humans.

THE EVALUATION OF INDOOR AIR QUALITY IN FLUOROSIS PREVALENCE AREAS CAUSED BY COAL BURNING
S Cao, R Ji and Y Li

Coal containing high levels of fluoride and sulfur is widely used for domestic cooking and heating in fluorosis-prevalent areas of China. Fluorosis caused by coal burning is found in 14 provinces. Since the local residents usually burn coal for cooking and heating inside houses in open pits without chimneys, the result is a peculiar indoor air pollution, and food, vegetable and drinking water contamination. The indoor concentrations of fluoride and sulfur dioxide are 10.5-304.0 μg/m³ and 0.2-122.7 mg/m³, respectively. Their average daily concentrations exceed by many times the national atmospheric health standards for residential areas. The intake of total fluoride is 2.43-9.65 mg/person/day in some fluorosis areas. The intake through respiratory tract is much higher than the standards allow. The results show that the fluoride in air is a major pathogenic factor for the dental fluorosis, which in turn is related to total fluoride intake.

Institute of Environmental Health and Engineering, Chinese Academy of Preventive Medicine, CHINA.

RESEARCH PROGRESS ON ENDEMIC FLUOROSIS IN CHINA
Z D Wei

Endemic fluorosis is a widely prevailing disease in China. Its prevention and treatment have made rapid progress in recent years. In the 1970s, the close relationship between fluorosis and the environment was indicated when hygienists discovered fluorosis caused by grain dried by burning coal, completely independent of the drinking water in Guizhou. Fluoride was found to originate from a variety of sources, and the concept of total fluoride intake was investigated. A special office responsible for the prevention and treatment of endemic disease was established by the government. In the 1980s, a large-scale program, involving 18 provinces and cities, was organized to establish hygienic standards of drinking water fluoride, and starting with multiple elements, hygienists discovered endemic regions of fluoride-arsenic and fluoride-aluminum intoxication. Also, a government program aided in the improvement of stoves and the decrease in fluoride intake in the Sanxia area. In the 1990s, prevention and treatment of endemic fluorosis are being promoted by the primary health care community, not only to decrease the total fluoride intake, but also to enhance the body's resistance to the disease. Finally, emphasis is being placed on the need for assessment of research progress at regular intervals, and efficient management.

Department of Hygiene, Guiyang Medical College, Guiyang, Guizhou 550004, CHINA.
RESULTS OF BONE DENSITOMETRY USING DPX IN PATIENTS WITH IATROGENIC FLUOROSIS

J Franke and S Hauch

Iatrogenic (medically induced) fluorosis is the most severe form of an inadequate and/or uncontrolled fluoride therapy of osteoporosis.

Our earlier studies showed a distinct increase in bone density on normal X-rays and histologically - a hyperostosis, combined with a high bone turnover and a massive surface and volume osteoidosis.

We have now quantified the bone density at various sites in 10 patients with medically-induced fluorosis using a DPX-L bone densitometer (Lunar).

Overall we found a distinct increase in bone mineral density (BMD) in the spine. In the lumbar spine the BMD was 138-228% of the age-matched normal values in a.p. direction. In the lateral direction of the lumbar spine we found an increase of 208-515%, even 2415% in an isolated measurement of the mid region of a lumbar vertebra. Towards the periphery of the skeleton the increase in BMD is not so pronounced. In the femoral neck regions we measured the following values: Ward's triangle: 93-155% (average 110%), greater trochanter: 90-136% (average: 109%), femoral neck: 95-123% (average 108%).

In the total body scans we found a mild increase of the BMD of 111% on the average, mainly conditioned by the increase of the central part of the skeleton.

The often observed stress fractures on the diaphyses of long bones in iatrogenic fluorosis show an effect of high-dose, short-term fluoride exposure on the peripheral skeleton. Under these conditions, however, there is no significant increase of the BMD as found in long-term, low-dose fluoride exposures in industrial fluorosis.

Department of Orthopaedic Surgery, Medical Academy Erfurt, Erfurt, GERMANY.

PHYSICOCHEMICAL PROPERTIES OF HETEROGENEOUS FLUORIDATED APATITES

M Okazaki, J Takahashi and H Kimura

In biological systems, fluoride uptake often occurs intermittently. Heterogeneous fluoridated hydroxyapatites may therefore be formed, and the phenomenon of the formation may become very complex. In this study, a simple two-step supplying system was adopted. Two types of fluoridated hydroxyapatites (H-FAp and F-HAp) were synthesized at 80°C. This involved the use of two solutions which were supplied at 250 mL/h with a microtube pump into 1 L of mechanically stirred 1.3 mol/L CH₃COONH₄ solution. H-FAp apatites were made by supplying 0.5 L of 100 mmol/L Ca(CH₃COO)₂H₂O solution throughout the supplied period and 0.25 L of 60 mmol/L NH₄H₂PO₄ solution for the initial half of the period, followed by 0.25 L of 60 mmol/L NH₄H₂PO₄ solution containing 20 mmol/L HF for the final half of the period. For F-HAp apatites, the order of supply of fluoride was reversed. The pH was maintained at 7.4 with occasional addition of concentrated NH₄OH solution. The suspension was stirred for 3 h, then kept at 25°C for 24 h. Each slurry was filtered, washed with distilled water, then dried at 110°C.
Both heterogeneously synthesized fluoridated hydroxyapatites showed typical calcium phosphate patterns. Total fluoride contents of both fluoridated hydroxyapatites were almost the same: half of the maximum fluoride content, equal to that of fluorapatite. However, the tendencies of physicochemical properties were quite different. The peak breadth of X-ray diffraction of H-FAp was wider than that of F-HAp; apparent solubility of the former was less than that of the latter at 37°C and pH 4.0; and wavelength dispersive spectroscopy attached to the SEM showed different F spectra.

These results suggest that two different types of heterogeneous fluoridated hydroxyapatites may be formed, hydroxyapatite covered with fluorapatite (H-FAp) and fluorapatite covered with hydroxyapatite (F-HAp). In addition, in the presence of carbonate ions a similar experiment was carried out, and the results showed the same tendency.

Osaka University Faculty of Dentistry, Osaka 565, JAPAN.

The following are abstracts of papers presented at the XIXth Conference of the International Society for Fluoride Research at Kyoto, Japan, September 1992, in the session on Biological Effects of Fluoride.

THE ORIENTATION OF CAPSULAR AND INTERCAPSULAR LINEARLY ORIENTED POLYSACCHARIDES OF BONE AND ARTICULAR CARTILAGE IN EXPERIMENTAL OSTEOFLUOROSIS

M Bély

The oriented ultrastructure of capsular and intercapsular, linearly arranged polysaccharides shows an induced and enhanced birefringence after an anisotropic staining procedure with aldehyde-bisulphite-toluidine blue (ABT) reaction.

The ABT reaction gives a higher contrast than the routine PAS reaction and induces in linearly oriented polysaccharides a longitudinally negative birefringence and a negative dichroism. The increase in staining intensity and the anomalous polarization colours of the birefringence allow the detection of a minute amount of vicinal OH-groups which show no light absorption with the PAS reaction. In detecting the changes of birefringence - determined by the linearly oriented polysaccharides - the ABT reaction is suitable for characterisation of structural changes in bone tissue and articular cartilage.

Daily administration of 0.5 mg and 5 mg NaF for three months produced a significant decrease in the regularity of the capsular and intercapsular linearly oriented polysaccharides of preexisting bone tissue and articular cartilage.

Alteration in the regularity of linearly oriented polysaccharides (mostly connected to the glycoprotein binding components of the matrix) is a part of complex disturbances of the fluorotic bones and articular cartilage.

National Institute of Rheumatology, Budapest, HUNGARY.
THE MECHANISM OF CHANGES IN METABOLISM AND TRANSPORT OF GlUCOSE CAUSED BY FLUORIDE ADMINISTRATION TO RATS

T Sakurai, K Suzuki, T Taki and Y Suketa

We found that hyperglucose urine and hyperglycemia were caused by a single large dose of fluoride (35 mg as NaF/kg, ip) to rats. In our previous paper renal and hepatic glucose-6-phosphatase activities were reported to be remarkably elevated by the fluoride dose. The evidence demonstrated a trigger of hyperglycemia derived from fluoride ingestion. Glucose mobilization is controlled by fluoride metabolism and transport.

In this paper, in vitro effects of fluoride on glucose transport were examined using renal brush border membranes and basolateral membranes. Glucose reabsorption in kidney is driven by Na+-glucose cotransporter and by active glucose transport coupling with Na+, K+-ATPase (driving force of Na+-glucose cotransporter). The renal Na+-glucose cotransporter activity of rat was slightly suppressed by a higher concentration of fluoride (20 mM), whereas the corresponding Na+, K+-ATPase activity was almost completely inhibited by 10mM fluoride. From the result, the renal brush border membranes were found to be hard damaged by fluoride or to be resistant against it. On the other hand, the Na+, K+-ATPase itself and the related protein in the basolateral membranes were confirmed to have a high affinity for fluoride.

On the other hand, we have already found renal Na+, K+-ATPase activity to be suppressed by a single and a one-week oral administration of fluoride (50 mg as NaF/kg/day) to rats. Active transport of glucose in the renal basolateral membranes was suggested to be strongly suppressed after a single dose of fluoride (35 mg as NaF/kg, ip) to rats. Thus the in vivo effect of fluoride on the activity of renal Na+-glucose cotransporter and Na+, K+-ATPase is now being examined.

Department of Environmental Biochemistry and Toxicology, University of Shizuoka School of Pharmaceutical Sciences, 52-1 Yada, Shizuoka, Shizuoka 422 JAPAN.

RESTORATION OF CALCIUM DISTURBANCE IN KIDNEY OF FLUORIDE-TREATED RATS: ROLE OF PROTEIN KINASE C AND CALCIUM PROTEASE (CALPAIN)

Y Suketa, M Yamamoto, Y Ibuki and T Imagawa

In animal fluorosis, osteoporosis and mottled teeth are well known as metabolic disorders of calcium. We found that calcification of kidney was caused by a single large dose (ip) of fluoride to rats. Cytosolic calcium was increased 4-times that of controls 3 hours after the fluoride dose (NaF 35 mg/kg, ip) to rats. The increment of calcium in kidney is suggested to be due to either augmentation of calcium influx or suppression of calcium efflux. However, renal Ca²⁺-ATPase activity (Ca-pump) was elevated beyond expectation by the fluoride dose.

On the other hand, renal plasma membranes might be directly damaged by fluoride ion. Thus, to understand the effect of fluoride on calcium influx in the
kidney, incorporation of \(^{45}\)Ca-Calcium was examined using renal brush border membranes of rats. The incorporation was stimulated by fluoride concentration of less than 1 mM but was inhibited by more than 5 mM. The stimulation was suppressed by nifedipine. The stimulation of calcium incorporation by fluoride was concluded to be due to opening of calcium channel gate rather than any direct damage of brush border membranes by fluoride.

Moreover, elevations of calcium in several organel of rat kidney by fluoride administration were examined in relation to changes in protein kinase C activity using some specific inhibitors of calphostin C (for protein kinase C) and of K-5720 (for protein kinase A) and staurosporine. The elevations of calcium levels in cytosol and microsomes were enhanced beyond expectation by treatment of calphostin C or staurosporine, but were not influenced by treatment of K-5720. Protein kinase C might thus contribute to lowering of calcium influx.

Protein kinase C activity may be regulated by calcium protease (calpain). Hence mobilization of calcium protease (calpain) activity was examined in this experiment. The renal calcium protease activity was increased about 1.8 times that of controls 3 hours after a single dose of fluoride to rats. The increment was suppressed about 50% by treatment of staurosporine (6.6-66 \(\mu\)g/kg, ip). From the result, calcium protease activity can be considered to be controlled by protein kinase C and/or protein kinase A.

Department of Environmental Biochemistry and Toxicology, University of Shizuoka School of Pharmaceutical Sciences, 52-1 Yada, Shizuoka 422, JAPAN.

CHRONIC FLUORIDE TOXICITY: A BIOCHEMICAL AND SCANNING ELECTRON MICROSCOPIC STUDY OF ENAMEL SURFACE OF RABBIT TEETH

M Bhatnagar and A K Susheela

Promoting fluoride for caries prevention continues to be a subject of debate, in part because the effect of fluoride on teeth has not been adequately investigated. The present study was therefore carried out on rabbits administered 10 mg NaF/kg body weight for 18 and 23 months. The incisor and molar teeth were investigated for fluoride, calcium, phosphorus contents and Ca/P ratio were assessed. Both inner and outer surfaces of incisor teeth were examined under scanning electron microscope of 23 months fluoride treated rabbits. The results revealed accumulation of fluoride, depletion of calcium and phosphorus, and increased Ca/P ratio. The scanning microscopic observations revealed hypoplastic pitted and eroded enamel surfaces covered with granular deposits.

These observations suggest that long-term fluoride administration leads to severe structural alterations of the enamel surface, possibly acquired by defective mineralization.

Fluoride and Fluorosis Research Laboratories, Department of Anatomy, All India Institute of Medical Sciences, New Delhi 110 029, INDIA.
THE EFFECT OF ORGANIC AND INORGANIC FLUORINE COMPOUND ADMINISTRATION ON FLUORIDE LEVELS IN THE PLASMA AND IN THE SOFT AND HARD TISSUES OF RATS BREEDING ON A LOW-FLUORIDE DIET

M Tomita, T Sugimura, M Koyama and Y Kaneko

In studies on the metabolism and accumulation of fluoride (F) in the tissues of experimental animals given low-level fluoride compounds, it is effective to use a low-F basal diet. Moreover, reports on the fate of F in biological systems exposed to organic fluoride compounds are limited. The purpose of this study was to find the changes of F levels in rat tissues exposed to organic and inorganic fluoride compounds.

Methods: 1) Experimental animal diet; Commercially available diet (CD) (Oriental Yeast Co product code; MF, F=36 ppm), low F diet (Oriental Yeast Co, F=0.5 ppm). 2) Experimental animals; 3-week-old female Wistar rats were randomly divided into six groups. The CD-control group was given CD and tap water, the control group was given the low-F diet and distilled water until the end of the experiment. After 6 weeks of preliminary breeding with the low-F diet, the organic fluoride compound dose groups (7.5FU and 15FU groups) were given the low-F diet and 5-fluorouracil (5-FU) solutions for 6 weeks at levels of 7.5 and 15 mg F/L, respectively. In the same manner as the organic fluoride dose groups, the 3NaF and 7.5NaF groups were given NaF solutions at levels of 3 and 7.5 mg F/L, respectively. 3) F determination; i) direct measurement by F ion electrode (plasma); ii) F separation by microdiffusion and F measurement by gas chromatography (soft tissues; liver, kidney, brain); iii) direct extraction with a 2% trimethylchlorosylane-hexane solution and F measurement by gas chromatography (hard tissues; femur, incisor).

Results and Conclusions: 1) F intake estimated; the mean F intake of the CD-control group showed the highest amount (2.2 mg F/kg/day) of all the experimental animal groups (0.03 mg F/kg/day from the low-F diet, 0.29 ~ 1.54 mg F/kg/day from the dose compounds). 2) F levels in plasma; although the control group showed a very low F level (0.009 mg F/L), the CD-control group showed a higher F level (0.026 mg F/L) than the 7.5FU-, 3NaF-groups. 3) F levels in soft tissues; the F levels of the control group in the kidney were higher than those of the CD-control group. 4) F levels in hard tissues; the CD-control group showed high F levels (455 ppm for the femur, 193 ppm for the incisor). The control group showed low F levels (66 ppm for the femur, 14 ppm for the incisor) and the organic and inorganic fluoride dose groups clearly showed higher F levels than the control group.

There were significant differences of average ionic F levels in the plasma and the soft and hard tissues between the control group and both forms (organic, inorganic) of the F in the F-dose groups.

Department of Hygiene and Oral Health, School of Dentistry, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, JAPAN.
EFFECTS OF FLUORIDE ON SHEEP MOLARS

G E Milhaud

In sheep with fluorosis, molars are notably more affected than molars in bovines. The purpose of this study was to investigate this phenomenon.

Fourteen sheep were dosed orally with 3.5 mg of fluoride/kg/body weight for 4 months (from 5 to 9 months after birth). Seven sheep were slaughtered immediately after fluoride administration. The other 7 sheep were slaughtered 4 months later. Three control groups of 7 sheep each were slaughtered at 5, 9 and 13 months.

During fluoride administration, plasma fluoride concentration rapidly increased from 0.125 mg/L to values ranging from 0.40 mg/mL to 0.55 mg/L. No changes in tooth morphology (length, dentine and enamel thickness) were visible either at 9 months or at 13 months. But enamel lesions were observed in semi lunar pits and close to the root (thinness, irregularities). During fluoride administration, fluoride accumulation had increased in the enamel, mainly in the outer enamel of the first molar (from 4000 to 5500 μg/kg [dry weight]). It also increased in dentine where fluoride concentrations increased from values ranging between 500 and 1000 mg/kg [dry weight] to values ranging between 2500 and 4500 mg/kg [dry weight].

Hardness values were measured in enamel and dentine using a microhardness tester: the microhardness of dentine slightly decreased during fluoride administration (from 65 to 70 Vickers U to 55 to 60 Vickers U). The microhardness of enamel decreased more notably, not only in the enamel that formed during fluoride administration (from 240 to 250 Vickers U to 90 to 100 Vickers U) but also in the enamel already formed (from 240 to 250 Vickers U to 180 to 190 Vickers U).

‘In situ’ calcium measurements and microradiography revealed no significant changes in the pattern of mineralization of the tooth.

Histological examination helped determine the nature of lesions in the enamel formed during fluoride administration: irregular surface, mainly in semi lunar pits, and demineralized or hypermineralized zones. Scanning electron-microscopic examination indicated deep changes in the structure and direction of crystals.

In conclusion, molars that were in the process of mineralization during fluoride administration had thinning enamel, marked decrease in hardness and lower resistance to wear. To a lesser extent, the same phenomena were observed in the molar teeth that had mineralized before fluoride intake.

Ecole Nationale Vétérinaire d'Alfort, 94704 Maisons-Alfort, FRANCE.
The following are abstracts of Poster Presentations at the XIXth Conference of the International Society for Fluoride Research, Kyoto, Japan, September 1992.

STUDY OF REGIONAL FLUORIDE STANDARDS FOR AMBIENT AIR QUALITY IN BAOTOU

X Wu

In order to formulate regional fluoride standards for ambient air quality in Baotou, on-site exposure tests of different organisms were designed. The focal point of this research was to determine airborne fluoride levels that affected people, animals, and plants. The tests were conducted at sites that had different airborne fluoride concentrations. A quantitative relationship between fluoride levels and damage was found. Damage criteria were developed for seven plant, four goat husbandry, and three adult human groups. Using reference literature and economic profit and loss considerations, we have devised three levels of ambient air quality fluoride standards for protecting humans, plants, and animals.

Environmental Monitoring Station of Baotou, Inner Mongolia, CHINA.

CORRELATION BETWEEN THE FLUORIDE CONCENTRATION IN AIR AND CANADA POPLAR LEAVES

X Wu

The fluoride concentration in air is very low, generally in the ppb range, and therefore difficult to monitor. This article reports that the Canada poplar, which has been planted extensively in Baotou, acts as a fluoride guide plant. The fluoride content of Canada poplar leaves (F) is proportional to the fluoride concentration in air (C) and exposure time (T). The relation is:

\[ F = KCT \quad (K = \text{coefficient}). \]

Fluoride concentration in air was measured by the lime-filter method. From the analysis of sixty samples, the relationship between the leaf and air fluoride concentrations was determined statistically to be:

\[ Y = 0.71T + 3.64 \quad (Y = \text{F content of Canada poplar leaves}; \]

\[ T = \text{monitoring day number}; \]

\[ X = \text{average daily fluoride in air}. \]

The relative coefficient was 0.87 (n = 60).

This result indicated that it is possible to monitor fluoride in air with a guide plant. Furthermore, monitoring fluoride in air by a guide plant has the advantage of using an easily preserved sample and being economical and simple. Moreover, fluoride in air was found to show good inverse correlations with distance from the pollutant source.

Environmental Monitoring Station of Baotou, Inner Mongolia, CHINA.
FLUORIDE CONCENTRATION IN WATER FROM LAKES MAGADI, BOGORIA, AND ELEMENTAITA, KENYA

J K Gikunju,1 T E Maitho1 and P Lokken2

Measurements were made of the pH and fluoride concentration of water samples collected from Lakes Bogoria, Elementaita, and Magadi situated in the Kenyan Rift Valley of Kenya. All water samples were alkaline with mean pH ranging from 10.1 to 10.3. The mean fluoride levels of water from Lakes Magadi, Elementaita, and Bogoria, as determined by the Orion® 96-09 fluoride ion specific electrode, were 84, 463 and 738 mg F/L, respectively. Dilution per se was found not to influence pH and measurement of fluoride when TISAB (Total ionic strength adjustment buffer - Orion®) II and III were used, $p > 0.05$.

After addition of TISAB II and III, the water samples from the three lakes had pH values above 5.5, which is the upper pH limit recommended for fluoride analysis with the fluoride ion selective electrode. Dilution of the water did not alter the pH before addition of buffer, and therefore interfering ions could be responsible for the problem encountered in fluoride analysis in the alkaline water. Hence the commonly used methods for fluoride analysis in water might give erroneous or unreliable results when used on water with high pH as found in some of the Rift valley lakes.

Measurement of fluoride concentration in water samples using TISAB II and III were not significantly different, $p > 0.05$. However, water from the Rift Valley lakes may require suitable dilution before measurement of fluoride can be made; otherwise another TISAB solution should be developed for analysis of fluoride from the salty lakes.

Although the dilution of samples of alkaline water helps in obtaining appropriate pH after addition of buffer, it cannot be stated with certainty what causes the consistent drop and rise in fluoride concentration and finally a steady fluoride concentration.

1 Department of Public Health, Pharmacology and Toxicology, College of Agriculture and Veterinary Sciences, University of Nairobi PO Box 29053, KENYA.
2 Department of Pharmacology, Blindern Oslo 3, PO Box 1057, NORWAY.

EXPERIMENTAL STUDIES ON FLUORIDE-INDUCED DIABETIC HYPERGLYCEMIA

A Shashi

Experiments on white albino rabbits of both sexes receiving 5, 10, 20 and 50 mg of sodium fluoride per kilogram body weight per day for 4 months revealed specific changes in glycogen biosynthesis in pancreas. The glycogen concentration of pancreas showed a significant ($P < 0.001$) dose-dependent elevation after fluoride treatment. Females in all fluoride treated groups showed higher per cent change in the level of glycogen in comparison with the males. Light microscopy revealed necrosis of pancreatic cells. The islets of Langerhans were small and showed a rapid decline in the beta cell population with the development of fluorosis. This may result in low insulin production thereby producing defective carbohydrate metabolism. These results indicate that increased and prolonged fluoride intake could induce diabetic hyperglycemia in experimental animals.

Department of Zoology, Punjabi University, Patiala 147002, INDIA.
AN OVERVIEW OF FLUORIDE RESEARCH:
ANALYTICAL LEVEL AND MEDICOBIOLOGICAL APPROACH

K Yoshitake,1 G Yamamoto1 T Kimura2 and T Ando2

In the history of medicobiological researches on fluoride (F), knowledge has increased with the progress in F analysis. For the purpose of investigating epidemiology on mottled enamel, it is necessary to be able to measure 1 ppm F, while for the community fluorosis index (CFI) calculation, 0.3 ppm is the minimum level for analysis.

With the ability to detect a 0.05 ppm difference, the mechanism of F absorption from the gastrointestinal tract was studied. Our experimental results showed that the absorption of F from the stomach and small intestines occurs through passive diffusion and active transport. The antagonistic effect of the chloride ion on F absorption was also demonstrated.

Recently, at the level of 5 ppb in blood analysis, distribution and absolute concentration of three kinds of fluoride/fluorine in human whole blood and serum have been determined. The method is based on low-temperature oxygen plasma ashing, acid hydrolysis, and gas chromatography using chlorotrimethylsilane. Without any exposure to F, the sum of fluoride and fluorine (total F) content of serum was found to be 46 ng in 1 ml of blood of males, whereas it was 53 ng in the blood of females. A large part, 40% or more, of the total F is distributed in clots.

1 Department of Oral and Maxillofacial Surgery, 2 Department of Chemistry, Shiga University of Medical Science, Seta, Ohtsu 520-21 JAPAN.

AIR-POLLUTION FLUOROSIS IN THE REGION OF PINGXIANG,
JIANGXI, PEOPLE'S REPUBLIC OF CHINA

Y Chen, M Lin, Z He and X Xie

An investigation of endemic fluorosis in Pingxiang indicates that the type of endemic fluorosis in this region is caused by air-pollution with the main source of pollution coming from domestic coal burning. Another pollution source are the small brick kilns and tile kilns which have increased in number, especially in the last ten years. The fluorine content both in the coal and in the mud used to mix the coal in the region is quite high. During the process of burning coal or baking bricks and tiles, a large amount of insoluble fluorine is converted into water-soluble fluoride which pollutes the air and does harm to humans.

Jiangxi Institute of Labor Hygiene and Occupational Medicine, Nanchang, Jiangxi, CHINA.
STUDY OF THE EFFECT OF THE MICROELEMENT MOLYBDENUM ON ENDEMIC FLUOROSIS

Z M Yao and L H Chang

This paper reports an investigation of the effect of the microelement molybdenum on endemic dental fluorosis. In experiments with white rats, sodium molybdate moderated fluorine intoxication, and in children it ameliorated fluorotic staining of teeth. Higher levels of Mo in water containing upwards of 2.0 ppm F were associated with less dental fluorosis. With increased amounts of Mo in animal feed, urinary excretion of F was increased. The F content of teeth was reduced, but the pathology of the skeleton was unaffected. When fluorotic children were treated with sodium molybdate, hair F was reduced from 5.79 to 4.74 ppm (p 0.001) and the immune function was enhanced. In the PHA skin test, the diameter of the red spot increased from 5.56 to 11.56 mm, with a positive rate increase from 33.3% to 69.2%. Molybdenum is thus seen as interfering with F intoxication and therefore has potential value for the prevention and treatment of fluorosis.

The Institute of Prevention and Treatment of Endemic Diseases of Shanxi Province, Linfen, Shanxi 041000 CHINA.

FURTHER IDENTIFICATION OF A DFP-HYDROLYZING ENZYME IN PLANTS TISSUES

S Sakurai¹ and M H Yu²

We have previously reported that extracts of seedlings from mung bean (Vigna radiata) and several other plant species contained enzyme(s) capable of hydrolyzing diisopropyl-fluorophosphat e (DFP), a potent acetylcholinesterase inhibitor. The purpose of this study was to investigate occurrence of the enzymes in tissues of other plant species. Crude enzyme extracts were prepared from tissues of 36 species of fruits and vegetables and assayed for DFP-hydrolyzing activity. This activity was exhibited by 21 out of the 24 species of vegetables and by 4 out of the 12 species of fruits that were tested. The highest enzyme activity was found in mushroom extract, with a specific enzyme activity of 12.4 nmol F hydrolyzed/mg protein/min. These results revealed the presence of DFP-hydrolyzing activity in a large number of plant species. The possible use of plant enzymes in the detoxication and decontamination of organophosphates in the environment is discussed.

¹ Otsuma Women's University, Tokyo, JAPAN.
² Western Washington University, Bellingham WA, USA.
HIGH-RESOLUTION ELECTRON MICROSCOPY OF ENAMEL AND DENTIN CRYSTALS IN FLUOROTIC TEETH

Y Miake,1 T Yanagisawa,1 H Tohda1 and O Fejerskov2

The formation of a kind of hypoplasia caused by ingestion of fluoride containing water during the period of tooth formation is well known. In the past, our department has reported on ultrastructural changes brought about in teeth by various medications. This report presents results of high-resolution electron-microscopic investigations of changes caused mainly by fluoride.

Three kinds of teeth were used as experimental materials. First were fluorotic human teeth obtained from adults living in regions where the water supply contains 3.5 ppm fluoride. Second were teeth from young pigs and rats given fluoride; the pigs were given 2 mg/kg of fluoride in their diet daily for 10 months, and the rats were given 14 mg/kg of fluoride in their drinking water daily for 10 weeks. Third were formationally defective teeth obtained from rats to which 34 mg/kg of fluoride had been administered subcutaneously.

Microradiograms, and scanning-, transmission-, and high-resolution electron microscopic examinations were made of these teeth to investigate changes in cellular structures and in organic matter as well as hard-tissue crystalline structure. The thin outermost enamel surface layer was relatively highly mineralized and accompanied by a hypomineralized subsurface area. Crystals in the highly mineralized layer were either large with flattened-hexagonal configurations and central dark lines or small and nearly regular-hexagonal without central dark lines. From crystal-lattice striation intervals, it was possible to identify the large crystals as hydroxyapatite and the small crystals as fluorapatite. Large crystals were sparsely distributed in the hypomineralized area. Perforations were observed occasionally in the centres of crystals in fluorotic human teeth. Though both large and small crystals occurred in the transitional region between the highly mineralized surface layer and the hypomineralized subsurface area, small crystals were few in number.

Highly mineralized and hypomineralized layers were observed in dentin too. Though segmental collagen aggregates and fibrous long-spacing collagen occurred in the hypomineralized layer, depositions of either needle-shaped or thin, platelike crystals completely covered these abnormal kinds of collagen.

1 Tokyo Dental College, Chiba, JAPAN.
2 Royal Dental College, Aarhus, DENMARK.

ON ENDEMIC FLUOROSIS IN NONG-AN COUNTY, JILIN PROVINCE, CHINA

D Zhang,1 K Liao,1 A Gu,1 R Song,1 L Sha,1 B Du,1 F Zhao,1 M Zhu,2 B Zang,2 C Zhang,3 L Qi,3 Z Duan3 and L Zhang 4

We have been studying endemic fluorosis in the Yang shu-lin township of Nong-an county, Jilin Province, since 1965, when the first fluorosis patient was discovered in this township. By measuring the fluoride concentration in the water of 288 wells in Yang shu-lin, we found that most of the wells with high fluoride were located in four particular villages, where the fluoride generally ranged from 2.0 ppm to 5.0 ppm, but a maximum value of 9.27 ppm was recorded. Fluorosis examinations were conducted on 4318 residents of these four villages.

These examinations showed that 28.3% of the subjects had skeletal fluorosis. There was no sex difference in prevalence, but severe fluorosis was found mostly in women. The prevalence of skeletal fluorosis among multipara was 1.44 times that
among nonmultipara. The longer the duration of habitation and the higher the fluoride concentration in the wells, the greater the prevalence of fluorosis. However, groups of people living in different areas having the same fluoride concentration in well water did not exhibit the same incidence of skeletal fluorosis; in some cases, the disease was twice as prevalent in one area as in another.

Dental fluorosis was found in 74.4% of the examinees; of these cases, 14.7% suffered from the severe type. In one village, where the well water fluoride concentration was 4.5 ppm, dental fluorosis was found in 730 out of 904 people (80.8%). In some cases, fluorosis was observed even in deciduous teeth.

As a treatment and preventative measure for fluorosis, water containing a low fluoride concentration of 0.9 ppm was supplied to an area where the prevalence of fluorosis was high. Examination results showed that in an area where 72.8% of the residents suffered from skeletal fluorosis before low-fluoride water began to be supplied, the symptoms of fluorosis have either disappeared or been reduced in 82.6% of the affected people 20 years later. No new cases of skeletal fluorosis have been found within the last 20 years, and only four cases of the mild type of dental fluorosis have been found among the 47 children born during the same period.

Patients suffering from skeletal fluorosis showed an improvement in symptoms upon relocation to an area where the fluoride concentration in water was low, or upon treatment with certain types of herbal medicines.

1 Health and Anti-Epidemic Station, Changchun,
2 Changchun, Jilin Province Branch of Chinese Preventive Medicine Association,
3 Health and Anti-epidemic Station, Nong-an County,
4 Nanguan District Health Bureau, Changchun, CHINA.

RELATIONSHIP BETWEEN FLUORIDE AND IONIC FLUORINE CONTENT IN DRINKING GROUNDWATER AND FLUOROSIS ON THE SONGNEN PLAIN, CHINA

S L Xiong

A fluorosis region has been discovered on the Songnen Plain in China, where there is systematic hydrogeology in the recharge, runoff, and collection areas of the groundwater. The degree of fluorosis has been found to depend on the hydrogeological conditions. While in reports not considering hydrogeological conditions, water fluoride content and fluorosis prevalence have been directly related, we have obtained contrasting results. The morbidity of dental fluorosis decreased with increasing fluoride content in the runoff area, but decreased with decreasing content in the collection and mixture (combination of runoff and collection characteristics) areas. With skeletal fluorosis, a positive correlation appeared only in the collection area. With respect to ionic fluorine content, dental fluorosis was positively correlated in the runoff and collection areas, but skeletal fluorosis showed this relationship only in the collection area. In the runoff area, $\text{FeF}_2^+$ and $\text{FeF}_3$ content increased in accordance with the prevalence rate of dental fluorosis but decreased with that of skeletal fluorosis. Negative correlations were found for $\text{FeF}_2^+$ with the rates of both skeletal and dental fluorosis. In the collection area, positive correlations were found for $\text{FeF}_2^+$, $\text{FeF}_3^+$ and $\text{FeF}_3$ with the rate of dental fluorosis, and same was found for $\text{FeF}_2^+$ and $\text{FeF}_3$ with skeletal fluorosis. We conclude that fluoride and ionic fluorine were disturbed by other elements in the ground water, which affected their biological effects.

Research Center of Geochemistry for Human Health, Jilin Province, CHINA.
INVESTIGATION OF MAJOR TECHNOLOGIES AND FACILITIES FOR DRINKING WATER DEFLUORIDATION IN CHINA

C Huang¹ and J Liu²

Waterborne endemic fluorosis is common in all provinces, autonomous regions, and municipalities of China except Shanghai. A total of at least 37 million persons suffer from dental and 1.7 million from skeletal fluorosis in the whole country. In 1989, the authors investigated 71 community water defluoridation-desalination facilities in Shandong, Gansu, and Hebei Provinces, Inner Mongolia Autonomous Region, and Tianjin Municipality, together with 309 household defluoridators in 16 villages. The qualification rates of F⁻ (< 1.0 mg/L) and TDS (< 1000 mg/L) in community water supplies were 72.6 and 53.7%, respectively. Excessive levels were caused by: (1) unreasonable design and construction of equipment, (2) incorrect operation and management. We recommend an activated alumina process (with pH adjustment) for defluoridation and electrodialysis for desalination. The average qualification rate of F⁻ level in treated water of various types of household defluoridators was 49.2%. Administrative organizations should pay close attention to such devices and work out a series of reliable monitoring measures. Peasants must pay careful attention to constant reliable operations. All localities should ensure that defluoridators operate with a high rate of utilization. Otherwise they are not suitable for popularization.

¹ Institute of Environmental Health and Engineering, Chinese Academy of Preventive Medicine, 29 Nan Wei Road, Beijing 100050,
² Office of National Committee of Patriotic Health Campaign, Ministry of Public Health, Beijing 100725, CHINA.

EVALUATION OF THE EFFECT OF LOWER FLUORIDE LEVELS IN DRINKING WATER AFTER A TEN-YEAR PERIOD IN SHUNYI COUNTY, CHINA

L Hao and F Chen

The fluorine level of the natural drinking water in the villages of Shunyi county was greater than 1.0 mg/L. In 1978, the fluorine level of 2420 water samples from the shallow wells had been examined. Among these 51.0% were < 0.5 mg/L, 40.0% were 0.5-1.0 mg/L, and 9.0% were > 1.0 mg/L. By the health examination, 20520 of 34103 persons were affected by endemic fluorosis.

After 1981, the local government constructed 69 deep wells in order to supply appropriate fluorine levels.

We selected randomly 30 of these wells for observation of the fluorine level in dry and rainy seasons of every year. In a ten-year period, the average fluorine level in the wells was 0.66 mg/L (308 samples) and 0.60 mg/L (363 samples) in dry and rainy seasons, respectively. The range of the fluorine level was in accordance with the "Sanitary Standard For Drinking Water" (GB5749-85). At the end of the period, the incidence of endemic fluorosis among children in the villages was lowered from the previous 60.2% to 29.1%. An analysis of the economic benefit of 30 deep wells indicated that the ratio of investment to benefit was 1:81; clearly, the economic benefit was very significant.

Shunyi Health and Prevention Station, Shunyi 101300, Beijing, CHINA.
MOTION MODEL OF FLUORINE AND OPTIMIZED CONTENT CONTROL IN GROUNDWATER OF CHANGCHUN CITY AND SUBURBS, CHINA

Y Q Zhao

To improve drinking water conditions, we conducted tests using high-fluoride water in ChangChun and suburbs, China. This paper examines water usage, according to the system engineering theory with respect to the characteristics of the structure, the environment, and boundary conditions of the groundwater flow. On the basis of a hydrogeological model for elemental fluorine and fluoride, the motion model of fluorine and optimized content control, and a simulation model of the groundwater system, a management model was proposed whereby the output value of the well and benefits to the water user were taken as the economic constraint conditions. The relationship between groundwater mining yield and economic benefit provided new methods for scientific management of the region. In the hydraulic management model, the mining yield of 28 well groups is optimized to obtain the optimal mining plan. The above model is applicable to optimal control of content for each well and leads to improved economic and social benefits. The results, combined with engineering physics, prospecting, and hydrogeological research of regional groundwater, have enabled us to draw conclusions regarding the feasibility of tap water use and the use of groundwater resources.

The Water Resource Management Office of ChangChun City, CHINA.

EFFECTS OF VARIOUS FEEDS ON RATS WITH REDUCED BONE MINERAL DENSITY

J Senda,1 M Shimahara,1 N Hashiguchi,1 Y Ariyoshi,1 Y Yoshida,2 K Kono2 and T Dote2

Forty 14-week-old Wistar female rats were provided low calcium feed ad libitum to produce rats low in bone minerals. The rats were divided into 4 groups: a group fed a low-calcium diet, a group fed Funabashi Farms MM-3 feed, a group fed a low-calcium diet with fluoride added, and a group fed Funabashi Farms MM-3 feed with fluoride added. These feeds were provided for 8 weeks. Bone mineral density was determined before and at 1 month and 2 months. Bone mineral density was determined by double energy X-ray absorptiometry (Norland XR-26) with software for small animals. The determination on the lumbar spine was done in a supine position.

In the low-Ca diet group no changes in bone mineral density were observed at 1 month and 2 months. In the group provided Funabashi Farms MM-3 feed, a gradual rise in bone mineral density was observed at 1 month, and in the group provided Funabashi Farms MM-3 with fluoride added a linear rise up to the second month was observed.

1 Department of Oral Surgery, 2 Department of Hygiene and Public Health, Osaka Medical College, 2-7 Daigakumachi, Takatsuki, Osaka, JAPAN.
PROBLEM OF EXCESS FLUORINE IN WATER IN INDIA
AND ITS REMOVAL BY THE NALGONDA TECHNIQUE

D R Bulusu and W G Nawlakhe

A water quality survey of villages from various states of India was conducted. Out of 4252 water samples analysed, 821 samples showed excess fluoride in water. Among the various media tested and processes developed, the Nalgonda Technique for removal of excess fluoride from water is an economical and easily adaptable method for rural populations. The fill-and-draw system is best suited for rural water supplies. Cost estimates for various water demands ranging between 10 m³ and 480 m³ per day are made and presented.

National Environmental Engineering Research Institute, Nehru Marg, Nagpur 440 020, INDIA.

EFFECTS OF FLUORIDE ON EXPERIMENTAL POSTMENOPAUSAL
OSTEOPOROSIS WITH KIDNEY DYSFUNCTION

T Dote, Y Yoshida, K Kono, M Watanabe, Y Tanioka,
Y Bessho, Y Orita, J Yoshida and K Umebayashi

Renal function is one of the factors which influence the pathological condition of osteoporosis and the effects of therapeutic drugs. This study investigated two types of experimental models of postmenopausal osteoporosis: one with normal renal function and the other with renal dysfunction. We performed ovariectomy and administered a low calcium diet to female rats (group 1) and in addition did partial nephrectomy on them (group 2). Control rats were sham operated and administered the stock diet throughout the experiment. Bone mineral density (BMD: g/cm²) of the total lumbar spines was measured by dual energy X-ray absorptiometry (DEXA). BMD of group 1 was about 68% of the controls (p < 0.01), and that of group 2 was about 66% of the controls (p < 0.01). After treatment, group 1 and group 2 were administered the same stock diet ingested by the controls. Each group was divided into four sub-groups, and four different concentrations (0, 5, 10, 20 ppm) of sodium fluoride (NaF) solution were administered to the groups. BMD of each group was measured 6 times at monthly intervals after NaF administration. The BMD of group 2 increased significantly according to the concentration of NaF solution compared with group 1. Thus retention of F due to renal insufficiency was causing osteosclerosis. It was also evident that excretion of F was delayed by the partial nephrectomy in group 2, and the retention of F accelerated increase in bone density. Indexes of excretory function such as creatinine clearance and fluoride clearance decrease not only with the evidence of kidney disease but also with advancing age. Therefore, more attention to the dose and the side effects of drugs has to be paid for the treatment of postmenopausal osteoporosis, especially in the aged patients.

Department of Hygiene and Public Health, Osaka Medical College, 2-7 Daigaku-
machi, Takatsuki, Osaka, JAPAN.
RESEARCH ON CHRONIC EFFECTS OF OCCUPATIONAL EXPOSURE TO ORGANIC FLUORINE

G Z Zhang,1 Z Y Zhang,1 B C Xu,1 G F Sun2 and J K Guan2

Research on the effect of organic fluorine on the human body is limited. Here we report an epidemiological investigation on workers exposed to organic fluorine in a fluorine chemistry factory. Besides routine examinations, we also measured urine enzymes (NAG, ALP), pulmonary function, lipid peroxion (LPO), and thyroid function (T3, T4, TSH) in order to study whether there was chronic impairment from exposure to organic fluorine on workers and to look for sensitive indexes to monitor their health. The investigation showed: 1) Results of physical examination were as expected and included neurasthenic syndrome, chronic coughing, chest distress, short breath, asthenia and anorexia. 2) Urinary fluorine levels in the exposed group were higher than in the control group. Smoking increased the absorption of fluorine. The kind of work affected the level of urine fluorine. 3) The levels of urine NAG and ALP of fluorine workers were higher than those of control workers. This meant that kidney function of fluorine workers was impaired, but routine urine examinations of most workers were normal. Consequently, we felt that urine enzymes (NAG, ALP) could be used to monitor the health of workers exposed to organic fluorine. 4) The level of serum LPO in fluorine workers was higher than that in control workers and could barely be restored to normal if exposure was discontinued. 5) The thyroid function of the fluorine group decreased. The level of serum T3 and TSH was significantly different from those of control group. Decrease of T3 level might stimulate excretion of TSH. 6) Pulmonary function of the exposed group was lower than that of the control group. Abnormal rates of FEV1.0, FEV1.0/FEV, and FEF25-75% were more frequent. This indicated airway obstruction in fluorine workers.

In summary, from our investigation we conclude that chronic exposure to organic fluorine had adverse effect on the health of workers, not observed in usual clinical examinations but shown in subclinical biochemical measurement.

1 Research Department of Labor Health of Fuxin City, Liaoning Province, 2 Department of Preventive Medicine, China Medical University, Shenyang, CHINA.

EFFECTS OF ORGANIC FLUORINE EXPOSURE ON THE REPRODUCTIVE FUNCTION OF FEMALE WORKERS AND THE DEVELOPMENT OF THEIR OFFSPRING

Z Y Zhang,1 G Z Zhang,1 X J Liu,1 G F Sun2 and J K Guan2

Little is known about whether organic fluorine can affect the reproductive function of female workers and the development of their offspring. We made a retrospectively epidemiological investigation on menstruation, reproduction and development of the offspring of female workers exposed to organic fluorine in a fluorine chemistry factory. The results showed: 1) Occupational exposure to organic fluorine
induced abnormal menstruation including abnormal menstrual period and abnormal blood amount. 2) The occurrence of miscarriages, premature births and pregnancy complications among female fluorine workers was higher than among control workers.

The urine fluorine burden of female fluorine workers was higher. The mechanism by which organic fluorine affected reproductive function and offspring development is uncertain. Our results suggest that organic fluorine impaired embryonic cells, induced abnormal development of fetus, and gave rise to miscarriage and teratogenic effects. There were 3 hydrocrania cases among 8 dead fetuses. This indicated that embryos were affected during formation of the nervous system.

\[^{1}\text{Research Department of Labor Health of Fuxin City, Liaoning Province,}\]
\[^{2}\text{Department of Preventive Medicine, China Medical University, Shenyang, CHINA.}\]

STUDIES ON THE TREATMENT OF HYDROFLUORIC ACID SKIN BURNS

K Kono, \(^{1}\) Y Yoshida, \(^{1}\) M Watanabe, \(^{1}\) Y Tanioka, \(^{1}\) Y Orita, \(^{1}\) T Dote, \(^{1}\) Y Takahashi, \(^{1}\) Y Sumi \(^{1}\) and K Kiyokane \(^{2}\)

Hydrofluoric acid (HF) was applied to the skin of rats, and afterward biochemical observation of the tissues and sera was made to develop effective safety measures for an adequate method of emergency treatment for HF burns. The following four local treatments were studied: 1) After exposure to a 20% HF solution for 5 min, a lysozyme chloride ointment and antibiotic ointment were applied. 2) The afflicted area was flushed with running water for 10 min. 3) The burned area was immediately washed with running water, then lysozyme chloride ointment and gentamycin were administered several times a day. 4) After flushing with running water for 10 min, a 2.5% HFB-Jelly (calcium gluconate jelly) was applied several times a day. Flushing with running water was effective for HF burns. By applying HFB-jelly, concentrations of fluoride in the urine and the tissues surrounding the injured region were reduced. The results showed that irrigation with running water together with HFB-jelly applications was the most effective treatment for HF burns.

\[^{1}\text{Department of Hygiene and Public Health, \(^{2}\text{Department of Dermatology, Osaka Medical College, 2-7 Daigakumachi, Takatsuki, Osaka, JAPAN.}\}\]

EFFECTS OF FOOD INTAKE ON SERUM AND URINARY FLUORIDE CONCENTRATIONS AS AN INDICATOR OF OCCUPATIONAL FLUORIDE EXPOSURE

H Nagaie, Y Yoshida, K Kono, M Watanabe, Y Tanioka, Y Orita, T Dote, Y Takahashi, K Umebayashi and A Takasu

In exposure to environmental hydrofluoric acid (HF), the fluoride concentration in the urine and serum corresponds closely to average exposure. In this study, the effects of dietary fluoride intake, especially tea, on post-shift serum and urinary fluoride concentration among HF workers were investigated. Serum and urine samples from 142 HF workers who had been engaged in washing glass bulbs for TV picture tubes were studied. The time of tea intake before sampling the specimen
was checked by a questionnaire of the workers. Serum and urinary fluoride concentrations were measured by fluoride electrode methods. Mean fluoride concentrations in the green tea and black tea consumed in Japan were 0.52 to 2.77 ppm. Among healthy volunteers, within 1 hour after the intake of tea, serum and urinary fluoride concentrations increased considerably. Among HF workers, serum and urinary fluoride concentrations in the group that drank tea within 1 hour before sampling were significantly higher than in the non-tea-drinking group. The intake of tea and marine products having bones and shells, which are rich in fluoride, obviously affects fluoride levels in biological fluids. When judging relatively low concentrations of fluoride in the environment, it is important to eliminate the effect of fluoride-containing foodstuffs on fluoride in serum and urine as an indicator of HF exposure.

Department of Hygiene and Public Health, Osaka Medical College, 2-7 Daigakumachi, Takatsuki, Osaka, JAPAN.

EFFECTS OF ANTACID (CIMETIDINE) ON FLUORIDE ABSORPTION FROM THE GASTRO-INTESTINAL TRACT

Y Bessho, Y Yoshida, K Kono, M Watanabe, Y Tanioka, Y Orita, T Dote, J Yoshida and K Unebayashi

Fluoride is absorbed mainly from the stomach and duodenum in a short period of time. The luminal pH of the stomach is considered an important variable in the absorption of fluoride. In the present study, the changes in serum fluoride after the administration of black tea containing sodium fluoride (F: 5 mg) with or without Cimetidine (400 mg/person), a specific histamine H2-receptor antagonist, were investigated. Six healthy adult males who were studied were requested to limit their intake of foodstuffs such as tea and marine products, which contain large amounts of fluoride, from the day before the experiment. The serum fluoride concentration quickly increased after the intake of fluoride. It reached the maximum level after 30 min in both groups. In that time, the mean serum fluoride concentration was 0.169 ppm in the Cimetidine group and 0.209 ppm in the non-Cimetidine group, respectively. The results therefore indicate that the absorption of fluoride is inhibited by Cimetidine.

Department of Hygiene and Public Health, Osaka Medical College, 2-7 Daigakumachi, Takatsuki, Osaka, JAPAN.

FLUORIDE CONCENTRATION IN URINE AND SERUM AMONG INHABITANTS OF A FISHING VILLAGE

Y Tanioka, Y Yoshida, K Kono, M Watanabe, Y Bessho, T Dote, Y Orita, Y Takahashi and Y Tanimura

It is well known that tea, fish, seashells and other marine products contain fluoride in quantity. In a fishing village, we took fasting blood samples and urine specimens from 212 male and female inhabitants (aged 35 to 90). Serum fluoride concentration (SF) was examined in relation to age and time of year. Over time, hematological examination and chemical analysis of serum were investigated for any relation between SF and liver damage, diabetes mellitus, hyper-lipidemia, and so on.
The mean concentrations of serum and urinary fluoride were 15.66 ± 9.24 ppb and 0.69 ± 0.32 ppm, respectively. In serum, fluoride increased with age, and this is why renal function declined and excretion of fluoride decreased. No significant change of SF was found. SF in subjects over age 70 was higher than in a rural control area. In relation to blood tests, low SF in liver damage and high SF in hyperlipidemia were recognized. There was no difference in SF of other age groups between the fishing village and the rural area. We consider this to be the result of standardization of food intake brought about by traffic network and the food distribution system. The high SF among the elderly in the fishing village is apparently caused by long-term intake of marine products and diminution of fluoride excretion.

Department of Hygiene and Public Health, Osaka Medical College, 2-7 Daigakumachi, Takatsuki, Osaka, JAPAN.

URINARY FLUORIDE EXCRETION AFTER DIETARY INTAKE IN HEALTHY JAPANESE ADULTS

M Watanabe, Y Yoshida, K Kono, Y Tanioka, Y Orita, T Dote, Y Bessho, Y Takahashi and T Tagawa

This study was undertaken to analyze fluoride (F) concentrations in Japanese foodstuffs and daily diets and to measure urinary F excretion in healthy adults after dietary intake. F concentrations in the foodstuffs and urine were analyzed directly by the F ion-specific electrode method or its combination with the steam distillation method after ashing. The F concentration in boiled rice and noodles, which are the staple foods of most Japanese, was 0.33 and 0.57 ppm, respectively. The daily dietary F intake, exclusive of green tea, which contains a high content of F (1.5 ppm), ranged from 0.87 to 1.54 mg/day. The excretion ratio of F in the urine during a following 24-hour period was 33 to 44% in healthy adults. The results suggest that urinary F excretion depends mainly on the degree of digestion of each dietary item and the chemical nature of F in foodstuffs.

Department of Hygiene and Public Health, Osaka Medical College, 2-7 Daigakumachi, Takatsuki, Osaka, JAPAN.

EFFECTS OF LONG-TERM LOW-FLUORIDE INTAKE ON BONE IN GROWING, SENESCENCE-ACCELERATED MICE (SAM-P/6)

T Imai, Y Takeki and M Niwa

Despite continuing interest in the efficacy of fluoride as a therapeutic agent for osteoporosis, little information is available on its mechanism of action. The present studies were performed to examine long-term effects of low concentrations of sodium fluoride in drinking water on bone metabolism in growing, senescence-accelerated mice (SAM-P/6) as a spontaneous experimental model of senile osteoporosis. SAM-P/6 mice (obtained from Dr. T Takeda, Kyoto University) at 4 weeks of age were used. They were born and reared under SPF conditions, and maintained on a commercial diet (F2, Funabashi, containing 1.1% calcium, 0.88% phosphorus, 0.8μg/g fluoride) and water, ad libitum. The water supply of the controls was tap water (less than 0.05 mg/L fluoride), while that of treated mice was 1.5 mg/L NaF solution. After 11 months, serum was obtained and the femur excised. Calcium and
magnesium were measured by atomic absorption spectrophotometry. Phosphorus and alkaline phosphatase levels were evaluated colorimetrically. Fluoride was measured with a fluoride electrode. Femurs were fixed in neutral formaldehyde, decalcified, and embedded in paraffin. Coronary and tranverse sections were made for the epiphyseal portion and the shaft, respectively. Hematoxylin and eosin stain was used for decalcified sections. Body weight was not significantly different between any groups with or without treatment with sodium fluoride. There were no differences in serum levels of calcium, fluoride and alkaline phosphatase between the controls and the fluoride groups, although serum levels of phosphorus were higher in the fluoride groups than in the controls. Calcium and fluoride content per dry weight of femoral bone were higher than in the controls in the two groups of mice treated with sodium fluoride. Bone magnesium and phosphorus contents did not differ between the control and fluoride groups. Histologic studies of the femurs revealed that bone formation tended to increase in the fluoride groups. The results of this study suggest that in growing SAM-P/6 mice, long-term, low-concentration sodium fluoride intake affects skeletal metabolism.

The Nippon Dental University, Tokyo, JAPAN.

DEPENDENCE OF CELL FLUORIDE SENSITIVITY TO THE PERIOD OF THE CELL CYCLE

T Sato and M Niwa

Significant differences in fluoride sensitivity have been observed between cell types. Previously, we have reported that the sensitivity of cultured cells to fluoride is likely to be related to their rate of growth. The purpose of this study was to use synchronized cultures to clarify the mechanism responsible for differences in sensitivity to fluoride between cells. Fluoride sensitivity of cells during different phases of the cell cycle was examined by means of four human diploid fibroblasts:

1) NDU-1 cells (fetal lung),
2) FR 30 cells (fluoride-resistant cells produced from NDU-1 cells),
3) GF-12-Y cells [adult gingiva, population doubling level (PDL): 10-15], and
4) GF-12-E cells (PDL: 30-35).

The sensitivity of the cells to fluoride was estimated by measurement of the fluoride concentration necessary to inhibit DNA synthesis and/or cell growth. The lethal effect of fluoride on cells was also determined.

The sensitivity to fluoride was measured in both nonsynchronized and synchronized (G1/G0 phase and S/G2 phase) cultures. In non-synchronized cultures, the sensitivity of cells in decreasing order was NDU-1 > GF-12-Y >GF-12-E, FR 30 cells. DNA synthesis of synchronized cells at the S/G2 phase was depressed to about 50% of control cells for all four cell types when cultured with 0.79 mM fluoride for 8 hr (time of S phase). A lethal fluoride concentration was not observed in the cells in the G1/G0 phase.

These results indicate that the fluoride sensitivity of cells is dependent on the phase of the cell cycle. The different sensitivities to fluoride that have been observed between nonsynchronized cells is likely to be related to the fact that the cells were in different phases of the cell cycle.

The Nippon Dental University, Tokyo, JAPAN.
EPIDEMIOLOGICAL STUDY ON THE PREVALENCE OF DENTAL FLUOROSIS IN CHILDREN AFTER DRINKING WATER IMPROVEMENT AND DEFLUORIDATION

S Weibin and W Xuesong

The authors investigated and epidemiologically analyzed the prevalence of dental fluorosis in 4192 children, aged 8-12 years, living in 55 slightly, moderately, and seriously affected villages in 15 representative communities after 5-10 years of water improvements in Shandong. The results indicated that both alteration of water resources and defluoridation of drinking water were reliable and accessible measures for endemic fluorosis control, provided the fluoride level of daily drinking water was less than 1.0 mg/L. The reduction in the prevalence rate of dental fluorosis was closely related to duration of the water supply improvement process. Such prevalence rates in children aged 8 years could be reduced to a normal level after six years of improvements. The older the child, the later was the appearance of the normal level of dental fluorosis. This direct relationship provided an empirical basis for an accurate and prompt examination of the effectiveness of measures for water supply improvements. Thus, the reduced rate of dental fluorosis among 8-year-old children in these areas may be regarded as an early indication of a preventive effect. The durations of water supply improvements resulting in reduction to normal levels of dental fluorosis in slightly and moderately (as well as seriously) affected areas were 5 and 6-7 years, respectively. The stipulation of a fluoride level in drinking water of less than 1.0 mg/L and a prevalence rate of less than 30% where control measures were obtained is considered to be reasonable.

Shandong Institute of Endemics Prevention and Control, Jinan 250014, CHINA.

STUDY ON THE COMBINED EFFECT OF FLUORIDE AND SULPHUR DIOXIDE FROM INDOOR COAL BURNING ON EXPERIMENTAL ANIMALS THROUGH NATURAL INHALATION

C K Liang et al

The actual exposure patterns among peasants using burning pits and stoves in fluorosis prevalent areas were duplicated in experiments carried out in three groups of Sprague-Dawley exposed as follows:

1) High F (0.631 mg/m³) and high SO₂ (40.97 mg/m³)
2) High F (0.583 mg/m³) and low SO₂ (5.086 mg/m³)
3) Low F (0.008 mg/m³) and low SO₂ (0.191 mg/m³), as a control group.

Each group of animals was exposed to the above three conditions separately for 5 months and fed food and water with low fluoride content. The results showed that dental fluorosis was induced among the rats exposed in the first two groups. The high F and high SO₂ group was more severely effected than the high F and low SO₂ group. The dental tissue of the rats in the control group was normal.

Institute of Environmental Health and Engineering, Chinese Academy of Preventive Medicine, 29 Nan Wei Road, Beijing 100050, CHINA.
The following are abstracts of recently published research.

PREVALENCE OF RESPIRATORY DISORDERS AMONG ALUMINIUM POTROOM WORKERS IN RELATION TO EXPOSURE TO FLUORIDE

Vidar Søyseth and Johny Kongerud
Oslo, Norway

Abstract from British Journal of Industrial Medicine 49 125-130 1992

In a survey of 370 aluminium potroom workers in western Norway, bronchial responsiveness, lung function, and respiratory symptoms were studied in relation to occupational exposure to air contaminants in the potroom. Increased prevalences of respiratory symptoms, work related asthmatic symptoms, and abnormal lung function were found in subjects exposed to total fluorides above 0.5 mg/m³. No significant association between bronchial responsiveness and exposure to fluoride was found and the prevalence of respiratory symptoms was independent of the degree of dust exposure. These findings indicate that work related asthmatic symptoms in potroom workers may be related to exposure to fluorides.

Key words: Aluminium potroom workers; Respiratory disorders.
Reprints: Dr J Kongerud, Department of Thoracic Medicine, Rikshospitalet, University of Oslo, N-0027 Oslo 1, Norway.

LOW CONCENTRATIONS OF SODIUM FLUORIDE INHIBIT Ca²⁺ INFLUX INDUCED BY RECEPTOR-MEDIATED PLATELET ACTIVATION

Y Ozaki, K Satoh, Y Yatomi and S Kume
Yamanashi, Japan

Abstract from Biochimica et Biophysica Acta 1147 (1) 27-34 1993

Sodium fluoride (NaF) alone below the concentration of 10 mM had no effect on platelet intracellular Ca²⁺([Ca²⁺]i). When platelets were incubated with low concentrations of NaF (< 10 mM) prior to thrombin stimulation, the second phase of [Ca²⁺]i elevation which is attributable to Ca²⁺ influx was suppressed, while the initial rapid peak of [Ca²⁺]i which is attributable to internal Ca²⁺ release was unaffected. Ca²⁺ influx assessed by the addition of extracellular Ca²⁺ to cells preactivated by thrombin in the absence of extracellular Ca²⁺ was also inhibited by NaF in a dose-dependent manner. NaF was also effective in inhibiting thrombin- or U-46619-induced Mn²⁺ entry. This inhibitory effect of NaF on Ca²⁺ influx occurred after a lag of at least 30 s. However, Ca²⁺ influx induced by ionomycin-induced Ca²⁺ depletion or by thapsigargin, a Ca²⁺-ATPase inhibitor, was only partially suppressed by NaF treatment. It is suggested that Ca²⁺ entry induced by receptor-mediated activation is NaF-sensitive and that the depletion of Ca²⁺ storage sites by artificial procedures facilitates the opening of Ca²⁺ channels via NaF-insensitive pathways.

Key words: Calcium ion influx; Receptor-mediated platelet activation; Sodium fluoride.
Reprints: Y Ozaki, Yamanashi Medical College, Shimokato 1110, Nakakoma, Yamanashi 40938, Japan.
THROMBIN AND NaF, BUT NOT EPINEPHRINE, RAISE CYTOSOLIC FREE Na+ IN HUMAN PLATELETS

V Stamouli, C Vakirtzilemonias and W Siffert
Frankfurt, Germany

Abstract from Biochimica et Biophysica Acta 1176 (3) 215-221 1993

We have investigated changes in [Na+]i in SBFI-loaded platelets stimulated at 37°C with thrombin, epinephrine, and NaF. Basal [Na+]i was 4.9 ± 1.3 mM (n = 70). Stimulation of platelets with thrombin (0.1 U/ml) in the presence of 1 mM extra-cellular Ca2+ rapidly raised [Na+]i by 27.3 ± 6 mM (n = 16). Part of this increase (approx. 20-30%) is caused by Na+/H+ exchange, the rest is predominantly due to Na+ influx. Epinephrine (20 μM) failed to change [Na+]i both in the absence and presence of fibrinogen. This is in agreement with earlier reports showing that epinephrine also fails to activate Na+/H+ exchange in human platelets. NaF which activates platelets via a direct effect on GTP-binding proteins induced a slow rise in [Na+]i to 9.5 ± 2.5 mM (n = 4) and 33.0 ± 3.6 mM (n = 12) at 10 and 20 mM NaF, respectively. This effect was completely blocked by SK&F 96365, a blocker of receptor-mediated Ca2+ entry. Hence, the NaF-induced increase in [Na+]i is exclusively due to the opening of non-selective cation channels. This latter finding agrees with earlier observations which showed that NaF does not induce activation of Na+/H+ exchange in platelets.

Key words: Epinephrine; Hypertension; Platelet; Sodium; Sodium ion-proton exchange; Thrombin.
Reprints: W Siffert, Max Planck Institute of Biophysics, Kennedyallee 70, W-6000 Frankfurt 70, Germany.

THE INFLUENCE OF FLUORIDE ON PROTEOGLYCAN STRUCTURE USING A RAT ODONTOBLAST INVITRO SYSTEM

R J Waddington, G Embery and R C Hall
Cardiff, Wales

Abstract from Calcified Tissue International 52 392-398 1993

Using an in vitro rat incisor odontoblast system, the effect of fluoride on proteoglycans was investigated at both the metabolic and structural level. Incisors were removed from 4-week-old rats, split longitudinally, and the pulps removed. Teeth were incubated at 37-degrees-C, 5% CO2 in Eagle's Minimum Essential Medium containing S-35-sulfate for 7 hours in the presence of 0 mM, 3 mM, or 6 mM sodium fluoride. Teeth were demineralized in EDTA, proteoglycan was extracted from the residue with 4 M guanidinium chloride, and further purified by anion exchange chromatography. Uptake of radiolabel was monitored by liquid scintillation counting. The resultant products were examined by cellulose acetate electrophoresis, SDS-PAGE, chondroitinase digestion, and amino acid analysis. Differential effects of
fluoride were observed in both metabolism and biochemical characterization of proteoglycans following incubation at the two concentrations. Fluoride decreased uptake of the radiolabel but led to an accumulation of glycosaminoglycan within the proteoglycan of the matrix. Chondroitin sulfate was the predominant glycosaminoglycan identified, with the additional presence of dermatan sulfate and heparan sulfate identified. Dermatan sulfate levels increased in 3 mM-treated teeth. Fluoridetreated proteoglycans had a reduced molecular weight (200-90K to 180-79K), this reduction is primarily a result of smaller glycosaminoglycan chains, with limited reduction in the size of the core protein of 6 mM-treated teeth occurring. Such alterations in the biochemical metabolism and hence structure and function of proteoglycan may be implicated in the hypomineralization seen in fluorosis.

Key words: Fluoride; Odontoblasts; Proteoglycans.
Reprints: R J Waddington, School of Dentistry, University of Wales Cardiff College of Medicine, Heath Park, Cardiff CF4 4XY, Wales.

DENTAL FLUOROSIS, DENTAL CARIES AND FLUORIDE EXPOSURE AMONG 7-YEAR-OLDS

P J Riordan
Como, West Australia.

Abstract from Caries Research 27 71-77 1993

Mild dental fluorosis is frequently linked to fluoridated water, but discretionary fluoride sources may also be important. The aim of this study was to record age of weaning and fluoride exposure from water, toothpaste and supplements, and to relate these to the presence of caries and fluorosis in children born in 1983. In Perth (Western Australia) 14 school classes were selected. The 350 children (mean age 7.5 years) ultimately included gave fluoride exposure data for the period birth to 4 years of age. Caries (DMFT, WHO criteria, no radiographs) and dental fluorosis (TF index, dry permanent incisors) were registered clinically. Most (89%) children had lived at least 2.5 years in a fluoridated area. Supplement use was minimal and unrelated to caries or fluorosis. Mean age of weaning of those who had been breastfed was 7.7 months; by 9 months, 74% had been weaned. Eighty-five percent liked toothpaste, 60.7% had swallowed it, and the mean age of starting to use it was 1.5 (SD 0.96) years. Caries prevalence was 0.1 and mean DMFT was 0.13. The prevalence of fluorosis was 0.48; 63% of fluorosis was TF score 1. Residence in a fluoridated area for greater-than-or-equal-to 2.5 of the first 4 years of life had an odds ratio (OR) of 4.9 for fluorosis. Weaning before 9 months of age, swallowing toothpaste and liking toothpaste were also statistically significant risk factors. Major risk factors for more severe fluorosis (TF greater-than-or-equal-to 2) were early weaning and swallowing toothpaste (ORs 2.77 and 2.64, respectively). Residence in a fluoridated area (OR 2.2) was not a statistically significant risk factor. These findings confirm a high prevalence of mild fluorosis among children who have been
exposed to fluoride in their earliest years. High attributable proportions associated with age of weaning and toothpaste use suggest that elimination of fluoride from formula and a reduced fluoride concentration in toothpaste would contribute significantly to reducing the prevalence of more visible fluorosis.

Key words: Epidemiology; Infant formula; TF Index; Toothpaste.
Reprints: P J Riordan, Health Department of Western Australia, Community Dental Service, POB 50, Como, WA 6152, Australia.

MEASUREMENT OF INTRINSIC BONE QUALITY
IN VIVO BY REFLECTION ULTRASOUND
CORRECTION OF IMPAIRED QUALITY WITH SLOW-
RELEASE SODIUM FLUORIDE AND CALCIUM CITRATE

P P Antich, C Y C Pak, J Gonzales, J Anderson, K Sakhaee and C Rubin
Dallas, Texas, USA

Abstract from Journal of Bone and Mineral Research 8 301-311 1993

The intrinsic (material) quality of cancellous and cortical bone was evaluated in vivo from the measurement of reflection ultrasound velocities in the ulna. In cancellous bone, the reflection ultrasound velocity was inversely correlated with age in normal women \( r = -0.48, p = 0.001 \), with a significantly lower mean value in 32 normal postmenopausal women than in 14 premenopausal women (3124 versus 3341 m/s, \( p < 0.0001 \)). In 32 untreated osteoporotic women the cancellous bone velocity was lower than in normal postmenopausal subjects (2906 versus 3124 m/s, \( p = 0.0001 \)). Following treatment with slow-release sodium fluoride plus calcium citrate (mean 2.4 years in 33 osteoporotic patients with no fracture during treatment), the cancellous bone velocity was significantly higher than in untreated osteoporotic women (3082 versus 2906 m/s, \( p = 0.0002 \)) and was not significantly different from that in normal postmenopausal women. The cortical bone velocity displayed similar trends, but the changes did not attain statistical significance. The measurements were repeated approximately 9 months later in 9 untreated and in 20 treated patients; in 5 additional patients, the measurements were made both before and after 9 months of treatment with slow-release sodium fluoride and calcium citrate. The cancellous bone velocity increased significantly \( (p = 0.046) \) in these patients, from 3008 m/s before treatment to 3112 m/s after the first 9 months of treatment. The velocity rose significantly from 3037 to 3167 m/s \( (p = 0.017) \) in patients treated for a short time (12-30 months at first measurement), but it did not change in untreated patients or those treated for more than 30 months. Thus, the material quality of cancellous bone decreases with normal aging and is reduced further with the osteoporotic process. This impaired quality may be corrected by treatment with slow-release sodium fluoride plus calcium citrate.

Key words: Fracture rate; Postmenopausal osteoporosis.
Reprints: P P Antich, Department of Radiology, SW Medical Center, University of Texas, Dallas, TX 75235, USA.
DISTRIBUTION OF FLUORIDE IN CORTICAL BONE OF HUMAN RIB

Nagoya, Japan and Leeds, England

Abstract from Calcified Tissue International 52 278-282 1993

We describe a detailed study of fluoride distribution with age in the human cortical rib bone. Human ribs were obtained from 110 subjects (M:68, F:42) aged 20-93 years. The fluoride distribution from the periosteal to endosteal surfaces of the ribs was determined by sampling each specimen using an abrasive micro-sampling technique, and the samples were analyzed using the fluoride electrode, as described by Weatherell et al. The concentration of fluoride was highest in the periosteal region, decreased gradually towards the interior of the tissue where the concentration of fluoride tended toward the plateau, and then rose again towards the endosteal surface. Patterns of fluoride distribution changed with age, and the difference between periosteal and endosteal fluoride levels increased with age. Although average fluoride concentrations increased with age in both sexes, there was a significant difference between males and females at the age of about 55 years (P < 0.05).

Key words: Aging; Bone; Fluoride; Human; Sex.
Reprints: K Ishiguro, School of Dentistry, Aichi Gakuin University, 1-100 Kusumoto cho, Chikusa ku, Nagoya 464, Japan.

EFFECT OF FLUORIDE ON THE ATP REQUIREMENT FOR GLYCEROLIPID BIOSYNTHESIS IN ADIPOSE TISSUE OF 4 MAMMALIAN SPECIES

D C Rule
Laramie, Wyoming, USA

Abstract from Comparative Biochemistry and Physiology B - Comparative Biochemistry 104 (3) 469-473 1993

1. Maximal glycerolipid biosynthesis activity was determined with 700 g homogenates of human, ovine, rat and bovine adipose tissue, and in the presence of varied concentrations of ATP and fluoride (as KF).
2. Minimal responses to either ATP or KF were observed with human homogenates.
3. Increasing ATP, from 4 to 20 mM, markedly increased glycerolipid biosynthesis in ovine and rat homogenates, but not in those of the bovine; similar responses were observed with 4 mM ATP plus 12.5 mM KF for rat and ovine.
4. Results indicated that KF inhibited ATP degradation in adipose tissue homogenates and that the severity of ATP degradation differed with species.

Key words: Brain; Invitro; Muscle; Phosphatase; Purification.
Reprints: D C Rule, Department of Animal Science, University of Wyoming, Laramie WY 82071, USA.
CORRELATION AND CAUSE: AN ISSUE IN THE EPIDEMIOLOGY OF FLUOROSIS

John Colquhoun
Auckland, New Zealand

Abstract from Trace Elements: Roles, Risks and Remedies
Proceedings of the New Zealand Trace Elements Group Conference
Massey University, Palmerston North 1992

The "ecological fallacy" has been invoked to caution researchers against attributing a cause after discovering correlations between drinking water fluoridation and the incidence of hip fractures. However, the same caution has not been observed when attributing a benefit to teeth from fluoride intake. This paper compares the strength of these reported associations, as well as the evidence for a causative link between fluoride and bone fragility, and between fluoride and tooth decay.

Key words: Bone fragility; Epidemiology; Fluoridation; Hip fracture; Osteoporosis; Tooth decay.
Reprints: Dr J Colquhoun, 216 Atkinson Road, Titirangi, Auckland, New Zealand.

INHIBITORY EFFECT OF FLUORIDE ON INSULIN RECEPTOR AUTOPHOSPHORYLATION AND TYROSINE KINASE ACTIVITY

F Vinals, X Testar, M Palacin and A Zorzano
Barcelona, Spain

Abstract from Biochemical Journal 291 (2) 615-622 1993

Fluoride is a nucleophilic reagent which has been reported to inhibit a variety of different enzymes such as esterases, asymmetrical hydrolases and phosphatases. In this report, we demonstrate that fluoride inhibits tyrosine kinase activity of insulin receptors partially purified from rat skeletal muscle and human placenta. Fluoride inhibited in a similar dose-dependent manner both beta-subunit autophosphorylation and tyrosine kinase activity for exogenous substrates. This inhibitory effect of fluoride was not due to the formation of complexes with aluminium and took place in the absence of modifications of insulin-binding properties of the insulin receptor. Fluoride did not compete with the binding site for ATP or Mn2+. Fluoride also inhibited the autophosphorylation and tyrosine kinase activity of receptors for insulin-like growth factor I from human placenta. Addition of fluoride to the pre-phosphorylated insulin receptor produced a slow (time range of minutes) inhibition of receptor kinase activity. Furthermore, fluoride inhibited tyrosine kinase activity in the absence of changes in the phosphorylation of pre-phosphorylated insulin receptors, and the sensitivity to fluoride was similar to the sensitivity of the unphosphorylated insulin receptor. The effect of fluoride on tyrosine kinase activity was markedly decreased when insulin receptors were preincubated with the copolymer of glutamate/tyrosine. Prior exposure of receptors to free tyrosine or phosphotyrosine also prevented the inhibitory effect of fluoride. However, the protective effect of tyrosine or phosphotyrosine was maximal at low concentrations, suggesting the interaction of these compounds with the receptor itself rather than with fluoride. These data suggest: (i) that fluoride interacts directly and slowly with the insulin
receptor, which causes inhibition of its phosphotransferase activity; (ii) that the binding site of fluoride is not structurally modified by receptor phosphorylation; and
(iii) based on the fact that fluoride inhibits phosphotransferase activity in the absence of alterations in the binding of ATP, Mn$^{2+}$ or insulin, we speculate that fluoride binding might affect the transfer of phosphate from ATP to the tyrosine residues of the beta-subunit of the insulin receptor and to the tyrosine residues of exogenous substrates.

Key words: Alpha-subunit; Beta-subunit; Binding region; Growth factor-I; Human-placenta; Monoclonal-antibody; Phosphorylation; Protein-kinase.

Reprints: A Zorzano, Department of Biochemistry and Physiology, Biology Faculty, University of Barcelona, Avda Diagonal 645, E-08028 Barcelona, Spain.

Letters to the Editor:

NON-SKELETAL FLUOROSIS: COMMENT ON SURVEY

The recent paper by Professor A K Susheela and her colleagues gives the important result that, in an endemic skeletal fluorosis zone, non-skeletal manifestations such as gastro-intestinal complaints are common (1). It seems to me that the unpublished raw data already collected might also provide the basis for a valuable sequel by the same authors.

I understand that in many Indian villages, families tend to drink from the same wells for a generation or more. If this is true for the four villages studied by Susheela et al, the existing data might allow histograms to be produced of the prevalence of dental fluorosis, skeletal fluorosis and non-skeletal manifestations as functions of actual fluoride concentration in drinking water, in intervals of (say) 0.5 ppm. This would offer a more accurate picture of dose-response relationships than can be derived from Figure 1, which gives only the prevalences of the diseases by village. Since three of the four villages have fluoride concentrations varying by a factor of 18 or more between water supplies, the average fluoride concentration in a village is unlikely to be a meaningful variable.

Mark Diesendorf PhD
PO Box 48, O'Connor
ACT 2601, Australia

Reference

WATER FLUORIDE AND GOITRE

The high prevalence of goitre in areas in which fluoride is present in high quantities has long been known. Kaj Roholm (1937), in his extensive review, reported on animal experiments. Maumenee in 1854 had reported that fluoride fed to dogs (10 g in all over 4 months) produced a "struma like swelling of the throat", while Pighini in 1923 had reported a similar result on white rats (2-3 mg daily for 6-8 months). Roholm also drew attention to a 1932 report: "that in rats, the drinking water at Utrecht (Holland) can cause struma, and also dental changes resembling mottled enamel. This dental disease is said to occur endemically round Utrecht."

Rose and Marier (1977), in their review for the National Research Council of Canada, noted that increased prevalence of goitre had been reported in areas of endemic hydrofluorosis (Day and Powell-Jackson 1972, Teotia and Teotia 1975). Rose and Marier also drew attention to a Medical Research Council (UK) report that: "in some areas, even moderate concentrations of fluoride could block iodine absorption. It is known that iodine concentrations are lower in soft than in hard waters .... If fluoride is added to soft waters .... a proportion of the population may come to have suboptimal iodine intake. The effects might be subtle and slow to develop, and would certainly not be picked up by the crude screening used at present" (Crawford 1972). This relationship between the softness of water and adverse effects was dealt with by Jolly et al (1968) in their much-cited paper.

Waldbott et al (1978) also reviewed the evidence linking fluoride with goitre production. Waldbott reported a case of his own in which malfunction of the thyroid was cleared after cessation of exposure to fluoridated water.

An examination of frequently used American textbooks shows no indication that fluoride needs to be considered in the diagnosis of goitre. They illustrate of the omission of fluoride as anything other than a substance that is good for teeth.

Richard G Foulkes BA MD
Box 278, Abbotsford
BC V2S 4N9
Canada

References

1135-1138.
Jolly SS, Singh BM, Mathur OC, Malhotra KC (1968). Epidemiological, clinical and
biochemical study of endemic dental and skeletal fluorosis in Punjab. British Medical
Canada, Ottawa.
Teotia SPS, Teotia M (1975). Dental fluorosis in areas with a high natural content of
calcium and magnesium in drinking water - an epidemiological study. Fluoride 8 (1) 34-38.
Coronado Press, Lawrence, Kansas.
FLUORIDE, official journal of the International Society for Fluoride Research (ISFR), publishes quarterly reports on biological, chemical, ecological, industrial, toxicological and clinical aspects of inorganic and organic fluoride compounds. The International Standard Serial Number (ISSN) is 0015-4725.

SUBSCRIPTION: US$50 (or equivalent) per year in advance. Send to the Treasurer, ISFR, 216 Atkinson Road, Titirangi, Auckland 7, New Zealand.

MANUSCRIPTS, including papers presented at ISFR conferences, are accepted for publication after appropriate evaluation and recommendation by qualified reviewers. Send to Editor, Fluoride, 216 Atkinson Road, Titirangi, Auckland 7, New Zealand.

INSTRUCTIONS TO AUTHORS

1. General. The submitted paper, with a copy, should be written concisely in English. Either American or British spelling is accepted. Double space with generous margins. Measures should be in metric system.

2. Title. A concise but informative title should be followed by the name(s) of the author(s). The address where the research was carried out should appear at the bottom of the first page.


4. Key words. List the major themes or subjects.

5. Introduction. State the reason for the work with a brief review of previous work on the subject.

6. Materials and Methods. Condense. However, if the methodology is new or developed by the author(s) it can be more detailed.

7. Results. List the direct conclusions of the work.

8. Discussion. Deal with general conclusions, referring to other work on the subject. In short papers Results and Discussion may be combined.

9. Abbreviations or Acronyms. Define, either in brackets or in footnotes, when they first appear.

10. Acknowledgements. Keep brief. They may include funding source, technical assistance, text editing and useful comments.

11. References. See current issues of journal for usual style, which identifies references by bracketed numbers in the order in which the references first occur. Other styles may be accepted, provided they are accurate and consistent.

MEMBERSHIP. Researchers are invited to join ISFR. Applications for membership should be sent to the Secretary, Professor Gene W Miller, Biology Department, Utah State University, Logan, Utah 84322-5305, USA. Application forms are available from either the Secretary or Treasurer.