

Volume 31
Number 1
Pages 1-58
February 1998

FLUORIDE
JOURNAL
of the
International Society
for
Fluoride Research



Photo by Fabian Bachrach

WALDBOTT
COMMEMORATION
ISSUE

THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

President:

Dr Miklos Bély, National Institute of Rheumatology, Budapest, Hungary

Vice President:

Prof N B K Yoshitake, Shiga University of Medical Science, Shiga-Ken, Japan

Secretary:

Prof Gene W Miller, Biology Dept., Utah State University, Logan UT 84322-5305, USA

Treasurer:

Dr John Colquhoun, 81A Landscape Road, Mt Eden, Auckland 1004, New Zealand

Editorial Board:

Dr D J Ballentyne, University of Victoria, Victoria BC, Canada

Dr Miklos Bély, National Institute of Rheumatology, Budapest, Hungary

Prof A W Burgstahler, University of Kansas, Lawrence KS, USA (Editor)

Prof Shouren Cao, Chinese Academy of Preventive Medicine, Beijing, China

Dr M Chikuma, Osaka University of Pharmaceutical Sciences, Japan

Dr John Colquhoun, School of Education, University of Auckland, New Zealand (Editor)

Dr John A Cooke, University of Natal, Durban, South Africa

Dr Edward Czerwinski, Krakow Academy of Medicine, Poland

Prof Mark Diesendorf, University of Technology, Sydney, NSW, Australia

Prof G Embery, University of Wales Medical College, Cardiff, Wales UK

Dr Richard G Foulkes, Abbotsford BC, Canada

Prof J Franke, Heinrich Mann Hospital, Bad Liebenstein, Germany

Prof G Neil Jenkins, Newcastle upon Tyne, England

Prof Rongdi Ji, Chinese Academy of Preventive Medicine, Beijing, China

Dr Y Kaneko, Showa University School of Dentistry, Tokyo, Japan

Prof K Kono, Osaka Medical College, Osaka, Japan

Prof Jerzy Krechniak, Medical University, Gdansk, Poland

Dr K A V R Krishnamachari, National Institute of Nutrition Hyderabad, India

Prof Lennart Krook, Cornell University, Ithaca NY, USA

Dr John R Lee, 9620 Bodega Hwy, Sebastopol CA, USA

Prof C James Lovelace, Humboldt State University, Arcata CA, USA

Dr Zygmunt Machoy, Pomeranian Medical Academy, Szczecin, Poland

Prof G W Miller, Utah State University, Logan UT, USA (Co-Editor)

Prof F Murray, Murdoch University, Murdoch WA, Australia

Dr James C Pushnik, California State University, Chico CA, USA

Dr B P Rajan, Madras Dental College, Madras, India

Dr Bruce Spittle, University of Otago Medical School, Dunedin, New Zealand (Co-Editor)

Dr Jörg Spitz, Dept. of Nuclear Medicine, Wiesbaden, Germany

Prof Guifan Sun, China Medical University, Shenyang, China

Prof A K Susheela, All India Institute of Medical Sciences, New Delhi, India

Prof S P S Teotia, LLRM Medical College, Meerut, India

Prof H Tsunoda, Iwate Medical University, Morioka, Japan

Prof Zan-Dao Wei, Guiyang Medical College, Guizhou, China

Dr Sally Wheeler, Hawkesbury Agricultural Research Unit, Richmond NSW, Australia

Prof Y Yoshida, Osaka Medical College, Osaka, Japan

Prof N B K Yoshitake, Shiga University of Medical Science, Shiga-Ken, Japan

Prof Ming-Ho Yu, Western Washington University, Bellingham WA, USA (Co-Editor)

FLUORIDE

QUARTERLY JOURNAL OF THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

Managing Editor:
Dr John Colquhoun
Auckland, New Zealand

Scientific Editor:
Prof A W Burgstahler
Lawrence, Kansas, USA

Co -Editors:
Prof G W Miller Logan, Utah, USA
Dr Bruce Spittle Dunedin, New Zealand
Prof Ming-Ho Yu Bellingham, Washington, USA

CONTENTS

WALDBOTT COMMEMORATION

| | |
|---|------|
| EDITORIAL: CENTENNIAL COMMEMORATION John Colquhoun | 1 |
| GEORGE L WALDBOTT – A PRE-EMINENT LEADER IN FLUORIDE RESEARCH A W Burgstahler, USA | 2-4 |
| MEMORIES OF GEORGE L WALDBOTT – AN EXTRAORDINARY PHYSICIAN John R Lee, USA | 4-6 |
| THE FLUORIDE RESEARCH LEGACY OF GEORGE L WALDBOTT Gene W Miller, USA | 6-7 |
| GEORGE L WALDBOTT – A TRULY GREAT PHYSICIAN OF OUR CENTURY Hans C Moolenburgh, The Netherlands | 7-8 |
| MY FATHER'S DEDICATION TO FLUORIDE RESEARCH Elizabeth Ramsay, Israel | 8-9 |
| GEORGE L WALDBOTT – AN EXCEPTIONAL PHYSICIAN A K Susheela, India | 9-12 |
| ENVIRONMENTAL TOXICOLOGY – WHAT I OWE TO DR WALDBOTT Ming-Ho Yu, USA | 12 |

SPECIAL COMMEMORATION RESEARCH REPORT

| | |
|--|-------|
| THE PRESKELETAL PHASE OF CHRONIC FLUORIDE INTOXICATION George L Waldbott, USA | 13-20 |
| FLUORIDE PUBLICATIONS OF GEORGE L WALDBOTT, MD | 21-25 |

RESEARCH REPORTS AND REVIEWS

| | |
|---|-------|
| TOXIC EFFECTS OF FLUORIDE ON BEATING MYOCARDIAL CELLS CULTURED <i>IN VITRO</i> F Y Wang, D X Chang and R M Wang, China | 26-32 |
| FLUORIDE INTAKE, DISTRIBUTION, AND BONE CONTENT IN DIABETIC RATS CONSUMING FLUORIDATED DRINKING WATER I Boros, P Keszler, Gy Cskós and H Kalás, Hungary | 33-42 |
| INTERACTIONS BETWEEN FLUORIDE AND BIOLOGICAL FREE RADICAL REACTIONS R Rzeuski, D Chlubek and Z Machoy, Poland | 43-45 |

ANNOUNCEMENT XXIInd ISFR Conference, Bellingham, USA, August 24-27 46

Continued next page

ABSTRACTS**GASTRODUODENAL MANIFESTATIONS IN PATIENTS
WITH SKELETAL FLUOROSIS.**

S Dasarathy, T K Das, I P Gupta, A K Susheela and R K Tandon, India 47

**ACUTE FLUORIDE TOXICITY FROM INGESTING HOME-USE DENTAL
PRODUCTS IN CHILDREN, BIRTH TO 6 YEARS OF AGE**

J D Shulman and L M Wells, USA 47-48

**OUTBREAK OF ACUTE FLUORIDE POISONING CAUSED
BY A FLUORIDE OVERFEED, MISSISSIPPI, 1993**

A D Penman, B T Brackin and R Embrey, USA 48-49

**TESTING THE POTENTIAL OF SODIUM FLUORIDE
TO AFFECT SPERMATOGENESIS IN THE RAT**R L Sprando, T F X Collins, T N Black,
J Rorie, M J Ames and M O'Donnell, USA 49**CHROMOSOMAL ABERRATIONS AND MICRONUCLEI IN LYMPHOCYTES
OF WORKERS AT A PHOSPHATE FERTILIZER FACTORY**

Z Q Meng and B Zhang, China 50

**EFFECTS OF FLUORIDE ON STRUCTURE AND
FUNCTION OF CANINE GASTRIC MUCOSA**

G M Whitford, D H Pashley and R H Garman, USA 50

**ARTHROPODS AS INDICATORS OF THE EFFECTS OF FLUORIDE
POLLUTION ON THE SUCCESSION FOLLOWING SAND MINING**

K E Madden and B J Fox, Australia 51

**UPTAKE OF FLUORIDE INTO DEVELOPING SHEEP TEETH,
FOLLOWING THE 1995 VOLCANIC ERUPTION
OF MOUNT RUAPEHU, NEW ZEALAND**

G E Coote, T W Cutress and G W Suckling, New Zealand 51-52

**PREVENTION OF INDUSTRIALLY-INDUCED
CATTLE AND SHEEP FLUOROSIS**

G Milhaud and H J Van Weering, France 52

SULFURYL FLUORIDE

Caroline Cox, USA 53

**EFFECT OF DRINKING WATER FLUORIDATION
ON THE PREVALENCE OF OTOSCLEROSIS**

E Vartiainen and T Vartiainen, Finland 53

**RISE AND FALL OF CARIES PREVALENCE IN GERMAN TOWNS
WITH DIFFERENT F CONCENTRATIONS IN DRINKING WATER**

W Kunzel and T Fischer, Germany 54

**DENTAL CARIES AND DENTAL FLUOROSIS AT VARYING
WATER FLUORIDE CONCENTRATIONS**

K E Heller, S A Eklund and B A Burt, USA 54

DISCUSSION SECTION (LETTERS TO EDITOR)**DOSE FOR INCIPIENT ACUTE FLUORIDE INTOXICATION:
TRUE SCIENCE AND FALSE SCIENCE**

Toshu Murakami, Japan 55-56

REPLY TO CRITIQUE W G Young, Australia 57

REJOINDER Bill Wilson, New Zealand 57-58

CENTENNIAL COMMEMORATION



Photo by Fabian Bachrach

George L. Waldbott

The year 1998 marks the 100th anniversary of the birth of George L. Waldbott, MD, founder of the International Society for Fluoride Research and the journal *Fluoride*. Born in Speyer, Germany, on January 14, 1898, Dr Waldbott earned his medical degree at the University of Heidelberg in 1921. He then emigrated to the United States and interned in 1923-1924 at Henry Ford Hospital in Detroit, Michigan. In the following decades as a physician in clinical practice he specialized in the treatment and study of allergic and respiratory diseases. Beginning in the 1950s he increasingly turned his attention to adverse health effects of environmental pollutants, especially fluoride. This work he continued until his death on July 17, 1982.

In this issue of *Fluoride* we begin our commemoration of the centennial of Dr Waldbott's birth with tributes by seven individuals who were closely associated with him. These testimonials are followed by a previously unpublished article by Dr Waldbott on one of his favorite topics – the preskeletal phase of chronic fluoride intoxication. Finally, for the benefit of our readers, we conclude the commemoration with a list of Dr Waldbott's fluoride publications, which actually comprise only about half of his total medical research output.

John Colquhoun

GEORGE L WALDBOTT – A PRE-EMINENT LEADER IN FLUORIDE RESEARCH

"The seeds of great discoveries are constantly floating around us, but they only take root in minds well prepared to receive them."

American physicist Joseph Henry (1797-1878)

Throughout his long and distinguished medical career, Dr Waldbott embodied the spirit of Joseph Henry's maxim by the remarkable acumen he displayed for identifying exposure to particular toxic agents in relation to various illnesses. Taking a cue from the pioneering research of the Danish physician and health officer Kaj Roholm (1902-1948) on the symptoms of incipient stages of skeletal fluorosis,¹ he was able, beginning in the 1950s, to link these same adverse effects in some of his patients to fluoride in their drinking water and other sources of intake. By simply eliminating their excessive ingestion of fluoride, these patients gradually recovered and became well.

During this early period of his fluoride research, Dr Waldbott undertook a comprehensive survey of the biomedical literature of fluoride through which he made contact with leading fluoride investigators worldwide. He also found, much to his chagrin, that despite publishing his reports in highly respected peer-reviewed – but mostly European – medical journals, the clinical details of his investigations were blocked from appearing in leading US medical journals. In an effort to surmount this impediment, he organized the first international symposium on the toxicology of fluorine compounds, which was held in Bern, Switzerland, October 15-17, 1962, after being cancelled by the George Eastman Dental School host in Rome where it was originally scheduled.²

Encouraged by the success of this symposium, which was attended by over 30 researchers from 11 countries,³ Dr Waldbott arranged for a similar conference in Detroit in 1966 sponsored by the newly formed American Society for Fluoride Research, which then became the International Society for Fluoride Research. Again there was strong opposition, this time from the American Dental Association and the American Association for the Advancement of Science.⁴ Not to be deterred, Dr Waldbott went ahead with the International Society for Fluoride Research, which held its first meeting the following year in Frankfurt, Germany. In the ensuing years 20 additional ISFR conferences have been held in over 10 countries throughout the world, the last four since 1990 taking place in the USA, Japan, China, and Hungary.⁵ The XXIIInd Conference is scheduled for August 24-27, 1998, in Bellingham, Washington, USA.

My acquaintance with Dr Waldbott began in 1964 when he kindly responded to my requests for reprints of his fluoride research publications. The following year I met him for the first time while I was in Detroit attending a national meeting of the American Chemical Society. There I was also introduced to his gracious wife Edith, and their warmth and hospitality made a lasting impression. During this visit I read and helped correct the galleys of Dr Waldbott's new monograph *A Struggle With Titans*,⁶ which gave me a clearer and deeper insight into the enormity of the problems he was facing in attempting to make the truth about fluoride toxicity better known and understood.

Following this visit I began to assist Dr and Mrs Waldbott in editing and revising papers submitted to the ISFR journal, which started to appear in July 1968 under the title *Fluoride Quarterly Reports* (changed in 1970 to simply *Fluoride*). Later, H Lewis McKinney, one of my university colleagues, and I collaborated with Dr Waldbott in writing *Fluoridation: The Great Dilemma*, published in 1978.⁷ We were both greatly impressed by Dr Waldbott's extraordinary knowledge of the biomedical literature of fluoride as reflected by the wide range of editorial topics he wrote about in *Fluoride*. Moreover, even during the last years of his life, he remained actively engaged in his clinical practice, almost to the time of his death in July 1982.⁸

In assessing the legacy of Dr Waldbott's prodigious work on fluoride, we are saddened by the fact that it has been disparaged or ignored by powerful vested interests, official and unofficial, who regard it as a major threat to the security of their positions. Many of them have waged a relentless campaign of defamation and slander, falsely calling the ISFR an unscientific organization and incorrectly alleging that papers published in *Fluoride* are not peer reviewed. Evidently acting on their advice, the US National Library of Medicine has excluded *Fluoride* from *Index Medicus*,⁹ the leading biomedical referencing source for libraries and researchers. Yet *Fluoride* has long been and still is the only international scientific journal devoted to uncensored publication of legitimate research concerning all aspects of biological effects of fluoride. The result has been not only to marginalize much of that research but also to impede the free flow and exchange of information about such investigations that are crucial not only to the success of science but also to the vital needs and interests of everyone in society.

Meanwhile, the exciting vistas in fluoride research that Dr Waldbott penetrated are open for all to explore. And then time, in its own inexorable way, will finally and surely allow many additional "seeds of great discoveries" about fluoride, like those he made, to "take root in minds well prepared to receive them."

Albert W Burgstahler
Department of Chemistry
The University of Kansas
Lawrence, KS 66045, USA

REFERENCES AND NOTES

- 1 Roholm K. *Fluorine Intoxication. A Clinical-Hygienic Study*. H K Lewis, London 1937 Chapter XIII.
- 2 For details see pp 344-345 of reference 7 below.
- 3 Gordonoff T (Ed). *Toxicologie des Fluors (The Toxicology of Fluorine)*. Symposium. Bern October 15-17, 1962. Schwabe, Basel 1964. See p 206 for recorded list of participants.
- 4 Greenberg DS. Fluoridation: a meeting in Detroit raises some questions. *Science* 153 1499-1500 (23 September) 1966. Comment letter: Burgstahler AW. Detroit fluoride conference. *Science* 154 590 (4 November) 1966.
- 5 Editorial: Conferences of the International Society for Fluoride Research: Past and future. *Fluoride* 30 (1) 4 1997.
- 6 Waldbott GL. *A Struggle With Titans: Forces Behind Fluoridation*. Carlton Press, New York 1965.
- 7 Waldbott GL, Burgstahler AW, McKinney HL. *Fluoridation: The Great Dilemma*. Coronado Press, Lawrence, Kansas 1978.

- 8 For detailed biography see In Memoriam: George L. Waldbott, M.D. Jan. 14, 1898-July 17, 1982. *Fluoride* 15 (4) 165-168 1982.
- 9 Statement: "Information Concerning Review of Publications for Indexing by the National Library of Medicine," Executive Editor, *Index Medicus*, National Library of Medicine, Bethesda, Maryland, December 1988. In part this statement reads: "The Library is also working with professional societies to obtain their advice on which titles are useful to researchers, clinical practitioners, and educators in subject areas. Reports from the professional societies will assist the [Literature Selection Technical Review] Committee when it periodically reviews the coverage in each subject." It is noteworthy that these two sentences were added to the June 1988 version of this memorandum - shortly after numerous requests by ISFR members had been submitted for inclusion of *Fluoride* in *Index Medicus*.

MEMORIES OF GEORGE L WALDBOTT AN EXTRAORDINARY PHYSICIAN

In 1976, I had the opportunity and privilege of sharing a hotel room for three days with Dr Waldbott in Long Beach, California. We had been called to testify as expert witnesses in a court case involving industrial fluoride exposure and the subsequent illnesses of a man who had been employed at a major petroleum refinery in southern California. The worker was in his early fifties and had been disabled for over three years with a variety of serious medical problems. In brief, he had suffered brain damage (primarily affecting his previous excellent mathematical ability, memory, and sense of balance), degeneration of the cartilage in his knees, back and neck pains, severe emphysema, prostatism, and frequent (16-18 time a day) bowel movements.

His medical records revealed that he had received a detailed and expensive medical examination at the University of California Medical Center in San Francisco. There his mental deterioration, arthritis, and back and neck pains were attributed to "age", although at the time he was less than 50 years old. His prostatism was also attributed to age, and his emphysema was claimed to be due to his having smoked cigarettes for a short time many years before. His unusually frequent bowel movements were termed "short transit time syndrome" of unknown origin. His blood and urine fluoride levels were found to be normal - a year and a half after he had left his job! The opinion of the UC medical expert was that the symptoms were not the result of work-related exposure to fluoride.

The medical records also included several X-rays of his knee that had been operated on for the degenerated cartilage. One X-ray, however, showed a metal staple as might have been used for a fracture of his proximal tibia. But the patient assured us that he never had a knee fracture and no staple had been used in his surgery. That X-ray picture was of someone else's knee!

Our subject had been employed at a major gasoline-producing refinery where hydrogen fluoride was used to combine "light" and "heavy" hydrocarbon and alkene fractions to form high-octane gasoline. Periodically, the equipment was purged of the fluoride-contaminated tar-like material that accumulated in it. The purging was done by a pressurized steam hose in a depressed pit, and the cloud of steam emerging from the equipment contained toxic levels of hydrogen fluoride/hydrofluoric acid. The man had been doing this work on a regular basis

for a number of years and had worn a rubber suit and hood for protection against the steam and products spewed into the air. Not provided, however, were a mask and an auxiliary air supply for breathing. Often, while performing this cleaning operation, he would suffer painful breathing and become faint. A rope around his waist was used to pull him out of the pit when he became excessively woozy or fainted.

Later he found out that men performing this work usually lasted only two to three years before becoming ill and being replaced. Never having been warned of the toxic nature of his work, he had, with dogged persistence, lasted over four years before his illnesses forced him into early retirement. The question was: should the employer be liable for the man's medical problems? Both Dr Waldbott and I had examined the man and had studied his medical records. Our opinion was that, yes, the employer should be liable.

This was my first meeting with Dr Waldbott, whom I found to be a courtly, older gentleman with a slight accent hinting of his European birth and education. Underlying his calm and gentle manner was an intelligence strong as steel, precise and logical, but pleasant and not overbearing. His medical knowledge was extraordinary, and in our discussions of medical topics he examined alternative hypotheses equably and explained his conclusions in terms of biochemistry and mechanism of action. I could not recall meeting any doctor with a more brilliant mind, yet one whose thinking was so free of excessive ego or rancor. Inherent to him was the conviction that problems or differences in points of view could be resolved by clear dispassionate logic and evidence. He was not upset that some supposed authorities held fluoride positions that differed from his. Instead, he saw such a situation as an opportunity for presenting evidence as clearly and fairly as possible until a commonality of understanding was reached.

Every day we would gather at the court house awaiting our turn to testify, but each day our testimony was put off for some unknown reasons. Finally, on the third day, we were told that the case had been settled by the petroleum company admitting its liability. The lawyer explained that the man's disability payments would be paid by the company instead of by workmen's compensation insurance - a Pyrrhic victory, one might say, but a victory nonetheless. Later we were to find that the lawyer had sued the wrong party; the suit should have been brought against the supplier of the hydrogen fluoride who had failed to specify the toxic nature of the product. I suspect such a suit would have been successful, and the award for damages could have been considerable.

But for me, the reward I found greatest was the opportunity to spend three days with perhaps the best physician in the USA - three days of exposure to an extraordinary man whose precepts for practicing medicine have been a beacon of sound reasoning throughout the years of my own career that followed.

Shortly after I returned home, Dr Waldbott wrote to me suggesting that we write an article describing this occupational hazard to the industry and thereby perhaps prevent future occurrences of such fluoride poisoning among petroleum workers. Though Dr Waldbott provided the major research effort and was the principal author of the article, he insisted that it include me as a co-author. The report was eventually published in the peer-reviewed journal *Clinical Toxicology*,

volume 13, number 3, 1978, under the title "Toxicity from Repeated Low-Grade Exposure to Hydrogen Fluoride - Case Report."

I also became aware that Dr Waldbott had founded the International Society for Fluoride Research with the goal of providing an open forum for all fluoride researchers, regardless of viewpoint. This goal continues to hold, despite the fact that some so-called authorities (who seem to remain blind to research they find opposing their fixed mind set) only rarely attend ISFR conferences. The legacy of Dr Waldbott's dedication to fairness and openness in scientific research will prevail, however. Truth in science is not determined by politics or propaganda. Eventually it emerges in spite of such impediments through the patient efforts of those who share Dr Waldbott's precepts and ideals.

John R Lee, MD
9620 Bodega Highway
Sebastopol, CA 95472, USA

THE FLUORIDE RESEARCH LEGACY OF GEORGE L WALDBOTT

In an editorial, published in only the third issue of *Fluoride*, January 1969, Dr Waldbott drew attention to the pioneering fluoride research of Dr Kaj Roholm of Denmark. In particular, he cited Roholm's astute recognition of the dominant role fluoride played in Belgium's notorious Meuse Valley air pollution disaster of December 3-5, 1930. Commenting on Roholm's investigation, he wrote:

"Roholm is still recognized as the world's foremost expert on fluoride. For some unknown reason, however, his account of the Meuse Valley disaster fell into oblivion shortly after it appeared [in 1937]. Had the medical profession and scientists interested in air pollution given it the attention which it deserved, the worst U.S. air pollution disaster in Donora, Pa, 18 years later, might have been averted."

Dr Waldbott's closing paragraph pointedly began: "Although more than 3 decades have elapsed since Roholm's article appeared, most of the data which he presented are as new to physicians today as they were in 1937."

As a respiratory and allergy disease specialist, Dr Waldbott spent much of his professional life endeavoring to acquaint the medical profession with how pollutants like fluoride affect human health. He personally initiated and, out of his own funds, founded *Fluoride* for publication of scientific investigations on biological effects of fluoride. He personally contacted scientists throughout the world and encouraged them to publish their fluoride research in *Fluoride*. He invited them to join the International Society for Fluoride Research and to participate in its international conferences. He also used his own funds to sponsor and enable scientists to attend these meetings. His engaging interest, keen intelligence, extensive knowledge, and contagious enthusiasm about fluoride research were compelling, and although the conferences often had limited attendance, they were always scientifically stimulating and challenging.

Drawing on the broad background he had acquired in all areas of fluoride, Dr Waldbott's questions and comments at the conferences were trenchant and

thought-provoking. He had strong convictions but was always sensitive to those around him. He was fearless, however, in his efforts to inform members of the medical profession and other disciplines about the health effects of environmental pollutants.

It is not often that an individual of such extraordinary intellect and dedicated achievement comes into our lives. His memory and work will long remain an inspiration and a beacon for future endeavors in fluoride research.

Gene W Miller
Department of Biology
Utah State University
Logan, UT 84322-5305, USA

GEORGE L WALDBOTT: A TRULY GREAT PHYSICIAN OF OUR CENTURY

When the Municipal Council in Haarlem decided in October 1968 to fluoridate our water supplies, I was thoroughly alarmed. From a physiological standpoint, adding a well-known enzyme poison to our drinking water seemed like folly to me, but I had no scientific evidence to offer against the measure, only logic. I therefore began to look for reliable literature on the subject. At first I discovered a statement by a German association of scientists who condemned the measure as dangerous and unscientific, but their arguments seemed to me to be mainly theoretical, just like mine. A lucky chance then brought me into contact with the pioneering fluoride work of Dr George L Waldbott.

It was with mounting admiration for this independent-minded physician of unimpeachable integrity that I read his stirring book *A Struggle With Titans*. It more than confirmed all my concerns about the danger of using fluoride in medicine. Waldbott's work immediately was of great practical value to me. Although a strong resistance among the population had prevented Haarlem from being fluoridated, the adjoining municipality of Heemstede had not been able to evade the measure. The people in Heemstede were opposed to it, but the city derived its water from Amsterdam, which had started to fluoridate in February 1972.

This situation placed me in a unique position, since half of my patients lived in nonfluoridated Haarlem and the other half in fluoridated Heemstede. It was then that my admiration for Dr Waldbott approached overwhelming awe. One after another, I saw all the so-called side-effects he had described appear in my Heemstede patients. I say "so-called" side effects because, as I realized later, these were, in fact, the main effects of fluoridation.

I saw an explosion of patients with stomatitis, gastric distress, troublesome skin irritation, blurred vision, migraine-like headaches, and loss of mental acuity. Colicky babies crying loudly all through the night were driving their parents frantic. Later on, patients began to complain of joint stiffness, muscular weakness, and excessive fatigue. The list is far from complete, but the cure was simple: nonfluoridated water for all drinking and cooking for ten days brought complete relief!

I taught my colleague practitioners in the fluoridated regions what to look for, and soon they made the same observations. We were all extremely grateful to Dr Waldbott for having shown us the way. In 1973 our research group sent me to Oxford to attend the Conference of the International Society for Fluoride Research. There I met Professor Sinclair, our gallant host, Professor Burgstahler from Kansas, Professor Jolly from Punjab, and Dr Zanfagna from Massachusetts. Binding all these scientists together was Dr Waldbott, whom I also met for the first time.

A few years later, in February 1976, I organized the VIIth ISFR Conference in Zandvoort near Haarlem with Dr Waldbott as chairman. There Dean Burk presented his research in collaboration with John Yiamouyiannis on the link between fluoridation and increased cancer mortality. Evidently the findings presented at this conference helped keep fluoridation at bay in the Netherlands, whose water supplies to this day remain unfluoridated.

With the passage of time after 1982, I more or less expected that the ISFR would die together with Dr Waldbott, but the opposite has happened. His spiritual child has grown by leaps and bounds and has continued to attract scientists from all over the world.

George Waldbott is an ongoing positive influence in my life. He taught me a new way of medical thinking; the modern version of the steady drip hollowing out the stone. Even a very small dose of a poison can, through repetition, cause detrimental effects of an extremely grave nature.

As a consultant in food and chemical intolerances, I continue to discover many children with untoward effects from fluoride ingested through toothpaste, tablets, or dental sealants. Each time one of these little ones is cured I mentally take off my cap to my great teacher. Thanks to George Waldbott, I have been able to heal hundreds of my own patients and, as I made his insights popular, even thousands of others in my country. Moreover, during the time fluoridation existed in Holland many people were able to diagnose their own illness from it and make themselves well.

George Waldbott has been an enormous blessing in my life and in the lives of many of my patients. I will live in his debt and honor his memory as long as I live.

Hans C Moolenburgh, MD
Oranjeplein 11
2012 LN Haarlem
The Netherlands

MY FATHER'S DEDICATION TO FLUORIDE RESEARCH

I wish to express my warm and deep appreciation for the special honor being paid to my father, Dr George L Waldbott, in this issue of *Fluoride* commemorating the 100th anniversary of his birth. He is already held in high esteem, but now even more so, because this journal, which he founded 30 years ago, continues to grow with an enviable standard of scientific excellence and reach a broader spectrum of the international scientific community.

Apart from the journal, however, I also recall how tireless and devoted my father was in pursuing research while still providing high-quality medical care for his many, very grateful patients in his allergy practice. To them and all who knew him, he was always most understanding, gracious, and warm-hearted.

In the arena of fluoride research, my father's keen sense of scientific integrity was deeply offended when, all too often, he encountered apparent cover-ups or direct distortions of critical findings in the medical literature and in his correspondence with editors of leading journals. He simply could not rest after reading such statements until he had exposed and corrected their scientific inaccuracies.

As many readers are aware, the essentially closed and one-sided forum on fluoride research in America in the 1960s prompted my father to invite scientists from different parts of the world to participate in scientific conferences in what became the International Society for Fluoride Research. His primary purpose was to provide an opportunity for scientists engaged in fluoride research to present and discuss their findings in a strictly scientific setting. The *Fluoride* journal was the natural outgrowth of the ISFR and the important need to disseminate the research presented at these meetings.

My congratulations to all who continue this effort at a time when the fluoride controversy is still very much with us. Vested interests who insist fluoride as a deterrent to dental caries is safe and beneficial for all are seldom prepared to acknowledge valid research to the contrary.

Elizabeth Ramsay
HaChasida 23/1, Gilo
Jerusalem 93850
Israel

GEORGE L WALDBOTT - AN EXCEPTIONAL CLINICIAN

In late 1974, when I was about to begin a sabbatical year at the University of Southern California in Los Angeles, I decided to start my journey from New Delhi a few days early in order to attend a conference of the International Society for Fluoride Research scheduled in November at Williamsburg, Virginia. Since India has serious health problems caused by high levels of fluoride in drinking water, I thought this meeting would be a good opportunity to share results of our research on these matters.

In 1972 my laboratory at the All India Institute of Medical Sciences began investigating the effect of fluoride on skeletal muscle tissue in patients afflicted with skeletal fluorosis. These patients were then under the care of Professor S S Jolly of the government Medical College Hospital in Patiala, Punjab. We embarked on this research because, if the normally high calcium content of skeletal muscle tissue is depleted by elevated fluoride, the muscle cannot contract properly. Our studies revealed that fluoride-induced skeletal muscle damage is primarily myogenic and not due to neuronal involvement. When I wrote to Dr Waldbott about my interest in presenting this work at the 1974 ISFR conference, he was most receptive to the idea.

Arriving at the motel in Williamsburg on the eve of the conference, I found a welcoming note with directions to the hall where Dr and Mrs Waldbott were hosting a reception dinner for the participants. At the dinner they had reserved a place for me next to them. This was the first time I met Dr Waldbott and his ever-helpful wife Edith. I already knew that he was one of the world's leading allergy specialists, so I was eager to meet him. This meeting was the beginning of a long association that lasted until his death in 1982 and has continued with the ISFR.

During our discussions Dr Waldbott made frequent reference to our research at the All India Institute of Medical Sciences. He said he thought our contributions on skeletal muscle involvement in fluoride intoxication represented "a monumental piece of work." My presentation at the conference was published afterward in *Fluoride*.¹

Besides skeletal muscle tissue, my laboratory has also been investigating changes in gastric and intestinal mucosa in response to complaints of persons living in endemic fluorosis areas, who reported loss of appetite, nausea, stomach pain and gas, a bloated feeling, flatulence, and constipation alternating with intermittent diarrhoea. These studies were conducted using a careful protocol to eliminate other, nonfluoride causes of these non-ulcer dyspeptic complaints. Our diagnoses were confirmed through endoscopy and scanning electron microscopy of biopsy material obtained from gastric and duodenal regions, revealing a "cracked clay appearance of the mucosa," loss of microvilli from the mucosal surface, and even disappearance of mucus. Moreover, these same changes were found in patients with skeletal fluorosis whose drinking water contained 2.5 ppm or more fluoride as well as with patients undergoing fluoride therapy for otosclerosis for 3 to 12 months. Usually the non-ulcer dyspeptic symptoms disappeared in 10 to 15 days when the patients were diverted to safer drinking water containing less than 0.8-1.0 ppm fluoride. These observations have been reported in a series of articles published since 1992.²⁻⁶

Dr Waldbott's previously unpublished article, "The Pre-skeletal Phase of Chronic Fluoride Intoxication," which appears in this issue of *Fluoride* (pp 13-18), reveals he had examined and successfully treated patients with fluoride-induced non-ulcer dyspeptic complaints that were initially undiagnosed by other clinicians. He was one of the very few clinicians who understood the connection between fluoride toxicity and gastrointestinal symptoms and was able to relieve them. In the 1980s we confirmed this correlation of gastric disorders with excessive fluoride intake, and it is now widely used in India for detection of fluorosis. Additional tests include assessing fluoride levels in the drinking water, blood, and urine. Diverting patients from fluoride-contaminated water to a source of safe drinking water relieves the gastrointestinal complaints in 10-15 days, providing they are caused by fluoride. Thus, simply providing safe drinking water with the least amount of fluoride has proved to be one of the best approaches to treating fluoride-induced non-ulcer dyspepsia.

As noted by Dr Waldbott, Kaj Roholm found in the 1930s that 55 out of the 68 cryolite workers (80.9%) had either acute or subacute gastric symptoms. Data from our current studies in an aluminium plant in India confirm these findings.

Although Dr Waldbott's own clinical observations on fluoride intoxication date back to the mid-1950s, the manner in which he made them is worth bringing to the attention of practising clinicians throughout the world. His patients who suffered from gastrointestinal symptoms while living in a fluoridated area obtained relief when they moved to a nonfluoridated area, only to find their illness recur after moving back into a fluoridated area. Fluoride-induced non-ulcer dyspepsia leads to reduced haemoglobin, anaemia, weakness due to non-absorption of nutrients as the microvilli are lost, and a range of other health problems including depression. Fluoride intoxication and related health disorders need to be viewed in their totality to understand the seriousness of the resulting problems facing millions of people around the globe.

Dr Waldbott's article also reveals how many practising clinicians feel they need to advise patients not to disclose their diagnosis and their recommendation to avoid fluoridated water for fear of action against them by their colleagues. Again, we see Dr Waldbott's undaunted courage and conviction in seeking to alleviate the misery and suffering of those who experienced severe health problems due to drinking fluoridated water or exposure to fluoride dust and fumes in industrial environments.

I look forward to the day when adding fluoride to drinking water will be recognized for the harm it causes and will therefore be considered a criminal offense. Sometime soon, I hope, fluoridation will be banned on the basis of such pioneering research as by Dr Waldbott, his colleagues, friends, and associates around the world, whom he inspired and encouraged to continue and expand their investigations on the effects of fluoride on the human body.

A K Susheela, Professor
Director, Programmes
Fluorosis Research and Rural
Development Foundation
C-13, Qutab Institutional Area
(Nutrition Foundation of India Building)
New Delhi 110016, India

REFERENCES

- 1 Kaul RD, Susheela AK. Symposium on the non-skeletal phase of chronic fluorosis: The muscle. *Fluoride* 9 (1) 9-18 1976.
- 2 Susheela AK, Das TK, Gupta IP, Tandon RK, Kacker SK, Gosh P, Dekka RC. Fluoride ingestion and its correlation with gastro-intestinal discomfort. *Fluoride* 25 (1) 5-22 1992.
- 3 Susheela AK, Kumar A, Bhatnagar M, Bahadur R. Prevalence of endemic fluorosis with gastrointestinal manifestations in people living in some North-Indian villages. *Fluoride* 26 (2) 97-104 1993.
- 4 Das TK, Susheela AK, Gupta IP, Dasarathy S, Tandon RK. Toxic effects of chronic fluoride ingestion on the upper gastro-intestinal tract. *Journal of Clinical Gastroenterology* 18 (3) 194-199 1994. (Abstract in *Fluoride* 28 (1) 48 1995)
- 5 Susheela AK. Epidemiological studies of health risks from drinking water naturally contaminated with fluoride. In: Reichard G, Sapponic GA (Eds). *Assessing and Managing Health Risks from Drinking Water Contamination: Approaches and Applications*. (Proceedings of the Rome Symposium, September 1994). The International

Association of Hydrogeological Sciences (IAHS) Publication No. 233, United Kingdom 1995 pp 123-134.

- 6 Dasarathy S, Das TK, Gupta IP, Susheela AK, Tandon RK. Gastroduodenal manifestations in patients with skeletal fluorosis. *Journal of Gastroenterology* 31 (3) 333-337 1996. (Abstract in this issue, page 47)

ENVIRONMENTAL TOXICOLOGY: WHAT I OWE TO DR WALDBOTT

We all respect and admire George L Waldbott as the founder of the International Society for Fluoride Research and as the editor for many years of its official journal *Fluoride*. As a member and past officer of the Society, I share with others a deep indebtedness to Dr Waldbott for the large body of very important work he did on fluoride.

For me personally, however, I remember Dr. Waldbott particularly well for another reason. One of the books he wrote made a strong impression on me and greatly influenced my career activities.

During the early 1970s, at the newly established Huxley College of Environmental Studies at Western Washington University, I was developing a new course titled "Pollution and Health." In looking for a suitable textbook for this course, I came across Dr Waldbott's monograph entitled "Health Effects of Environmental Pollutants," which had just been published by the C V Mosby Company. I adopted the book and used it as a textbook for several years.

This book not only helped me teach the course, but it also greatly influenced other aspects of my career. In particular, it introduced me to the study of environmental toxicology, a branch of science that was attracting a growing interest among researchers. From that time on, environmental toxicology became the primary focus of my teaching and research.

Mainly because of the basic knowledge I had gained from Dr Waldbott's book and related research, I was able to co-author with Wayne G Landis a book entitled *Introduction to Environmental Toxicology: Impacts of Chemicals Upon Ecological Systems*, published in 1995 by Lewis Publishers, Boca Raton, Florida. This text is now used in colleges and universities in the United States and several other countries. At present I am writing another book entitled *Fundamentals of Environmental Toxicology*.

Although many years have passed since I first became aware of Dr Waldbott's book, my appreciation of his influential work continues to deepen as I recall my earlier days of teaching and research at Western Washington University.

Ming-Ho Yu
Center for Environmental Science
Western Washington University
Bellingham, WA 98225-9181, USA

THE PRESKELETAL PHASE OF CHRONIC FLUORIDE INTOXICATION

George L Waldbott MD[†]
Warren, Michigan, USA

Chronic poisoning from most toxic agents is rarely diagnosed by physicians in its initial stage. Most systemic poisons induce vague, subtle symptoms before the appearance of features characteristic of a particular kind of poisoning. For instance, the radial nerve paralysis or the lead line on the gums which are typical of lead poisoning are preceded by numerous vague symptoms such as lack of appetite, general fatigue, gastric pains, and bowel disorders. Similarly, the bone changes characteristic of chronic cadmium poisoning become apparent only after a prolonged, slowly progressive illness with changes in kidney function.¹

Roholm, the most astute observer on the health effects of fluoride, was first to outline a wide variety of vague manifestations in conjunction with skeletal fluorosis in cryolite workers (Table 1).² He also stated that these symptoms are transitory and tend to disappear:

"The rule is that for a period of some few days to some few weeks after starting at the factory the worker suffers from these acute gastric attacks, whereafter they disappear, especially the nausea and vomiting. Thereafter some of the workers tolerate the dust without observing the symptoms; others will still have transitory symptoms after holidays, or if the dust quantity temporarily becomes especially high. Cardialgia is less distinctly connected with working in the dust. Sometimes the aforesaid gastric symptoms are accompanied by a slight, passing pressure or pain in cardia ..." ²

Table 1. Frequency of complaints in cryolite workers with skeletal fluorosis

| Symptoms | Men | Women | Total | % of all workers examined (68) |
|---|-----|-------|-------|--------------------------------|
| Gastric (loss of appetite, gastric pain; nausea, vomiting) | 42 | 13 | 55 | 80.9 |
| Intestinal (chronic diarrhoea, constipation) | 15 | 8 | 23 | 33.8 |
| Circulatory and respiratory (dyspnea, cough, expectoration, palpitation) | 23 | 12 | 35 | 51.5 |
| Neuromuscular (joint pains, stiffness, indefinite or localized rheumatic pains) | 18 | 6 | 24 | 35.3 |
| Neurological (tiredness, headache, asomnia, vertigo) | 12 | 3 | 15 | 22.1 |
| Dermatological (rash) | 8 | 0 | 8 | 11.8 |

Murray and Wilson³ also refer to vague gastrointestinal and arthritic symptoms in conjunction with exposure to fluoride in their account of nine members of the household of a farm family residing close to a fluoride-emitting factory. Frada and Montesana recorded disturbances in the bowels in conjunction with skeletal fluorosis in residents of northern Sicily where the water contained

[†] Deceased: July 17, 1982.

between 3 and 6 ppm of fluoride naturally.⁴ Srikantia and Siddiqui emphasized polydipsia and polyuria in endemic skeletal fluorosis in India.⁵

I have presented a more detailed description of the above-named clinical syndrome.⁶⁻⁸ Because of the increasing significance of fluoride as an industrial pollutant of air and water and because of the difficulty encountered in the recognition of the early stage of chronic fluoride poisoning a review of the available data is indicated.

In 1953, Mrs S S aged 40, a resident of Bay City, Michigan, was referred to me by her physician for allergic studies in order to determine the cause of a gastrointestinal disorder accompanied by migraine-like headaches. My tests revealed that her illness was not due to allergy. She wondered why her condition always tended to clear up when she was away from Bay City but became aggravated upon her return. She questioned whether the Bay City water might be involved. At that time neither she nor I were aware that Bay City's water had been fluoridated since 1951, when her illness began.

In September, 1954, Mrs M E J, age 35, a patient from Highland Park, Michigan, consulted me because of a serious, progressive illness.⁹ The description of its initial stage resembled that of Mrs S S, of Bay City. Studies at Harper Hospital, (Detroit, Michigan), laboratory tests, and consultations with nine specialists ruled out illnesses with which these specialists were acquainted. The only positive findings were mottled teeth which led to the information that as a child she had resided in an area in China where water naturally contained fluoride in appreciable concentrations. Following her discharge from the hospital she eliminated fluoridated water for drinking and cooking at her home in Highland Park (then fluoridated for 2 years). She recovered completely without medication. Controlled studies with minute doses of fluoride in water proved that she was unusually susceptible to ill effect from fluoride.

A few weeks after fluoridation had been discontinued in Saginaw, Michigan, in November 1954, I had an opportunity to examine thirty patients, nine of whom appeared to be afflicted with the same condition.⁶ Their attention was drawn to fluoride in drinking water because during the weeks since fluoridation had been abandoned they had, for reasons unknown to them, markedly improved or completely recovered from an otherwise progressive chronic illness.

In November 1955, I encountered additional cases during a visit to Charlottesville, Virginia, shortly after fluoridation had been discontinued there. Most impressive was the case of Mr J A H, age 67, whose illness had baffled leading clinicians at two medical centers. He had not obtained a diagnosis of his illness nor had he received benefit from treatment. Like the others, he was not aware that fluoride was being added to his drinking water until he unexpectedly recovered his health following discontinuance of fluoridation in that city.

In contrast to the Saginaw cases in which the illness was recognized after cessation of fluoridation in Windsor, Ontario, it was detected after fluoride was introduced into the water supply. The local health department, fearing adverse publicity by citizens, did not announce in advance the date when fluoridation

would begin. This afforded an excellent opportunity - better than any double blind test - to determine incontrovertibly whether illness from fluoridated water is genuine or imaginary. Two weeks after its inception, when the press announced the event to the public, eight individuals were able to diagnose their own illness. Upon eliminating fluoridated water for drinking and cooking their food, they recovered their health.

During the past two decades I have had experience with more than 400 similar cases.⁶⁻¹⁶ At least twenty were hospitalized for detailed studies. In others laboratory and double blind tests have been utilized in my clinic. The salient symptoms have been sharp pains in the stomach area associated with nausea, spasticity of the bowels (ileitis, colitis), polydipsia and polyuria, arthritic pains, especially in the lower spine, migraine-like headaches and painful paresthesia in arms and legs with loss of muscular power. Some patients have had ulcers in the mouth, some have had frequent episodes of lower urinary tract disease. Most patients have shown a distinct mental deterioration, mainly loss of memory and of ability to concentrate. Most striking and consistent has been extreme exhaustion, which eventually caused many of them to be bedridden.

One of the most impressive cases in my series is that of N K T, age 45, who consulted me in early April 1975 because of a variety of complaints which his physicians had not been able to diagnose. He had been in perfect health until 1954 when he moved to Milwaukee (fluoridated August 1953) where he developed headaches and low back pain which gradually became so severe that in 1956 he had to seek lighter work as a salesman. The condition became aggravated by nausea and pain in the abdomen, and in fall 1957 he had two episodes of convulsions for which his physicians could find no explanation. Shortly thereafter he moved to Okauchee, Wisconsin (nonfluoridated)* where, unexpectedly, all his symptoms cleared completely. Upon resuming residence in Milwaukee in late 1959 his illness promptly recurred. In 1961 he took another job in Antioch, Illinois, where he remained in good health. Here he consumed the water of a shallow private well about 10 meters deep the composition of which could not be ascertained because it was abandoned after he returned to Milwaukee** where he accepted employment as a salesman in 1968. Almost immediately he began to be aware of persistent backaches, headaches, extreme tiredness, and excessive thirst. His mental faculties gradually deteriorated and he became forgetful and unable to comprehend. He experienced general muscular weakness, tinnitus in both ears, pain, and edema in hands and ankles, and bleeding of the gums. After five months he was obliged to stop working. He received a variety of treatments including traction of the spine, muscle relaxing drugs, and analgesics. Diagnostic studies by his physician revealed, according to the copy of the physician's record, only a minor hearing loss in his left ear. Another attempt at working on a job operating heavy equipment had to be relinquished because of his gradually worsening backaches.

* Okauchee water contained 0.15 ppm fluoride, 37 ppm calcium, 17 ppm magnesium and 164 ppm hardness.

** Milwaukee water contained 0.95 ppm fluoride, 16.1 ppm calcium, 0.6 ppm magnesium, and 80 ppm hardness.

In October 1969 he moved to Woodruff, Wisconsin (nonfluoridated)* where his illness again cleared up dramatically without medication. Three years later, on December 1, 1972, he once more took up residence in Milwaukee. Within days the backaches returned, associated with pains and swelling in most other joints, paresthesia in the fingers and muscular fibrillation. Because of the muscular weakness, it became difficult for him to open doors; he had to sleep for at least 12 out of 24 hours. There was a slight, temporary rise in blood pressure (160/90). His condition improved somewhat by taking large amounts of vitamin C (1500 mg daily) and bone meal tablets, but during October 1973 the arthritis gradually became much more pronounced, especially in his knees, and failed to yield to salicylates prescribed by his physician. He also had slight hayfever and considerable nasal discharge. Because of several episodes of acute abdominal pains, he had a cholecystectomy and appendectomy, but abdominal symptoms (pains, bloating, diarrhea) persisted. According to the records obtained from his physician, however, the physical and laboratory findings were unremarkable.

In late 1974, while his health continued to deteriorate, his attention was directed to fluoridated water: a general skin rash on his infant son cleared up promptly and completely as soon as distilled water was substituted for Milwaukee's water in the baby's formula, a measure recommended by a friend. Although skeptical about this apparent cure, the father, urged by his friend, decided to substitute distilled water likewise for himself. The bowel disturbances, especially the abdominal pains, were promptly alleviated. Within 10 days all other symptoms had disappeared. Since using distilled fluoride-free water he has remained in perfect health operating heavy construction equipment and performing difficult physical work without any of the previous adverse reactions. In June, 1970, his symptoms recurred temporarily. Subsequently on July 20 he learned that, due to failure of the deionization equipment, the water contained 0.54 ppm fluoride.

In order to confirm the relation of the illness to fluoridated water, I reproduced the disease by a controlled procedure advocated by the editor of the *Journal of the American Medical Association* in a letter to me dated April 2, 1958: "... Place the patient on a fluoride free water supply until the symptoms have subsided and then, unbeknown to the patient, add 2.2 parts per million of sodium fluoride to the water." This concentration is equivalent to 1 ppm fluoride in water, the optimal concentration recommended for fluoridation of drinking water.

I was able to confirm my initial observations by studying the same disease among residents in the environs of fluoride-emitting aluminum and fertilizer factories in Ontario¹⁵ and British Columbia, Canada; Bolzano, Italy; and Southern Ohio, USA. Most of these individuals identified the gastrointestinal symptoms with the ingestion of food grown in their own garden which had been contaminated by airborne fluoride (Table 1). In some, respiratory symptoms prevailed, presumably precipitated by airborne fluoride and perhaps by other contaminants. Data on the 112 cases from the four areas are presented in Table 2.

* Woodruff water contained 0.10 ppm. fluoride, 42 ppm calcium, 17 ppm magnesium, and 176 ppm hardness.

Table 2. Symptomatology in 112 cases of neighborhood fluorosis

| | Ontario | Ohio | Italy | British Columbia | TOTAL (%) |
|---|----------|------|-------|---------------------|-----------|
| | 28 cases | 36 | 24 | 24 | 112 |
| <u>Musculo-skeletal</u> | | | | | |
| Arthritis -esp. cervical & lumbar spine | 26 | 7 | | 9 | 42 (38) |
| Myalgia, Myasthenia, parasthesias | 25 | 13 | 13 | 6 | 58 (52) |
| Spasticity in extremities | 5 | | | 1 | 6 (5) |
| <u>Gastro-Intestinal</u> | | | | | |
| Epigastric pain, nausea, vomiting | 16 | 17 | 16 | 8 | 57 (51) |
| Distention, diarrhea, spastic constipation | 14 | 10 | 18 | 10 | 52 (46) |
| Acute abdominal episodes | 7 | 1 | | 2 | 10 (9) |
| Stomatitis | 3 | 6 | 10 | 2 | 21 (19) |
| <u>Neurological</u> | | | | | |
| Migraine-like headaches | 17 | 9 | 17 | 13 | 56 (50) |
| Scotomata, blurred vision | 8 | 3 | 13 | 3 | 27 (24) |
| Convulsions | | 1 | 1 | 2 | 4 (4) |
| Muscular fibrillation | 6 | | | 10 | 16 (14) |
| <u>Respiratory</u> | | | | | |
| Nasal & conjunctival | 24 | 11 | 14 | 3 | 52 (46) |
| Emphysema; asthma | 2 | 4 | 1 | 3 | 10 (9) |
| Epistaxis | 5 | 4 | 2 | 1 | 12 (11) |
| <u>Skin</u> | | | | | |
| Dermatitis | 1 | 3 | | 1 | 5 (4) |
| Chizzola maculae* | 7 | 16 | | 18 | 41 (37) |

*Lesions resembling traumatic suffusions described by Waldbott and Steinegger¹⁶

I have also studied other cases of chronic fluoride intoxication which were dominated by one major symptom such as convulsions,¹¹ urticaria,¹² and, in infants, gastrointestinal hemorrhages¹⁴.

In 1973, Petraborg, a Minnesota physician, interviewed and examined 28 individuals in Milwaukee. The medical history of their illness, the uniformity of their complaints, and his personal examinations convinced him that fluoridated water had indeed caused a serious progressive illness.^{17,18}

Another group of 12 physicians in Haarlem, Holland, studied 60 patients, 30 of whom underwent carefully controlled double blind tests.¹⁹ Other physicians have diagnosed the disease on their own, among them Dr William P Murphy, a 1934 Nobel Prize winner in medicine, but have hesitated to report these cases in medical journals for reasons discussed below. On at least three occasions to my knowledge, physicians have treated illnesses by advising patients to abandon fluoridated water but have expressly requested the patient not to disclose the diagnosis.

DISCUSSION

The following questions arise in conjunction with the description of this disease: 1. Why must fluoride and no other toxic agent be considered the cause of this disease? 2. What accounts for the wide spectrum of vague symptoms? 3. What, if any, specific laboratory criteria are available to permit the establishment of the diagnosis? 4. Why is the illness not diagnosed more frequently in view of the wide-spread consumption of fluoridated water?

1. *Other Possible Causal Sources:* The prompt disappearance of the symptoms upon eliminating fluoridated water for drinking and cooking and their return on its resumption could conceivably be attributed to a psychosomatic background of the clinical picture described above. However, in most instances - certainly in the Saginaw and Windsor group - no one was aware that their water had been fluoridated when the disease terminated or began, respectively. In others, the disease was reproduced by blind and double-blind procedures. Furthermore, most individuals had undergone extensive diagnostic tests by competent diagnosticians and had been treated unsuccessfully with numerous therapeutic measures to which the disease failed to respond. Why would the disease respond to avoidance of fluoridated water but not improve following the many other therapeutic measures which had been previously instituted? For most patients under my own care, unbiased specialists were consulted who ruled out other known diseases. Moreover, the clinical events beginning usually with minor gastrointestinal complaints, excessive thirst, paresthesias muscle pains and fibrillation, headaches, and other inconspicuous neurological manifestations and the progressive nature of the illness, resulting eventually in complete disability, represent a clear-cut syndrome which undoubtedly constitutes an attenuated form of acute fluoride intoxication.⁸ The latter is also dominated by gastrointestinal hemorrhages and neuromuscular manifestations, particularly convulsions.¹¹ Some of the symptoms observed in my cases such as the skin lesions, the early changes in the retina or gastric hemorrhages and the skin lesions could not possibly be induced by psychosomatic features.

2. *Wide Spectrum of Symptoms:* In order to explain the wide spectrum of the symptomatology of this illness, we must recall that the fluorine ion is extremely minute in size and has a high charge density which enables it to penetrate into every cell of the body and become attached to other ions, especially polyvalent ones.²⁰ Usually soft tissue organs do not accumulate the halogen which has a strong affinity for calcified tissue, i.e. bones and teeth. Nevertheless, the presence of sizeable amounts of fluoride has been reported in soft tissue organs,²⁰ particularly in nephritic patients whose kidneys retain more fluoride than kidneys of normal persons. The fact that fluoride permeates readily into every organ of the body where it interferes with many enzyme systems might conceivably account for its effect on the function of so many organs. When fluoride is ingested or imbibed with water the first major target reached is the stomach, the acidity of which produces the highly irritating hydrofluoric acid and thus accounts for the gastric symptoms. The production of ulcers in the mouth can be explained in a similar manner when acid foods are in contact with

fluoride excreted in the saliva. The lower urinary tract is involved in reabsorption of fluoride, especially when the urine is acid and hydrofluoric acid is likely to be formed.²¹ In individuals residing close to fluoride-emitting factories, respiratory symptoms are more prevalent than in hydrofluorosis cases because the respiratory tract is the first target for inhaled fluorides.²⁰

3. *Laboratory Features:* In most of my cases of preskeletal fluorosis, fluoride levels in the urine were not excessive, which indicates that the so-called optimal concentration (1 ppm) of fluoride in drinking water is sufficient to induce poisoning in persons intolerant to fluoride. High urinary fluoride excretion does not necessarily correlate with the degree of poisoning²² nor can poisoning be ruled out when urinary fluoride levels are low. Whereas I was unable to obtain data on fluoride plasma and bone levels, it is doubtful that these parameters are related to the incipient stage of fluorosis.

Occasionally in beginning fluorosis two laboratory findings merit special attention, namely, an increase in alkaline phosphatase and changes in the calcium and phosphorus levels of the blood,²³ but due to the inconsistency of these parameters they cannot be considered pathognomonic for fluorosis. In squirrel monkeys maintained on 1 and 5 ppm fluoride in their drinking water for 18 months, Manocha *et al*²⁴ found that the kidneys "showed significant cytochemical changes, especially in the animals on 5 ppm fluoride in their drinking water." Moreover, in the last 10 months of the study, water consumption by animals drinking the fluoridated water was higher than that of the controls on fluoride-free water. Kidney tests for changes in renal enzyme activity might therefore offer a means to detect the early stage of fluoride poisoning.

4. *Lack of Recognition of the Disease:* Many reasons account for the fact that reports of this disease which have appeared in the medical literature are rare. The absence of conclusive laboratory data, the slow and insidious onset of the disease, the wide spectrum of vague symptoms, the unawareness of physicians of possible side effects from fluoridated water and airborne fluoride all render the diagnosis difficult. Furthermore, physicians are constantly being assured that fluoridation is safe.²⁵ For the same reasons the untoward effects of many other environmental pollutants have escaped our recognition for many decades.

In view of the widespread industrial application of fluoride and its great significance as an environmental pollutant and because of the likelihood that the illness already described affects a sizeable segment of the population, careful review of all available data on this subject is indicated.

REFERENCES

- 1 Waldbott GL. *Health Effects of Environmental Pollutants*. Mosby, St Louis, 1973 (2nd Edition 1978).
- 2 Roholm K. *Fluorine Intoxication: A Clinical-Hygienic Study*. HK Lewis, London 1937 p 138.
- 3 Murray MM, Wilson DC. Fluorine hazards with special reference to some social consequences of industrial processes. *Lancet* 2 821-824 1946.
- 4 Fradà G, Mantesana G, Nalbene G. Recerche sull'idrofluorosi. *Minerva Medica* 54 451-59 1963.

- 5 Srikantia SG, Siddiqui AH. Metabolic studies in skeletal fluorosis. *Clinical Science* 28 477-485 1965.
- 6 Waldbott GL. Incipient fluorine intoxication from drinking water. *Acta Medica Scandinavica* 156 157-168 1956.
- 7 Waldbott GL. Fluoride in Clinical Medicine. *International Archives of Allergy and Applied Immunology* 20 Suppl 1 1962.
- 8 Waldbott GL. Acute fluoride intoxication. *Acta Medica Scandinavica* 174 Suppl 400 1963.
- 9 Waldbott GL. Chronic fluorine intoxication from drinking water. *International Archives of Allergy and Applied Immunology* 7 70-74 1955.
- 10 Waldbott GL. Incipient chronic fluoride intoxication from drinking water. II. Distinction between allergic reactions and drug intolerance. *International Archives of Allergy and Applied Immunology* 9:241-249 1956.
- 11 Waldbott GL. Tetaniform convulsions precipitated by fluoridated drinking water. *Confinia Neurologica* 17 339-347 1957.
- 12 Waldbott GL. Urticaria due to fluoride. *Acta Allergologica* 13 456-468 1959.
- 13 Waldbott GL. Allergic reactions to fluoride. *Journal of Asthma Research* 2 51-64 September 1964.
- 14 Shea JJ, Gillespie SM, Waldbott GL. Allergy to fluoride. *Annals of Allergy* 25 388-391 July 1967.
- 15 Waldbott GL, Cecilioni VA. "Neighborhood" fluorosis. *Clinical Toxicology* 2 387-396 December 1969.
- 16 Waldbott GL, Steinegger S. New observations in "Chizzola" Maculae. In: *Proceedings of the Third International Clean Air Congress of the International Union of Air Pollution Prevention Association. October 8-12, 1973, Dusseldorf, Federal Republic of Germany.* Verein Deutscher Ingenieure, Dusseldorf 1975 pp A63-A67.
- 17 Petraborg HT. Chronic fluoride intoxication from drinking water (Preliminary Report). *Fluoride* 7 47-52 1974.
- 18 Petraborg HT. Hydrofluorosis in the fluoridated Milwaukee area. *Fluoride* 10 165-169 1977.
- 19 Grimbergen GW. A double blind test for determination of intolerance to fluoridated water (Preliminary Report). *Fluoride* 7 146-152 1974.
- 20 Waldbott GL. Symposium on the non-skeletal phase of chronic fluorosis. *Fluoride* 9 5-8 1976.
- 21 Whitford GM, Pashley DH, Stringer GI. Fluoride renal clearance: a pH-dependent event. *American Journal of Physiology* 230 527-532 1976.
- 22 Dinman BD, Bovard WJ, Bonney TB *et al.* Prevention of bony fluorosis in aluminum smelter workers. Parts 1-4 *Journal of Occupational Medicine* 18 7-23 1976. Abstract in *Fluoride* 9 215-216 1976.
- 23 Waldbott GL. Urinary fluoride and calcium excretion in persons suspected of fluoride intolerance. *Henry Ford Hospital Medical Bulletin* 5 259-268 December 1957.
- 24 Manocha SL, Warner H, Olkowski Z. Cytochemical response of kidney, liver and nervous system to fluoride ions in drinking water. *Histochemical Journal* 7 343-355 1975.
- 25 Smith EH Jr. Fluoridation of water supply. *Journal of the American Medical Association* 230 1569 1974.

FLUORIDE PUBLICATIONS OF GEORGE L WALDBOTT, MD

- 1 Chronic Fluorine Intoxication from Drinking Water. *International Archives of Allergy and Applied Immunology* 7 (2) 70-74 1955.
- 2 Medical Evidence Against Fluoridation of Public Water Supplies. *Australian Dental Journal* 59 13-20 February 1955.
- 3 Intoxicación crónica por fluorina a partir del agua bebida. Pequeña epidemia en Saginaw, Michigan. *Folia Clinica Internacional* 5 (4) 144-150 1955.
- 4 Ärztliche Beweisführung gegen Fluorierung des Trinkwassers. *Zahnärztliche Reform* Heft 7/8 1955.
- 5 Incipient Fluorine Intoxication from Drinking Water. *Acta Medica Scandinavica* 156 (3) 157-168 1956 (Read before the Fourth International Congress of Internal Medicine in Madrid, Spain, September 24, 1956).
- 6 Incipient Chronic Fluoride Intoxication from Drinking Water. II. Distinction between Allergic Reactions and Drug Intolerance. *International Archives of Allergy and Applied Immunology* 9 (5) 241-249 1956 (Read before the European Congress of Allergy in Florence, Italy, September 15, 1956).
- 7 Fluoride Intoxication from Drinking Water (A report of 52 cases). *Wissenschaftliche Zeitschrift der Ernst Moritz-Arndt-Universität-Greifswald* 5 (3/4) 307-316 1955/1956. (500th Centennial Festschrift).
- 8 Allergic Manifestations to Fluoride-Containing Water; Its Differentiation from Chronic Fluoride Poisoning. (Abstract) Atti del III Congresso Europeo di Allergologica, Firenze 12-15 September 1956.
- 9 Tetaniform Convulsions Precipitated by Fluoridated Drinking Water. *Confinia Neurologica* 17 (6) 339-347 1957.
- 10 Sur l'intoxication chronique provoquée par l'eau boisson fluorée in excès. *Revue Médicale de Liege* 12 (15) 408-411 1957.
- 11 Dermatologische Manifestationen bei chronischer Fluor-Vergiftung durch Trinkwasser. *Der Hautarzt* 8 (8) 368-370 1957 (Read before the SW German Dermatological Association, Bonn, Germany, October 1956). Stellungnahme zu den Bemerkungen von H Hornung. 9 (10) 468-471 1958.
- 12 Fluoride Poisoning in the Fluoridated Cities. Part II of *The American Fluoridation Experiment* (book by F B Exner and G L Waldbott, edited by J Rorty. Devin-Adair Company, New York 1957). Revised Edition 1961 pp 155-194.
- 13 Urinary Fluoride and Calcium Excretion in Persons Suspect of Fluoride Intolerance. *Henry Ford Hospital Medical Bulletin* 5 (4) 259-267 December 1957.
- 14 Letter to the Editor. *Journal of the American Dental Association* 55 (12) 873 December 1957.
- 15 A Test for Urinary Fluoride and Calcium Excretion. *Harper Hospital Bulletin* (Detroit, Michigan) 16 (2) 96 March-April 1958 (Abstract).
- 16 The Medical Aspect of Fluoridation. *The Torch* 31 (2) 41-47 April 1958.
- 17 Allergic Reactions from Fluorides. *International Archives of Allergy and Applied Immunology* 12 (6) 347-355 1958 (Presented at the Midwest Forum on Allergy, Columbus, Ohio, November 2, 1957).
- 18 Fluorose, hervorgerufen durch Trinkwasser. *Deutsche Medizinische Wochenschrift* 84 (15) 728-730 April 10, 1959.
- 19 Urticaria Due to Fluoride. *Acta Allergologica* 13 456-468 1959.
- 20 Chronic fluoride poisoning from drinking water. *Nordisk Medicin* 61 971 1959 (Presented before the Swedish Medical Society in Stockholm, November 4, 1958).

- 21 The Action of Fluorides. *Canadian Medical Association Journal* 82 940 April 30, 1960 (Comment on letter by Nikiforuk).
- 22 Physiology of Fluoride. *Patna Journal of Medicine* 34 (6) 225-236 June 1960.
- 23 "The Physiologic and Hygienic Aspects of the Absorption of Inorganic Fluorides" – Comments on the Symposium. *Archives of Environmental Health* 2 (2) 155-167 1961. Correspondence. 3 (4) 459 1962.
- 24 Is Fluoridation of Water Really Safe? *New Medical Materia* November 1961 p 48. Correction and addition: April 1962 p 20.
- 25 Fluoride in Clinical Medicine (monograph). Supplement 1 to *International Archives of Allergy and Applied Immunology* 20 1-60 1962.
- 26 Chronische Fluorvergiftung durch Trinkwasser. *Medizinische Klinik* 57 (30) 1284-1287 July 27, 1962.
- 27 Fluoride in Food. *American Journal of Clinical Nutrition* 12 (6) 455- 462 June 1963. Correction: 13 (6) 393 December 1963.
- 28 Acute Fluoride Intoxication (monograph). *Acta Medica Scandinavica* 174 (Supplement 400) 1-44 1963. (Reply to review by B D Dinman: *Archives of Environmental Health* 12 (2) 269 1966).
- 29 Fluoride in Food. In: T Gordonoff (Editor). *The Toxicology of Fluorine*. Symposium held in Bern, Switzerland, October 15-17, 1962. Schwabe and Company, Basle/Stuttgart 1964 pp 185-193.
- 30 Allergic Reactions to Fluoride. *Journal of Asthma Research* 2 (1) 51-64 September 1964. Dr Waldbott's Reply re Fluoride Allergy. 3 (3) 249-251 March 1966.
- 31 Fluoride and Optic Neuritis. *British Medical Journal* 5414 945 October 10, 1964 (Comment on article by Geall and Beilin).
- 32 Guest Editorial: Fluoride in Medicine. *GP* (Kansas City, Missouri) 30 76-77 December 1964. Reprinted in *American Family Physician* January 1965.
- 33 Letter to the Editor: Fluoride's Side Effects. *Saturday Review* 62-63 May 1, 1965.
- 34 *A Struggle With Titans – Forces Behind Fluoridation* (monograph). Carlton Press, New York 1965, 383 pp.
- 35 Fluoride in Food. *Pakistan Dental Review* 16 (1) 36-45 January 1966.
- 36 Fluoridation – You are the Guinea Pig. *Physician's Committee of the Greater Detroit Citizens Opposed to Fluoridation*. Detroit, Michigan, September 1966.
- 37 Fluoride and Calcium Levels in the Aorta. *Experientia* 22 (12) 835-837 December 15, 1966.
- 38 J J Shea, S M Gillespie, and G L Waldbott. Allergy to Fluoride. *Annals of Allergy* 25 (7) 388-391 July 1967.
- 39 L'Aspect Médical de la Fluoruration. *La Presse Médicale* 75 (34) 1733-1734 July 22-29, 1967.
- 40 Fluoridation: A Sampling of Documented Facts. *Midwest Physicians' Committee Studying Fluoridation*. Dayton, Ohio, December 1967.
- 41 Introduction to Fluoride Quarterly Reports. *Fluoride* 1 (1) 1 July 1968.
- 42 Editorial: New Observations on Fluorosis. *Fluoride* 1 (2) 54-55 October 1968.
- 43 Editorial: A Gap in Fluoride Research. *Fluoride* 1 (2) 55 October 1968.
- 44 Hydrofluorosis in the U.S.A. *Fluoride* 1 (2) 94-102 October 1968.
- 45 Intoxicación crónica por fluor. *Folia Clinica Internacional* 18 (11) 587 1968.
- 46 Editorial: Roholm's Contribution to the Air Pollution Problem. *Fluoride* 2 (1) 1 January 1969.
- 47 Editorial: Neighborhood Fluorosis in the U.S.A. *Fluoride* 2 (1) 2-3 January 1969.
- 48 Editorial: Tokio Takamori. *Fluoride* 2 (2) 74-75 April 1969.

- 49 Hidrofluorosis en los Estados Unidos. *Folia Clinica Internacional* 19 (5) 278-286 1969.
- 50 Le Fluor et le Scientifique. *Bulletin de la Société Scientifique d'Hygiène Alimentaire* 57 (7, 8, 9) 207-216 1969.
- 51 Editorial: Cancer and Fluoride. *Fluoride* 2 (3) 136-140 July 1969.
- 52 Editorial: Liver Damage in Chronic Fluoride Intoxication. *Fluoride* 2 (3) 140-141 July 1969.
- 53 Biological Action of Fluoride. Report of a Meeting. *American Journal of Clinical Nutrition* 22 (10) 1407-1409 1969.
- 54 G L Waldbott and V A Cicilioni. "Neighborhood" Fluorosis. *Fluoride* 2 (4) 206-213 1969. A slightly different version: *Clinical Toxicology* 2 (4) 387-396 December 1969.
- 55 Letter to the Editor: Atmospheric Fluorides. *Journal of the Air Pollution Control Association* 19 (12) 956 1969.
- 56 Editorial: Fluoride Intake Through Food. *Fluoride* 3 (1) 1-3 January 1970.
- 57 Editorial: "Chizzola" Maculae. *Fluoride* 3 (1) 1-3 January 1970.
- 58 G L Waldbott and V A Cicilioni. "Chizzola" Maculae. *Cutis* 6 331-334 March 1970 (Awarded First Prize in the Sixth Annual *Cutis* Manuscript Contest).
- 59 Editorial: Fluorocitrate in Bones. *Fluoride* 3 (2) 47-48 April 1970.
- 60 Editorial: Airborne Fluorides and Human Health. *Fluoride* 3 (3) 109-112 July 1970.
- 61 Editorial: Retinitis Pigmentosa Due to Fluoride. *Fluoride* 3 (3) 112-113 July 1970.
- 62 Letter to the Editor: Effects of Fluorides on Man. *Journal of the Air Pollution Control Association* 20 (9) 614 1970.
- 63 Editorial: Fluoride in Osteoporosis. *Fluoride* 3 (4) 164-166 October 1970.
- 64 Chronic Fluoride Intoxication Due to Air Pollution. In: H M and W T Beery (Editors). *Proceedings of the Second International Clean Air Congress of the International Union of the Air Pollution Association*. Academic Press, New York 1971. (Presented at the Second International Clean Air Congress, Washington, DC, December 6-11, 1970).
- 65 Editorial: The Fate of Fluoride in the Human Organism. *Fluoride* 4 (1) 1-4 January 1971.
- 66 Special Report: Airborne Fluoride in the Lake St. Clair-Detroit River Areas. *Fluoride* 4 (2) 93-96 April 1971.
- 67 Editorial: Fluoride Intoxication in Humans. *Fluoride* 4 (3) 102-108 July 1971.
- 68 Fluoridation of community water supplies. *Journal of Allergy and Clinical Immunology* 48 (4) 253-254 October 1971 (Letter to the Editor in response to statement of the Executive Board of the American Academy of Allergy on the Question of Allergy to Fluoride as Used in the Fluoridation of Community Water Supplies).
- 69 Editorial: Tokio Takamori. *Fluoride* 4 (4) 152-153 October 1971.
- 70 Editorial: Takamori's Research. *Fluoride* 5 (1) 1-2 January 1972.
- 71 Report: The Fourth Annual I.S.F.R. Conference. *Fluoride* 5 (1) 2-3 January 1972.
- 72 Editorial: Sea Salt and Skeletal Fluorosis. *Fluoride* 5 (2) 56-57 April 1972.
- 73 Editorial: Halothane Hepatitis and Methoxyflurane Nephropathy. *Fluoride* 5 (3) 103-105 July 1972.
- 74 Editorial: Can Fluoride Cause Lung Cancer? *Fluoride* 5 (4) 169-171 October 1972.
- 75 Editorial: Is Fluoride an Essential Element? *Fluoride* 6 (1) 1-3 January 1973.
- 76 Editorial: Perfluorochemicals as Substitutes for Blood. *Fluoride* 6 (2) 71-72 April 1973.
- 77 Disasters (Chapter 1), Systemic Poisons: Fluoride and Cadmium (Chapter 13). In: G L Waldbott. *Health Effects of Environmental Pollutants*. C V Mosby Company, St. Louis 1973, Second Edition 1978.

- 78 Editorial: Fluoride and Osteoporosis. *Fluoride* 6 (3) 123-126 July 1973.
- 79 Editorial: Fluoroacetate Toxicity. *Fluoride* 6 (4) 187-188 1973.
- 80 Editorial: "Optimal" Fluoride in Drinking Water. *Fluoride* 6 (4) 188 October 1973.
- 81 G L Waldbott and S Steinegger. New observations in "Chizzola" Maculae. In: *Proceedings of the Third International Clean Air Congress of the International Union of Air Pollution Prevention Association. October 8-12, 1973, Dusseldorf, Federal Republic of Germany.* Verein Deutscher Ingenieure, Dusseldorf 1975 pp A63-A67.
- 82 Editorial: Fluoride and the Heart. *Fluoride* 7 (1) 1-3 January 1974.
- 83 Editorial: Sodium Fluoride in Osteoporosis. *Fluoride* 7 (2) 65-68 April 1974.
- 84 Editorial: The Pre-Skeletal Phase of Chronic Fluoride Intoxication. *Fluoride* 7 (3) 118-122 July 1974.
- 85 Letter to the Editor: Fluorides. *Environment* 16 (6) 42 July/August 1974 (Comment on article by M J Prival).
- 86 Editorial: Fluoride Versus Sulfur Oxides in Air Pollution. *Fluoride* 7 (4) 174-176 October 1974.
- 87 G L Waldbott and W Oelschlager. Fluoride in the Environment. *Fluoride* 7 (4) 220-222 October 1974.
- 88 How Safe is Fluoridation? A Brief Review of Supporting Evidence. *Fluoride* 8 (1) 41-47 January 1975.
- 89 Editorial: Serendipity and Research on Fluoride. *Fluoride* 8 (2) 58-60 April 1975.
- 90 Editorial: Inert Fluorocarbons. *Fluoride* 8 (3) 121-124 July 1975.
- 91 Editorial: Placental Transfer of Fluoride. *Fluoride* 8 (4) 178-181 October 1975.
- 92 Editorial: Target Organs in Fluorosis. *Fluoride* 9 (1) 1-4 January 1976.
- 93 Symposium on the Non-Skeletal Phase of Chronic Fluorosis: Introduction. *Fluoride* 9 (1) 5-8 January 1976.
- 94 Symposium on the Non-Skeletal Phase of Chronic Fluorosis: The Arteries. *Fluoride* 9 (1) 24-28 January 1976.
- 95 The Skeletal Changes in Chronic Fluorosis. *Fluoride* 9 (2) 71-72 April 1976.
- 96 Editorial: Fluoride Content of Teeth and Drinking Water in Relation to Dental Caries. *Fluoride* 9 (3) 124-126 July 1976.
- 97 Editorial: Industrial Fluorosis. *Fluoride* 9 (4) 170-172 October 1976.
- 98 Editorial: Fluoride and the Skin. *Fluoride* 10 (1) 1-4 January 1977.
- 99 G L Waldbott and M N Zacks. Blood Clotting in Patients with "Chizzola" Maculae. *Fluoride* 10 (1) 29-33 January 1977.
- 100 Editorial: Skeletal Fluorosis near Fluoride-Emitting Factories. *Fluoride* 10 (2) 45-47 April 1977.
- 101 Editorial: The Fluoride-Cancer Controversy. *Fluoride* 10 (3) 95-101 July 1977.
- 102 G L Waldbott and J Yiamouyiannis. Special Report. AAAS Fluoride Symposium in Denver. *Fluoride* 10 (3) 141-144 July 1977.
- 103 Editorial: Gastric Ulcer and Fluoride. *Fluoride* 10 (4) 149-151 October 1977.
- 104 Editorial: Fluoride in Soil. *Fluoride* 11 (1) 1-3 January 1978.
- 105 Editorial: Reliability of the Double-Blind Test in Fluoride Poisoning. *Fluoride* 11 (2) 43-45 April 1978.
- 106 Editorial Review: Nonskeletal Fluorosis. *Fluoride* 11 (3) 111-114 July 1978.
- 107 G L Waldbott and J R Lee. Toxicity from Repeated Low-Grade Exposure to Hydrogen Fluoride - Case Report. *Clinical Toxicology* 13 (3) 391-402 October 1978.
- 108 Editorial: Toxicity of Fluoride. *Fluoride* 11 (4) 163-165 October 1978.
- 109 G L Waldbott, A W Burgstahler, and H L McKinney. *Fluoridation: The Great Dilemma* (book). Coronado Press, Lawrence, Kansas 1978, 423 pp.
- 110 Editorial Review: Environmental Fluoride. *Fluoride* 12 (1) 1-3 January 1979.

- 111 Editorial: The Diagnosis of Incipient Fluorosis. *Fluoride* 12 (1) 3-4 January 1979.
- 112 Preskeletal Fluorosis Near an Ohio Enamel Factory: A Preliminary Report. *Veterinary and Human Toxicology* 21 (1) 4-8 February 1979.
- 113 Pre-Skeletal Neighborhood Fluorosis: An Epidemic Near an Ohio Enamel Smelter. *Veterinary and Human Toxicology* 21 (Supplement) 140-144 1979.
- 114 Editorial: Another Fluoride Fatality: A Physician's Dilemma. *Fluoride* 12 (2) 55-57 April 1979.
- 115 Editorial: Fluoride's Effect on Collagen – A Breakthrough. *Fluoride* 12 (3) 111-113 July 1979.
- 116 Editorial: Preskeletal Phase of Fluorosis. *Fluoride* 12 (4) 169-171 October 1979.
- 117 Health Impact of Fluoride in Air and Water – International Clinical Data. In: G K Chacko (Editor). *Health Handbook*. North-Holland Publishing Company, 1979 pp 49-74.
- 118 Editorial: Fluoride and Kidney Stones. *Fluoride* 13 (1) 1-3 January 1980.
- 119 Fluoridation: A Clinician's Experience. *Southern Medical Journal* 73 (3) 301-306 March 1980. Dr Waldbott Replies to Comment by J M Norton. 74 (4) 519 April 1981.
- 120 Editorial: Factors Affecting Tolerance to Fluoride. *Fluoride* 13 (2) 46-48 April 1980.
- 121 Letter to the Editor: Fluoride Research: Report of a Meeting. *Clinical Toxicology* 16 (3) 391-394 May 1980.
- 122 Editorial: Antidotes for Fluoride Intoxication. *Fluoride* 13 (3) 96-99 July 1980.
- 123 Letter to the Editor: Fluoride Intoxication. *Journal of the American Medical Association* 244 (4) 331 July 25, 1980.
- 124 L'Interesse dell'Allergologo nei Fluoruri. *Asma Allergia Immunopatologia* (Milano) 51 3-9 August 31, 1980.
- 125 Readers Forum: Comments on Fluoridation. *Journal of Nutrition Education* 12 (3) 145-146 July-September 1980.
- 126 Editorial: Standards for Fluoride Tolerance. *Fluoride* 13 (4) 145-147 October 1980.
- 127 Editorial: The Diagnosis of Fluorosis. *Fluoride* 14 (1) 1-3 January 1981.
- 128 Editorial: Food-Induced Skeletal Fluorosis. *Fluoride* 14 (2) 49-50 April 1981.
- 129 Mass Intoxication from Accidental Overfluoridation of Drinking Water. *Clinical Toxicology* 18 (5) 531-541 1981.
- 130 Editorial: Fluoride and Soft Tissue Calcifications. *Fluoride* 14 (3) 99-101 July 1981.
- 131 Editorial: Urinary Fluoride and Fluoride Toxicity. *Fluoride* 14 (4) 147-149 October 1981.
- 132 Editorial: Classification of Dental Fluorosis. *Fluoride* 15 (2) 52-53 April 1982.
- 133 Editorial: The Value of Urinary Fluoride Determinations. *Fluoride* 15 (3) 109 July 1982.
- 134 In Memoriam: Philip E. Zanfagna, M.D. *Fluoride* 15 (3) 109 July 1982.
- 135 Accidental Overfluoridation (Unfallverursachte Überfluorisierung). *Klinische Wochenschrift* 60 (15) 813-814 August 1982.
- 136 Editorial: Overfluoridation. *Fluoride* 15 (4) 169-172 October 1982.
- 137 Editorial: Fluoride Therapy and Osteoporosis. *Fluoride* 16 (1) 1-4 January 1983.
- 138 Editorial: A Clinician's Dilemma. *Fluoride* 16 (2) 70-71 April 1983.
- 139 Subacute Fluorosis Due to Airborne Fluoride. *Fluoride* 16 (2) 72-82 April 1983.
- 140 The Effect of Fluoride on the Human Organism (in Polish). *Czasopismo Stomatologiczne* 36 (9) 653-654 1983.
- 141 Editorial: Chronic Fluoride Intoxication. *Fluoride* 16 (4) 195-197 October 1983.
- 142 The Preskeletal Phase of Chronic Fluoride Intoxication. *Fluoride* 31 (1) 13-20 February 1998.

TOXIC EFFECTS OF FLUORIDE ON BEATING MYOCARDIAL CELLS CULTURED *IN VITRO*

FuYuan Wang,^a DeXin Zhang,^a and RuiMian Wang^b
Wuhan, China

SUMMARY:

Objective: To study the effect of fluoride on myocardial cells.

Method: Three days after seeding *in vitro*, five groups of neonatal rat myocardial cells were cultured for one day in media containing fluoride at concentrations of 0, 3.23, 6.46, 12.91, and 25.81 ppm respectively.

Results: Higher fluoride levels were associated with an increased beat arrest rate, media lactate dehydrogenase (LDH) activity and media nitric oxide (NO) level, and a decreased cell glycogen level and cell succinate dehydrogenase (SDH) activity. Scanning electron microscope study of the beat-arrested cells showed them to be rounder and smaller than the beating cells with the loss of microvilli, the shortening or loss of cell processes, and the appearance of pits and blebs on the cell surfaces.

Conclusion: Fluoride can be toxic to myocardial cells with possible mechanisms for this toxicity being impaired energy metabolism, cell membrane damage, increased NO production causing cell relaxation, and dissociation of the cytoskeleton. A threshold for fluoride toxicity was not established as significant toxicity was present at the lowest level studied, of 3.23 ppm of fluoride in the media.

Key words: Beat arrest rate; Fluoride toxicity; Myocardial cells; Rat.

INTRODUCTION

There are differing reports of the effect of fluoride on cardiovascular disease. A more rapid decline in cardiovascular mortality has been reported in association with fluoridation.¹ Another report, from Antigo, Wisconsin, USA, found a seven-fold increase in the annual cardiac death rate from 5 deaths per 100,000 in 1949, to 35 deaths per 100,000 population in 1960, when fluoridation was stopped.² When an adjustment was made for age the cardiac death rate increased ten-fold from 1.2 deaths per 100,000 in the decade prior to fluoridation to 12 deaths per 100,000 after the introduction of fluoridation. The increase in the annual cardiac death was interrupted when fluoridation was discontinued for five years but resumed again two years after the reintroduction of fluoridation. In China, significantly more cardiovascular disease³ and more electrocardiogram abnormalities⁴ have been found in an area with endemic fluorosis compared to an area without endemic fluorosis. Foetal myocardial cells have been found to be abnormal in an endemic fluorosis area.⁵ Experiments *in vitro* have shown that fluoride may have a positive or negative effect on the contractility of muscle.^{6,7}

Because of the potential for confounding factors to affect an epidemiological study, the difficulties involved in detecting toxic effects of fluoride on myocardial cell *in vivo*, and the only limited time for which myocardial cells can be maintained in a viable and stable state for *in vitro* experimentation, it was elected to use cultured neonatal rat myocardial cells *in vitro* to study the effect of fluoride on myocardial cells, concentration-response effects, and the mechanisms by which toxicity occurred.

^a Department of Hygiene. ^b Department of Histology and Embryology, Hubei Medical University, Wuhan, 430071 People's Republic of China.

MATERIALS AND METHODS

1. Primary culture of beating myocardial cells

The hearts of neonatal Sprague-Dawley rats were excised, washed with phosphate-buffered saline (PBS) at pH 7.2 and minced.⁸ The cells were isolated with 0.125% trypsin and purified with 6% bovine serum albumin (BSA) and cytosine-1 β -D-arabinofuranoside (ARA-C), the final concentration of which in the medium was 10 μ M. The muscle cells were seeded at a density of 10⁵ cells/mL in plastic culture dishes which had 24 wells with cover-slips. The muscle cells were maintained in medium 199 (Gibco) supplemented with 15% foetal bovine serum (Gibco) and antibiotics. The cultures grew at 37°C in a humidified incubator gassed with 95% air/5% carbon dioxide.

2. Fluoride exposure

Seventy-two hours after seeding the cultures were divided into five groups each containing seven cultures. Medium 199 was added to the medium of the control group and sodium fluoride to the other four groups to give final concentrations of fluoride for Group A of 3.23 parts per million (ppm) (mg/L), Group B 6.46 ppm, Group C 12.91 ppm, and Group D 25.81 ppm. The culture was then continued for 24 hours.

3. Beat arrest rate of myocardial cells

The number of beating cell clusters was counted under an inverted microscope at 72 hours after seeding before exposure to fluoride and 96 hours after seeding following 24 hours of exposure to fluoride. The beat arrest rate was calculated from the formula:

$$\text{Beat arrest rate (\%)} = \frac{A-B}{A} \times 100 (\%)$$

A: number of beating clusters at 72 hours, before exposure to fluoride

B: number of beating clusters at 96 hours, 24 hours after exposure to fluoride

4. Cytochemical reactions and image analysis

Twenty-four hours after exposure to fluoride, the Periodic Acid Schiff Reaction (PAS) and the Nachlass Nitro-blue Tetrazolium Reaction (Nitro-BT) were carried out on the cultures to detect glycogen and SDH activity in the myocardial cells.⁹ The optical density (OD) values of the reactions were determined with image analysis equipment (Kontron IBAS). Thirty microscopic visual fields in 7 cultures (4 or 5 visual fields in one culture) of each group were determined.

5. Analysis of media LDH and NO

Media from each group were collected at 72 hours and 96 hours to determine LDH activity and the NO level.¹⁰ The NO level was determined by measuring absorption at 490 nm and calculating using a curve calibrated from sodium nitrite standards.

6. Scanning electron microscopy (SEM)

At 96 hrs the control culture and Groups B and D were prepared with routing for SEM and observed under a scanning electron microscope (Hitachi S-450).

7. Statistical analysis

Statistical analysis was carried out using the Statistical Analysis System V6.03. Analysis of variance (F value) was used to test for significance of the experimental values obtained for the groups. The Student-Newman-Keuls test (q test) was used to test for significant differences between groups.

RESULTS

1. Beat arrest rate of myocardial cells

When examined under the inverted microscope 24 hours after seeding the myocardial cells were found to have developed into cylindrical or polygonal cells with some processes but the majority of them were not beating. At 72 hours the myocardial cells had increased in size and cell clusters had formed through active proliferation. As the cells came in contact with one another they connected through the long processes on the cells and most began to beat in synchrony. At 96 hours, after exposure to fluoride for 24 hours, the beat lost its regularity and beat arrest appeared. The beat arrest rate increased with increasing concentrations of fluoride in the media (Table 1). The median beat arrest concentration (BAC₅₀) for fluoride, using the modified Karber method of calculation, was 8.05 ppm, 95% confidence interval 7.23-8.91 ppm.

TABLE 1. Beat arrest rate of myocardial cells and media fluoride concentration

| Group | Fluoride in media (ppm) | No. of samples | No. of beating clusters | | Beat arrest rate (%) (Mean \pm SD) |
|---------|-------------------------|----------------|----------------------------|-----------------------------|---|
| | | | 72 hours (pre-exposure) | 96 hours (post-exposure) | |
| Control | 0 | 7 | 69 | 69 | 0 \pm 0 |
| A | 3.23 | 7 | 93 | 77 | 17.2 \pm 0.77 |
| B | 6.46 | 7 | 94 | 68 | 27.7 \pm 2.01 |
| C | 12.91 | 7 | 103 | 27 | 73.8 \pm 2.71 |
| D | 25.81 | 7 | 87 | 1 | 98.9 \pm 0.58 |

Comparing the media fluoride level and the beat arrest rate

$$r = 0.9688 \quad y = 5.855 + 3.9005x$$

2. Cytochemical reaction and image analysis

In the control group of beating cells, the presence in the cell bodies and processes, of glycogen was indicated by pink granules or coarse clumps (Figure 1), and SDH activity by purplish-blue granules (Figure 3). In the groups with fluoride, A - D, there was a progressive reduction in the intensity of the pink (Figure 2) and purplish-blue colouration (Figure 4), and optical density values. A significant negative correlation was present between the fluoride concentration and the glycogen content and the SDH activity (Table 2).

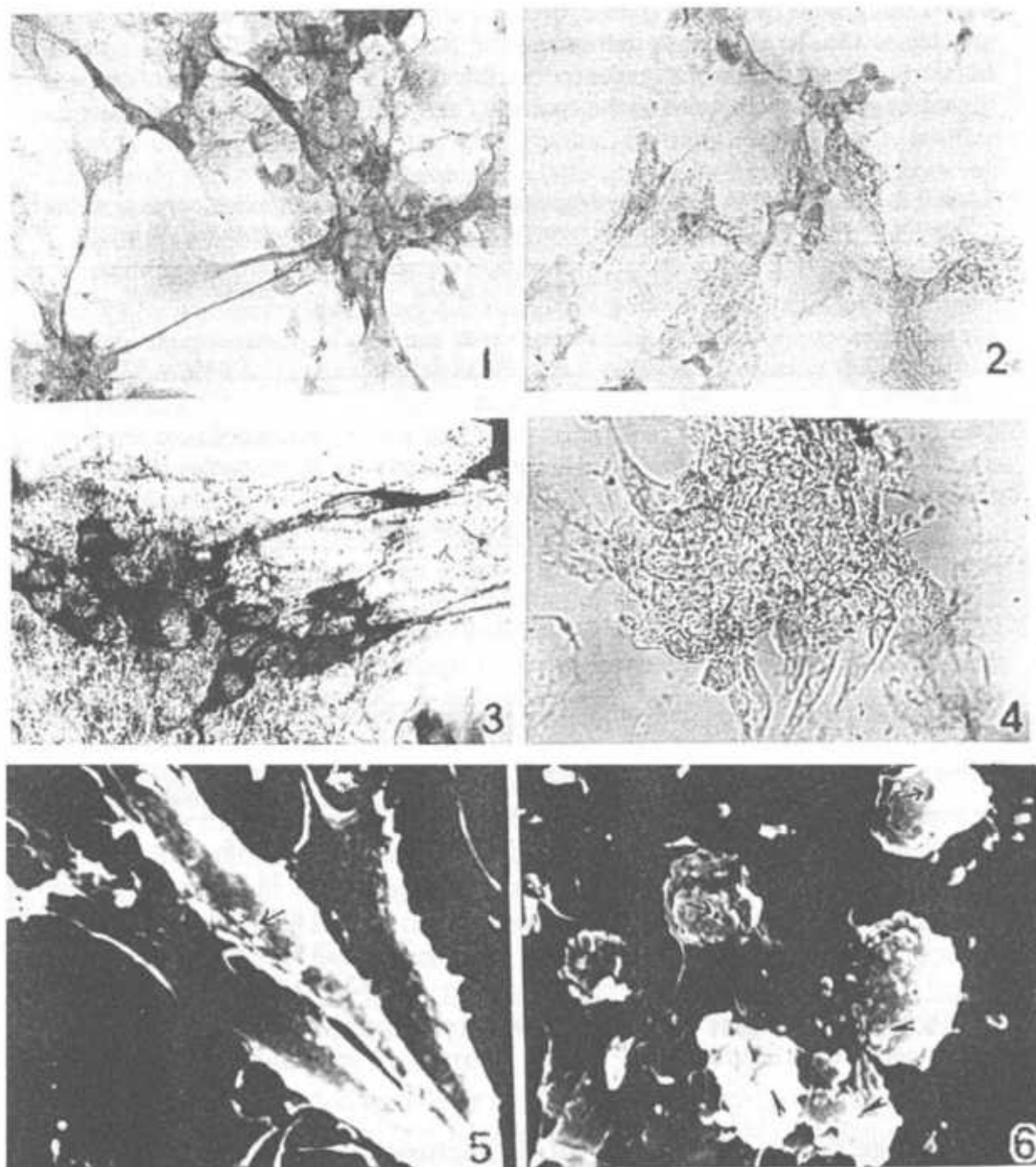
TABLE 2. Optical density values of glycogen content and succinate dehydrogenase activity after exposure to fluoride for 24 hours

| Group | Fluoride in media (ppm) | No. of samples | Glycogen content ^a (mean \pm SD) | Succinate dehydrogenase activity ^b (mean \pm SD) |
|---------|-------------------------|----------------|--|--|
| Control | 0 | 30 | 0.3066 \pm 0.0281 | 0.4569 \pm 0.0563 |
| A | 3.23 | 30 | 0.3017 \pm 0.0254 | 0.4486 \pm 0.0449 |
| B | 6.46 | 30 | 0.2656 \pm 0.0141* | 0.4048 \pm 0.0330 |
| C | 12.91 | 30 | 0.2277 \pm 0.0101* | 0.2828 \pm 0.0124* |
| D | 25.81 | 30 | 0.2164 \pm 0.0093* | 0.2252 \pm 0.0165* |

$$a \quad F = 140.33 \quad p < 0.0001 \quad y = 0.2996 - 0.004x$$

$$b \quad F = 242.75 \quad p < 0.001 \quad y = 0.4585 - 0.0010x$$

* compared to the control $p < 0.05$



Figures 1-6. Myocardial cells from rats cultured for 96 h

1. Beating cell clusters of control group. Positive PAS reaction (for glycogen). x 400. **2.** Beat arrested cell cluster of Group D. Weakened PAS reaction. x 400. **3.** Beating cell clusters of control group. Positive SDH reaction. x 400. **4.** Beat arrested cell cluster of Group D. Negative SDH reaction. x 400. **5.** Beating cell clusters of control group by SEM. Myocardial cells are cylindrical with processes (\hookrightarrow). Microvilli (\angle) distributed evenly in cell surface. X 6000. **6.** Beat arrested cells of Group D by SEM. Myocardial cells round and smaller. Pits (∇) instead of microvilli and blebs (\triangleright) appear on cell surface.

3. Lactate dehydrogenase activity and nitric oxide content in the media

After 24 hours of exposure to fluoride, the LDH activity in the media was higher in groups B,C and D compared to the control, and increased, in all groups, as the fluoride concentration in the media increased (Tables 3 and 4). Similarly after 24 hours of exposure to fluoride the nitric oxide content was higher in group D compared to the control (Table 3).

TABLE 3. Changes in lactate dehydrogenase activity and nitric oxide content of the media, between 72 hours and 96 hours after exposure to fluoride for 24 hours

| Group | Fluoride in media (ppm) | No. of samples | Change in Lactate dehydrogenase activity ^a (activity units) between 72 and 96 h (mean \pm S.D.) | Change in Nitric oxide content (nitrite N mg/L) between 72 and 96 h (mean \pm S.D.) |
|---------|-------------------------|----------------|--|---|
| Control | 0 | 7 | 23.3 \pm 22.2 | 0.0003 \pm 0.0035 |
| A | 3.23 | 7 | 104.9 \pm 49.1 | 0.0002 \pm 0.0015 |
| B | 6.46 | 7 | 282.6 \pm 56.0** | 0.0002 \pm 0.0090 |
| C | 12.91 | 7 | 272.4 \pm 54.3** | 0.0008 \pm 0.0223 |
| D | 25.81 | 7 | 470.1 \pm 62.0** | 0.0328 \pm 0.0105* |

^a F=9.94 p<0.0001 r=0.9730

* compared to the control p<0.05

** compared to the control p<0.01

TABLE 4. Lactate dehydrogenase activity of the media, at 96 hours after seeding, after exposure to fluoride for 24 hours

| Group | Fluoride in Media (ppm) | No. of samples | Lactate dehydrogenase activity ^a (activity units) at 96 h (mean \pm S.D.) |
|---------|-------------------------|----------------|--|
| Control | 0 | 7 | 483.86 \pm 28.15 |
| A | 3.23 | 7 | 551.71 \pm 74.35 |
| B | 6.46 | 7 | 713.86 \pm 77.12* |
| C | 12.91 | 7 | 782.29 \pm 51.36* |
| D | 25.81 | 7 | 982.57 \pm 68.20* |

^a F=9.94 p<0.0001 r=0.97 y = 520.79 - 18.80x

* compared to the control p<0.05

4. Damage to the cell surface ultrastructure

In the control group the beating myocardial cells had a cylindrical or polygonal shape with microvilli distributed evenly on the cell surfaces along with slender processes growing from the cell bodies connecting with the processes or cell bodies of nearby cells (Figure 5). In groups B and D the beat-arrested cells became smaller and rounder than the beating cells with the microvilli disappearing, the processes becoming shortened or absent, and the appearance of many pits or blebs on the cell surfaces (Figure 6).

DISCUSSION

Two types of myocardial cells were present in the cultures. One was pacemaker cells from the conductive system which contracted rhythmically with diastolic depolarization. The other type was the normal atrial and ventricular myocardial cells which were not able to contract or beat until able to make contact with the pacemaker cells or other beating cells.¹¹ When exposed to fluoride the myocardial cells may stop beating. In the present study a positive correlation and concentration-response relationship were found to exist between the concentration of fluoride and the beat arrest rate. Fluoride was found to be toxic or poisonous to the myocardial cells.

The myocardial cells in culture absorb glucose from the medium to produce energy for contracting and other metabolic functions or the synthesis of glycogen. The glycogen content reflects not only the availability of an energy store but the adenosine triphosphate (ATP) content in the cells. In measuring the activity of SDH, one of the dehydrogenases in the citric acid (tricarboxylic acid, Krebs's) cycle an indication is obtained of the ability to produce ATP. This study showed that a possible mechanism by which fluoride produced beat arrest in myocardial cells was by reducing the glycogen content and SDH activity in the cells indicating a reduced availability of energy in the form of ATP.

Lactate dehydrogenase is an enzyme in the cell cytoplasm. The raised levels of LDH in the media after the addition of fluoride indicates that the cell membrane is damaged by fluoride resulting in an increase in the permeability of the cell membrane and the escape of the enzyme from the cytosol. As the cell membrane of the myocardial cell is closely involved with excitation and contraction, damage to it may be another factor contributing to beat arrest.

Nitric oxide is formed from L-arginine by the enzyme NO synthase,¹² several isoforms of which have been purified and characterized from different cell types.¹³ In the myocardial cell a calcium-independent NO synthase isoform (iNOS) predominates.¹⁴ When iNOS is activated it produces NO which exerts a negative inotropic effect by increasing the level of the cyclic guanosine monophosphate (cGMP). In the present study the NO content in the medium increased when the fluoride content in the medium reached 25.81 ppm. In association with this a large number of myocardial cells stopped beating suggesting that the NO increase resulting from iNOS activated by fluoride may be another mechanism for beat arrest.

The marked alteration in cellular morphology associated with fluoride reflects damage to the cytoskeleton. In the course of plating, an adhesion plaque, made of vinculin and talin, appears at the inside of the cell membrane, which induces actin to assemble, producing fibres which support the cell and allow it to plate, spread, and extrude processes. A prerequisite for the assembly of actin is the presence of ATP¹⁵ which is reduced in availability by fluoride. The deficiency of ATP produced by fluoride may result in the dissociation of the skeleton of actin filaments bringing about injurious alterations in cell form and cell surface ultrastructure with finally cell death and shedding from the cover slip.

In conclusion, fluoride may be directly toxic or poisonous to myocardial cells. While there may be debate as to whether or not fluoride in small amounts may have a beneficial effect on the presence of dental caries, it is clear that in increasing amounts it is increasingly toxic to the heart. Exposure to excessive amounts of fluoride should be avoided. A threshold for fluoride toxicity was not established, as significant toxicity was present at the lowest level studied, of 3.23 ppm of fluoride in the media.

REFERENCES

- 1 Yang L. The toxicity of fluoride. *Foreign Medical Sciences (Section of Hygiene)* 5 277-279 1986 (in Chinese).
- 2 Jansen I, Thomson HM. Heart deaths and fluoridation. *Fluoride* 7 52-56 1974.
- 3 Liang Z, Wang Y, Zhang Z et al. Investigation and analysis of cardiovascular disease of residents in an area of endemic fluorosis and an area of non-fluorosis. *Hebei Medical Journal* 8 76-77 1986 (in Chinese).
- 4 Ji X, Li S, Li Y et al. A clinical survey of damage to the cardiovascular system in patients with fluorosis from coal smoke pollution. *Chinese Journal of Control of Endemic Disease* 8 18-21 1993 (in Chinese).
- 5 Wen P, Yang W. Effect of chronic fluorosis on myocardial cells of human foetuses. *Chinese Journal of Endemiology* 16 7-9 1997 (in Chinese).
- 6 Wang J, Ju D. Effects and mechanism of sodium fluoride on the physiological properties of rabbit atrial muscles. *Endemic Disease Bulletin* 5 50-53 1990.
- 7 Qin C, Wang J, Li X et al. The effect of fluoride on the electromechanical activities of myocardium. *Chinese Journal of Public Health* 9 17-20 1990.
- 8 Wang R, Rong C, Gui Z. Cytochemical studies on beating and beat arrested cardiocytes cultured *in vitro*. *Acta Anatomica Sinica* 20 (monograph) 48-52 1989.
- 9 Zhu F. *New Histochemistry of Enzymes*. People's Health Publishing House, Beijing 1983 p 62.
- 10 Termin A, Hoffmann M, Bing RJ. A simplified method for the determination of nitric oxide in biological solutions. *Life Sciences* 51 1621-1629 1992.
- 11 Ross G. *Essentials of Human Physiology*. 2nd ed. Year Book Medical Publisher, Chicago 1982 pp 149-152.
- 12 Lowenstein CJ, Dinerman JL, Snyder HS. Nitric oxide: a physiologic messenger. *Annals of Internal Medicine* 120 227-237 1994.
- 13 Knowles RG, Moncada S. Nitric oxide synthases in mammals. *Biochemical Journal* 298 249-258 1994.
- 14 Shindo T, Ikeda U, Ohkawa F et al. Nitric oxide synthesis in rat cardiac myocytes and fibroblasts. *Life Sciences* 55 1101-1108 1994.
- 15 Carlier MF, Pantaloni D. Nucleotide hydrolysis in actin and tubulin polymerization: liberation of Pi is the crucial step in the regulation of dynamic of microtubules and actin filaments. In: Rousset BAF (Ed). *Structure and Functions of the Cytoskeleton: Biological and Physiopathological Aspects*. John Libbey, London 1988 pp 33-41.

FLUORIDE INTAKE, DISTRIBUTION, AND BONE CONTENT IN DIABETIC RATS CONSUMING FLUORIDATED DRINKING WATER

I Boros, P Keszler, G Csikós and H Kalász*
Budapest, Hungary

SUMMARY: The aim of this study was to determine how metabolic and functional changes in diabetes affect the fluoride intake, distribution, and concentration in bone tissue; and whether alterations in fluoride metabolism in diabetes may influence the severity of the disorder. Two groups of rats received 0 (C) or 10 ppm (F10) fluoride via drinking water for three weeks, *ad libitum*. Two other groups were treated with a single dose of streptozotocin to induce diabetes, and also received 0 (D) or 10 ppm fluoride (DF10). The quantity of fluoride consumed via water by the DF10 animals was calculated daily and an equal amount was added to the drinking water of another group of non-diabetic animals (FF).

In the diabetic group (DF10) the intake of fluoride gradually increased, hyperglycemia was more severe, and renal hypertrophy was expressed less than in the diabetic group (D) which consumed deionized water. The femoral fluoride concentration increased in proportion to fluoride intake. The high fluoride intake of FF animals resulted, when compared to DF10 ones, in a further increase in the bone tissue and in relatively less elevation in plasma fluoride concentrations. It is concluded that (i) fluoride supply *via* drinking water may enhance the severity of diabetes in rats, and (ii) due to diabetic metabolic and functional imbalance, the fluoride metabolism may also change.

Key words: Bones, Diabetes, Distribution, Fluoride intake, Renal hypertrophy.

INTRODUCTION

Animal models of diabetes induced by alloxan or streptozotocin exhibit both renal hypertrophy and hyperplasia.^{1,2} Presumably, the increased glomerular filtration rate and elevation in glomerular pressure may contribute to diabetic nephropathy,³ which progresses to renal disease. Classic symptoms, e.g. increased thirst, elevations in food consumption and in fluid intake are present both in humans and animals. In addition, uncontrolled diabetes can lead to significant acid-base disturbances (ketoacidosis) as well.⁴ Diabetic patients and animals may also be at higher risk of dental disorders.^{5,6} Retardation in salivary flow (xerostomia) is another common symptom of the disorder.^{7,8}

It has often been considered that fluoride at a concentration of 1 ppm in drinking water is beneficial in reducing dental caries in humans under normal conditions and does not cause adverse effects.⁹ However, more definitive experiments are required to substantiate these claims. It is suggested that diabetes may influence the food and fluid intakes, and may also affect the body fluid and bone tissue fluoride concentrations, as well as the fluoride excretion. Only limited and contradictory data exist to show that fluoride retention and excretion is altered in diabetes.¹⁰⁻¹⁴ It was also reported that fluoride metabolism greatly depends on acid-base status, and the clearance of fluoride is directly related to the urinary pH.^{15,16} Reduction in renal clearance induced by

Department of Oral Biology, Faculty of Dentistry, and *Institute of Pharmacology, Semmelweis University Medical School, Budapest, Hungary.
Presented at the XXist Conference of the International Society for Fluoride Research, Budapest, Hungary, August 25-28, 1996.

acidosis may be responsible for increased enamel and plasma fluoride concentrations, elevated soft and bone tissue fluoride levels, and also for disturbances in enamel mineralization which are similar in appearance to true fluorosis.¹⁶⁻¹⁸

Based on these observations, the objectives of this study were to monitor the daily intake of fluoride, the plasma fluoride levels, and the fluoride concentrations in the femora of rats supplied with fluoride in drinking water for three weeks after a single IV administration of streptozotocin. Parameters were compared to non-diabetic, intact groups with or without fluoride treatment.

MATERIALS AND METHODS

Five groups of female young rats (Charles-River, Wistar, N=8/group) initially weighing 195 ± 14 g were housed in stainless steel individual cages. In two groups, after a 16 h fasting period diabetes was induced by a single *iv* dose of streptozotocin (Zanosar, Upjohn Co USA, 5 mg/100 g bw). The remaining groups were given citrate buffer (pH 4.5). Rats were provided a pelleted diet with 13 ppm of fluoride (Charles-River Wiga GmbH, Germany) and deionized water (groups C and D) or fluoridated water with 10 ppm fluoride as sodium fluoride (groups F10 and DF10) *ad libitum* throughout the three-week treatment period. One group of the non-diabetic rats (group FF) was given the same amount of fluoride *via* drinking water as DF10 animals, but water consumption was not limited. The 24 h intakes of food and water by each of the groups were determined twice a week and daily, respectively. Glucosuria was checked with conventional test strips at 72 h, midterm and on the last day before sacrifice. Euthanasia was performed between 9.00-11.00 am, and animals from the different groups were sacrificed in random order by bleeding through the femoral vein and bilateral pneumothorax under pentobarbital anesthesia preceded by 14 h fasting. Blood, kidneys, and femora were used for analysis. Citrated blood was centrifuged immediately after collection, and plasma was collected for fluoride analysis. For determination of blood glucose levels deproteinized (0.3 M perchloric acid) and centrifuged samples were assayed within 2 h. The femora were removed, cleaned of soft tissues and weighed. After drying at 100°C they were ashed at 500°C for 24 h. Aliquots of pulverized ashed samples were analyzed for fluoride. Plasma samples were analyzed for fluoride by using an ion-specific electrode (Radelkis, Hungary). Plasma fluoride concentration was recorded with the known addition technique¹⁹ with some modification for better recovery and accuracy.²⁰ Hard tissue fluoride concentrations were determined according to McCann.²¹ Blood glucose was detected by the glucose-oxidase method using Boehringer-kit (Test-Combination Glucose, Mannheim GmbH, Diagnostica, Germany). Results were expressed as mean values \pm SD. Differences between two means were analyzed for statistical significance using Student's t-test.

RESULTS

Table 1 shows the body weights of different groups at the termination of the experiment. As shown, the fluoride intake *via* drinking fluid did not influence the body weight of intact, non-diabetic groups F10 and FF. In contrast, the body weight in group D was reduced and a significant weight loss in group DF10 was recorded. It is also illustrated here that the daily water consumption of diabetic groups was progressively elevated.

TABLE 1. Final body weight (g), water consumption (mL/day/rat) and the control of glucosuria (Gu) at different time intervals

| Group | Body wt | Water cons. 1-4 day | Gu | Water cons. 5-7 day | Water cons. 8-14 day | Gu | Water cons. 5-21 day | Gu |
|------------------|----------|------------------------|----|------------------------|-------------------------|----|-------------------------|----|
| Non-diabetic: | | | | | | | | |
| C (non-F water) | 257 ± 14 | 38 ± 8.0 | Ø | 36 ± 7.0 | 34 ± 6.0 | Ø | 36 ± 9.0 | Ø |
| F10 (F water) | 247 ± 19 | 30 ± 7.0 | Ø | 25 ± 6.0 | 30 ± 6.0 | Ø | 31 ± 7.0 | Ø |
| Diabetic: | | | | | | | | |
| D (non-F water) | 229 ± 12 | 111 ± 37 | + | 161 ± 60 | 172 ± 77 | + | 188 ± 88 | + |
| DF10 (F water) | 170 ± 18 | 132 ± 25 | + | 217 ± 40 | 228 ± 23 | + | 256 ± 39 | + |
| Non-diabetic: | | | | | | | | |
| FF (= F to DF10) | 248 ± 12 | 32 ± 4.0 | Ø | 24 ± 3.0 | 25 ± 3.0 | Ø | 24 ± 4.0 | Ø |

Mean ± SD. Values between groups are significantly different; (C - DF10: $p < 0.001$)

The fluoride intake per day per rat at different time intervals is presented in Table 2. During the experimental period the fluoride intake from pellet of the controls showed only small variations. The total daily fluoride intake of group F10 was about twice of that of control. In group D the daily consumption of fluoride was in the same range as in group F10, as a consequence of polyphagia. Diabetic polydipsia occurred in group DF10 and resulted in an excessive fluoride intake with a mean of 3.16 ± 0.43 mg fluoride/day/rat at the last week of treatment. In this period the main source of fluoride seemed to be the fluoridated water, i.e., nearly 80% of total daily intake derived from the drinking water. In group FF compared to DF10 a nearly equal quantity of fluoride intake from water could be observed.

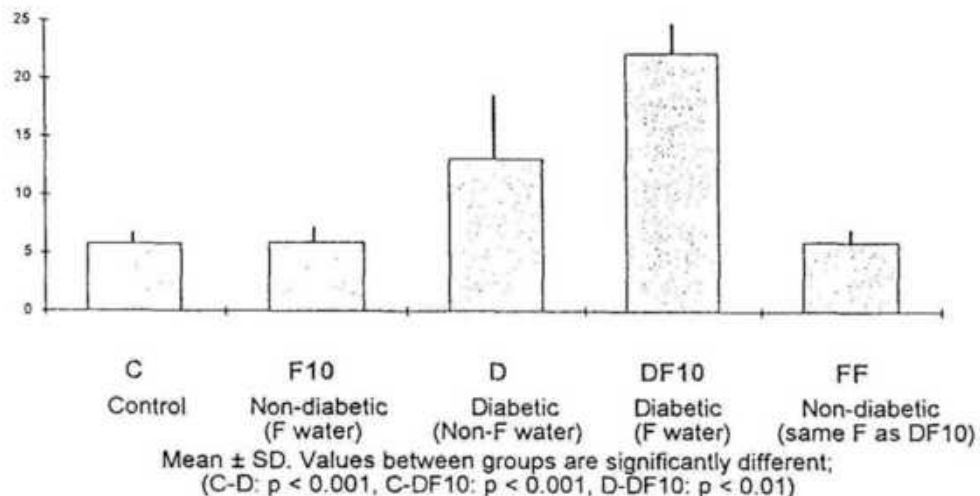
TABLE 2. The amount of ingested fluoride (mg F⁻/day/rat) at different time intervals

| Group | Ingested F | 1-4 days | 5-7 days | 8-14 days | 15-21 days |
|------------------|------------------|-------------|-------------|-------------|-------------|
| C (Control) | W (from water) | Ø | Ø | Ø | Ø |
| | P (from pellets) | 0.30 ± 0.03 | 0.26 ± 0.07 | 0.24 ± 0.03 | 0.25 ± 0.05 |
| | T (Total, W+P) | 0.03 ± 0.03 | 0.26 ± 0.07 | 0.24 ± 0.03 | 0.25 ± 0.05 |
| F10 (F water) | W | 0.30 ± 0.07 | 0.25 ± 0.06 | 0.30 ± 0.06 | 0.31 ± 0.07 |
| | P | 0.26 ± 0.03 | 0.24 ± 0.02 | 0.23 ± 0.03 | 0.21 ± 0.05 |
| | T | 0.56 ± 0.09 | 0.49 ± 0.05 | 0.53 ± 0.05 | 0.52 ± 0.11 |
| D (non-F water) | W | Ø | Ø | Ø | Ø |
| | P | 0.42 ± 0.10 | 0.46 ± 0.10 | 0.55 ± 0.13 | 0.57 ± 0.14 |
| | T | 0.42 ± 0.10 | 0.46 ± 0.10 | 0.55 ± 0.13 | 0.57 ± 0.14 |
| DF10 (F water) | W | 1.32 ± 0.23 | 2.17 ± 0.40 | 2.28 ± 0.23 | 2.56 ± 0.39 |
| | P | 0.32 ± 0.04 | 0.56 ± 0.05 | 0.56 ± 0.12 | 0.61 ± 0.05 |
| | T | 1.64 ± 0.28 | 2.73 ± 0.45 | 2.84 ± 0.21 | 3.16 ± 0.43 |
| FF (= F to DF10) | W | 1.35 ± 0.13 | 2.13 ± 0.23 | 2.53 ± 0.18 | 2.63 ± 0.23 |
| | P | 0.27 ± 0.04 | 0.28 ± 0.02 | 0.23 ± 0.02 | 0.29 ± 0.03 |
| | T | 1.62 ± 0.12 | 2.41 ± 0.17 | 2.76 ± 0.18 | 2.92 ± 0.22 |

The blood glucose fasting concentrations significantly increased in diabetic groups (Figure 1). Hyperglycemia was manifested more in DF10 than in D animals, but fluoride treatment of non-diabetic animals (groups F10 and FF) failed to cause any significant alteration.

FIGURE 1

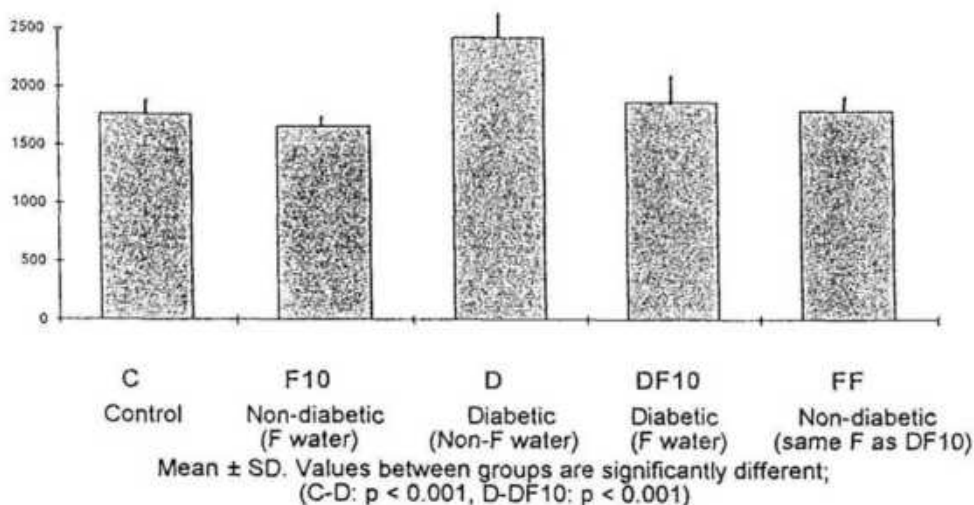
The terminal fasting blood glucose concentration (mmol/L)



Significant elevation of kidney wet weight in group D was also recorded (Figure 2). When data of kidney weights of the group DF10 were compared to controls, no significant alteration was detected, despite the fact that hyperglycemia was more remarkable.

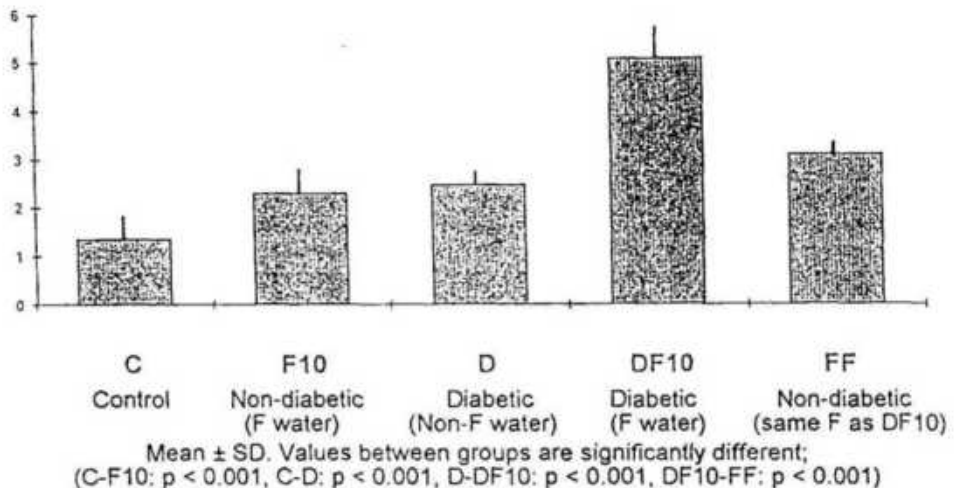
FIGURE 2

Kidney wet weight (mg)



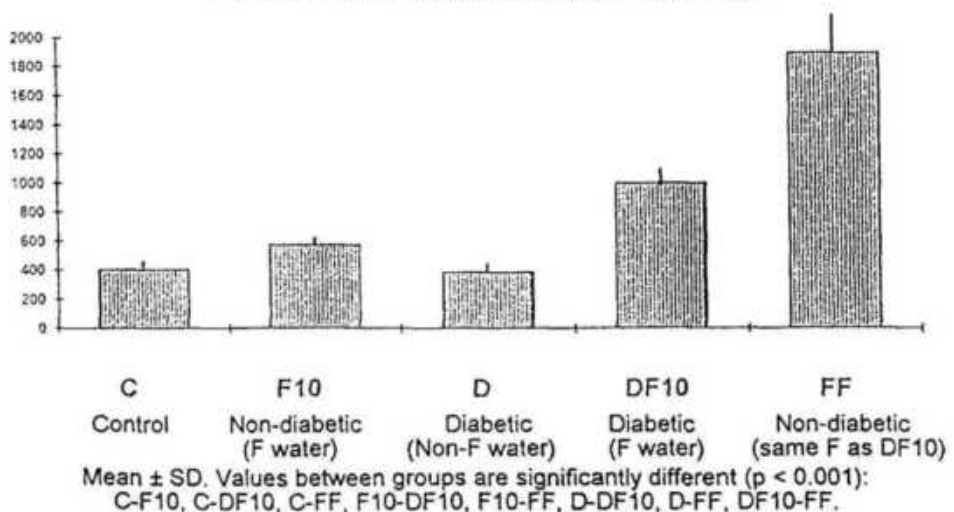
Plasma fluoride concentrations (Figure 3) reflected the daily intake of fluoride within groups F10 and D, which increased in accordance with the elevation of daily intake. Comparing the data of the group DF10 to group FF (both of which consumed the highest and nearly equal quantity of fluoride per day), significant differences were found, showing the most prominent elevation in plasma fluoride concentration of the group DF10.

FIGURE 3
Plasma fluoride concentration (micromol / L)



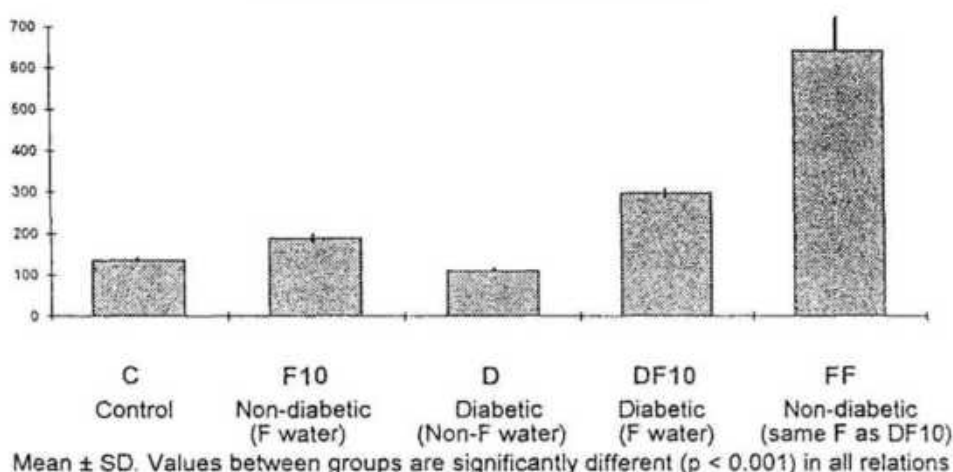
The femoral fluoride concentration was significantly increased in group F10 (Figure 4). This value was much higher in groups DF10 and FF with excessive

FIGURE 4
Femoral fluoride concentration (microgram / g)



fluoride intakes. When data of the group DF10 were compared to that of FF, considerable difference could be seen; intact non-diabetic animals were capable of storing more fluoride in bone at relatively lower plasma fluoride levels than the corresponding diabetic ones. Because of the weight reductions of ashed samples in the diabetic groups (C: 336 ± 17 , F10: 331 ± 23 , D: 287 ± 23 , DF10: 298 ± 15 , FF: 340 ± 23 mg ash), the femoral fluoride was also expressed as total fluoride/sample (Figure 5). The total fluoride in bone tissue was significantly elevated within groups F10, DF10, and FF in accordance with the consumption of fluoridated water. Fluoride content of group D was significantly reduced and it was also evident that total femoral fluoride content of group FF significantly exceeded that calculated for the group DF10.

FIGURE 5
Femoral fluoride content (microgram)



DISCUSSION

It is generally agreed that the use of fluoride via drinking water as a cariostatic agent must be well controlled, and all the conditions have to be investigated where adverse effects may be manifested. More studies are needed in diabetes which is one of the most chronic disorders, a condition where the metabolism of fluoride may be altered either by impaired metabolic and/or functional changes. The aim of this study was to observe the effects of fluoride supplied via drinking fluid in a caries preventive dose for rats²² on the kidney weight, the blood glucose level, the plasma and bone fluoride concentrations in young female diabetic rats and to compare their parameters to the intact non-diabetic animals either with different fluoride supplementation or without fluoride in the drinking water.

The dose of streptozotocin applied in this experiment is usually accepted as a dose that induces manifest but not severe diabetes.^{23,24} Glucosuria reflecting the manifestation of diabetic state appeared in groups D and DF10 after three days of streptozotocin injection and persisted throughout the treatment period. However, it is noteworthy that hyperglycemia was more evident, an increase in

kidney weight²⁵ failed to be detected, and initial body weight loss was present in DF10 animals. Earlier publications have reported that the more severe the diabetes is, the less is the increment in kidney size. It is concluded that the absence of elevation in kidney mass in group DF10 can probably be considered as a sign of a severe diabetic state.^{26,27} The data of Table 2 clearly show that DF10 animals supplied with fluoride *via* drinking water in a cariostatic dose have ingested greater quantities of fluoride from this source than did the F10 ones and they seemed to be at higher risk of severe diabetic hyperglycemia. The mechanism reflecting the high daily intake of fluoride that increased the magnitude of hyperglycemia has not yet been reported. Little is known as to the *in vivo* effects of large doses of fluoride in diabetes and to its effect on the metabolic processes.²⁸ However, the quantity of fluoride ingested per day by DF10 animals corresponds to the single acute toxic dose published for rats.²⁹ Based on data of acute fluoride poisoning changes in the liver³⁰ and kidney³¹ metabolism, stimulation of epinephrine secretion from the adrenal medulla³² or *in vitro* inhibition of insulin biosynthesis by fluoride³³ may be involved.

The plasma fluoride concentrations were elevated in all of the experimental groups in accordance with the increased fluoride intake. It was also evident that despite the nearly equal high quantity of daily fluoride intake, the plasma fluoride level detected in group DF10 significantly exceeded that of group FF. The plasma fluoride concentration within the group DF10 was comparable with the data of rats with incisor fluorosis.³⁴ Dental fluorosis as an adverse effect of fluoride can be related to either a transitory or a continuous elevation in plasma fluoride concentration achieved basically by high intake, but it can also be determined by other factors, *e.g.* impaired clearance of fluoride from plasma by bones, elimination *via* urine and by acidotic states.^{16-18,35-40} In this experiment the femoral fluoride concentration in group F10 was significantly elevated and consumption of fluoridated water and an average of about 0.5 mg daily fluoride intake of these young animals resulted in a $575 \pm 38 \mu\text{g F}^-/\text{g}$ ashed sample, which is comparable with earlier data.⁴¹ Non-diabetic animals that consumed higher amounts of fluoride (group FF) deposited more fluoride in their bones. In contrast, the femoral fluoride concentration as well as the fluoride content of ashed bone tissue of the group DF10 were significantly below that of the group FF. Thus, the bone fluoride uptake in fluoride-treated diabetic rats seems to be greatly reduced at the same high fluoride intake and it cannot only be related to the change in body and femoral weights. At this time there is no clear explanation for the great difference between the plasma and femoral fluoride concentrations of the DF10 and FF animals. The control of fluoride metabolism in diabetes seems to be rather complex and probably different from the non-diabetic condition.

Based on the data presented in this study, it is concluded that the untreated diabetic state may enhance the manifestations of adverse effects of long-term ingestion of fluoride *via* drinking water in different ways. Studies have detected high concentrations of fluoride in kidney and in urine during exposure,⁴² and due to elevated body fluid fluoride concentrations a urinary concentrating defect and prolonged decrease in glomerular filtration rate would occur in rats.^{43,44} In addition, it is also important to note that the urinary pH is a determinant of the

renal tubular excretion of fluoride.⁴⁵ At lower urinary pH values the excreted fluoride is only a low percentage of the total filtered amount.¹⁶ It is also supposed that water diuresis may influence the fluoride clearance.^{16,46} Thus, it is reasonable to assume that such mechanisms may be partly responsible for the elevation of plasma fluoride concentration in diabetic-fluoride treated animals and for the changes in bone tissue fluoride concentrations. However, the amount of fluoride present in bone depends on many other factors, e.g. the amount of fluoride ingested, duration of exposure, the continuity of fluoride intake, the bone type, and age.^{37,38,41,47-50} It is possible that in the diabetic state the changes in 1) the rate and extent of fluoride absorption from the gastrointestinal tract,^{16,51} 2) blood pH and hematocrit,¹⁶⁻¹⁸ 3) direction of fluoride movement between hydration shells of bones and the extracellular fluid,¹⁶ and 4) increments or reduction in release and/or plasma levels of hormones participating in bone metabolism⁵²⁻⁵⁶ may interact in this condition. Involvement of impaired hormonal control of bone metabolism in diabetes has further been demonstrated in diabetic rats by an increased serum alkaline phosphatase activity.⁵⁷

Data presented here call attention to the fact that more studies should be carried out to improve our understanding in this field. In order to learn about a possible hazard of fluoridation in juvenile diabetic individuals, there is a need for more detailed experimental and clinical investigations.

ACKNOWLEDGEMENT

Professor Hársing's contribution and personal inspiration to compile this manuscript before his unexpected death are gratefully acknowledged. This work was supported by grants from the University Research Council of the Semmelweis University Medical School, Hungarian Ministry of Welfare, and Zsigmond Diabetes Foundation, Budapest, Hungary.

REFERENCES

- 1 Seyer-Hansen K. Renal hypertrophy in streptozotocin diabetic rats. *Clinical Science and Molecular Medicine* 51 551-555 1976.
- 2 Seyer-Hansen K. Renal hypertrophy in experimental diabetes mellitus. *Kidney International* 23 643-646 1983.
- 3 Holstetter TH, Rennke HG, Brenner BM. The case of internal hypertension in the initiation and progression of diabetic and other glomerulopathies. *American Journal of Medicine* 72 375-380 1982.
- 4 Mac Garry ID, Foster DW. Regulation of ketogenesis and clinical aspects of ketotic state. *Metabolism* 21 471-489 1972.
- 5 Borghelli RF, Devoto FCH, Foglia VG, Erasquin J. Dental caries in diabetic and prediabetic rats. *Journal of Dental Research* 45 1105-1110 1966.
- 6 Bernick SM, Cohen DW, Baker L, Laster L. Dental disease in children with diabetes mellitus. *Journal of Periodontology* 46 241-245 1975.
- 7 Conner S, Iranpour B, Mills J. Alteration in parotid salivary flow in diabetes mellitus. *Oral Surgery* 30 50-59 1970.
- 8 Reuterving CO. Pilocarpine-stimulated salivary flow rate and salivary glucose concentration in alloxan diabetic rats. Influence of severity and duration of diabetes. *Acta Physiologica Scandinavica* 126 511-515 1986.
- 9 National Research Council. *Health Effects of Ingested Fluoride*. National Academy Press, Washington DC 1993 pp 1-11.
- 10 Sweeny EA, Shaw JH, Rubin RP. Effect of alloxan diabetes on fluoride retention and caries incidence in rats. *Journal of Dental Research* 41 866-874 1962.

- 11 Allmann DW, O'Connell K, Dunipace A, Shinbeckler T. Fluoride balance and retention in normal and diabetic rats (Abstract). *Caries Research* 18 50 1984.
- 12 Wagner MJ, Muhler JC. Retention of aqueous fluoride by alloxan diabetic rats. *Journal of the American Dental Association* 59 81-83 1959.
- 13 Largent EJ. *Fluorosis. The Health Aspects of Fluorine Compounds*. Ohio State University Press, 1961.
- 14 Dunipace AJ, Wilson CA, Wilson ME et al. (1996). Absence of detrimental effects of fluoride exposure in diabetic rats. *Archives of Oral Biology* 41 191-203 1996.
- 15 Jarnberg PO, Ekstrand J, Ehrnebo M. Renal excretion of fluoride during water diuresis and induced urinary pH changes in man. *Toxicology Letters* 18 141-146 1983.
- 16 Whitford GM. *The metabolism and toxicity of fluoride*. Karger, Basel 1989.
- 17 Whitford GM, Reynolds KE. Plasma and developing enamel fluoride concentrations during chronic acid-base disturbances. *Journal of Dental Research* 58 2058-2065 1979.
- 18 Whitford GM, Angmar-Mansson B. Fluorosis-like effects of acidosis, but not NH_4^+ , on rat incisor enamel. *Caries Research* 29 20-25 1995.
- 19 Hall LL, Smith A, De Lopez OH, Gardner DE. Direct potentiometric determination of total ionic fluoride in biological fluids. *Clinical Chemistry* 18 1455-1458 1972.
- 20 Boros I, Keszler P (1984). Serum fluoride concentration measurement with Radelkis OP-262. *Fogorvosi Szemle (Budapest)* 77 240-246 1984.
- 21 McCann HG. Determination of fluoride in mineralized tissues using the fluoride ion electrode. *Archives of Oral Biology* 13 475-477 1968.
- 22 Larson RH, Mellberg JR, Englander HR, Senning R. Caries inhibition in the rat by waterborne and enamel-bound fluoride. *Caries Research* 10 321-331 1976.
- 23 Ross J, Goldman JK. Effect of streptozotocin-induced diabetes on kidney weight and compensatory hypertrophy in the rat. *Endocrinology* 88 1079-1082 1971.
- 24 Steger RW, Lam AE, Weis RJ, Smith MS. Streptozotocin-induced deficits in sex behavior and neuroendocrine function in male rats. *Endocrinology* 124 1737-1743 1989.
- 25 Rasch R, Seyer-Hansen K. Streptozotocin diabetes as an animal model in kidney research. In: *Streptozotocin: Fundamentals and Therapy*. Elsevier/North-Holland Biomedical Press, Amsterdam 1981 pp 19-21.
- 26 Seyer-Hansen K. Renal hypertrophy in experimental diabetes: Relation to severity of diabetes. *Diabetologia* 13 141-143 1977.
- 27 Seyer-Hansen K. Renal hypertrophy in experimental diabetes: A comparison to compensatory hypertrophy. *Diabetologia* 14 325-328 1978.
- 28 Szymańska H, Gorzowski E, Nieczajew A, Kedzierska D. Disturbances of carbohydrate metabolism in the organism during chronic exposure to fluoride-fluorine diabetes. *Polskie Archiwum Medycyny Wewnętrznej* 51 57-65 1974.
- 29 Singer L, Armstrong WD, Ophaug RH. Effects of acute fluoride intoxication on rats. *Proceedings of the Society for Experimental Biology and Medicine* 157 363-368 1978.
- 30 Leemann W, Simon G, Almasy F. Über den Glykogengehalt der Leber mit Natrium-fluorid vergifteter Ratten. *Zentralblatt für Veterinarmedizin* 14 26-31 1967.
- 31 Suketa Y, Sato M. Changes in glucose-6-phosphatase activity in liver and kidney of rats treated with a single large dose of fluoride. *Toxicology and Applied Pharmacology* 52 386-390 1980.
- 32 McGown EL, Suttie JW. Mechanism of fluoride induced hyperglycemia in the rat. *Toxicology and Applied Pharmacology* 40 83-90 1977.
- 33 Lin BJ, Henderson MJ, Levine BB et al. Effects of iodoacetate and fluoride on islet respiration and insulin biosynthesis. *Hormone Metabolism Research* 8 353-358 1976.
- 34 Angmar-Mansson B, Whitford GM. Plasma fluoride levels and enamel fluorosis in the rat. *Caries Research* 16 334-339 1982.
- 35 Angmar-Mansson B, Ericsson Y, Ekberg O. Plasma fluoride and enamel fluorosis. *Calcified Tissue Research* 22 77-84 1976.

- 36 Angmar-Mansson B, Whitford GM. Single fluoride doses and enamel fluorosis in the rat. *Caries Research* 19 145-152 1985.
- 37 Ruzicka JA, Mrklas L, Rokytova K. Influence of water intake on the degree of incisor fluorosis and on the incorporation of fluoride into bones and incisor teeth of mice. *Caries Research* 7 166-172 1973.
- 38 Hodge HC. Metabolism of fluorides. *Journal of the American Medical Association* 177 313-316 1961.
- 39 Ekstrand J, Ehrnebo M, Boreus LO. Fluoride bioavailability after intravenous administration: Importance of renal clearance and urinary flow. *Clinical Pharmacology and Therapeutics* 23 329-337 1978.
- 40 Ekstrand J, Lange A, Ekberg O, Hammarström L. Relationship between plasma, dentin and bone fluoride concentrations in rats following long-term fluoride administration. *Acta Pharmacologica et Toxicologica* 48 433-437 1981.
- 41 Patz J, Karle EJ. Fluoridspiegel in Plasma and Femur nach einmaliger und mehrmaliger Verabreichung von Fluorid bei der Ratte. *Deutsche Zahnärztliche Zeitschrift* 31 185-188 1976.
- 42 Hongslo CF, Hongslo JK, Holland RJ. Fluoride sensitivity of cells of different organs. *Acta Pharmacologica et Toxicologica* 46 73-77 1980.
- 43 Whitford GM, Pashley DH. Fluoride depression of glomerular filtration rate in rats. *Journal of Dental Research* 55 B abs.193 1976.
- 44 Whitford GM, Stringer GI. Duration of fluoride-induced urinary concentrating defect in rats. *Proceedings of the Society for Experimental Biology and Medicine* 157 44-49 1978.
- 45 Whitford GM, Pashley DH, Stringer GI. Fluoride renal clearance: A pH dependent event. *American Journal of Physiology* 230 527-532 1976.
- 46 Chen PS Jr, Smith FA, Gardner DE et al. Renal clearance of fluoride. *Proceedings of the Society for Experimental Biology and Medicine* 92 879-883 1956.
- 47 Turner CH, Hasegawa K, Zhang W et al. Fluoride reduced bone strength in older rats. *Journal of Dental Research* 74 1475-1481 1995.
- 48 Armstrong WD, Singer L, Makowski WL. Placental transfer of fluoride and calcium. *American Journal of Obstetrics and Gynecology* 107 432-434 1970.
- 49 Weatherell JA. Fluoride and skeletal and dental tissues. In: *Handbook of Experimental Pharmacology*. Springer-Verlag, New York 1966 pp 141-172.
- 50 Smith FA, Gardner DE, Hodge HC. Age increase in fluoride content of human bone. *Federation Proceedings* 12 368 1953.
- 51 Whitford GM, Pashley DH. Fluoride absorption: the influence of gastric acidity. *Calcified Tissue International* 36 302-307 1984.
- 52 De Nicola AF, Fridman O, Del Castillo EJ, Foglia VG. Abnormal regulation of adrenal function in rats with streptozotocin diabetes. *Hormone Metabolism Research* 9 469-473 1977.
- 53 Heather E, Robinson H, Robinson TE. Impaired growth hormone secretion in streptozotocin diabetic rats. *Hormone Metabolism Research* 12 556-557 1980.
- 54 Tornello S, Coirini H, De Nicola AF. Effects of experimental diabetes on the concentration of corticosterone in central nervous system, serum and adrenal glands. *Journal of Steroid Biochemistry* 14 1279-1284 1981.
- 55 Tomita T, Sasaki S, Doull V et al. Pancreatic hormones in streptozotocin-diabetic rats. *International Journal of Pancreatolgy* 1 267-280 1986.
- 56 Tomlinson KC, Gardiner SM, Hebden RA, Bennett T. Functional consequences of streptozotocin-induced diabetes mellitus, with particular reference to the cardiovascular system. *Pharmacology Review* 44 111-112 1992.
- 57 Skillen AW, Hawthorne GC, Turner GA. Serum alkaline phosphatase in rats with streptozotocin-induced diabetes. *Hormone Metabolism Research* 19 505-506 1987.

INTERACTIONS BETWEEN FLUORIDE AND BIOLOGICAL FREE RADICAL REACTIONS

R Rzeuski, D Chlubek and Z Machoy
Szczecin, Poland

The term "free radical" means an atom, molecule or its fragment containing an unpaired electron. Oxygen free radicals include the superoxide radical ($\cdot\text{O}_2^-$) and hydroxyl radical ($\text{OH}\cdot$) which, together with hydrogen peroxide (H_2O_2) and singlet oxygen ($^1\text{O}_2$), are jointly called reactive oxygen species. They originate in cells in the microsomal and mitochondrial electron transport chains, in chloroplasts in the process of photosynthesis, and in numerous enzymatic reactions as well as autoxidation of many compounds.¹ Due to their high reactivity they may lead to chemical modification and impairment of the components of living cells (proteins, lipids, carbohydrates, nucleotides).²

Aerobic organisms mobilize mechanisms protecting them against the toxic action of oxygen radicals. Among these the most important involve enzymes, including superoxide dismutase (SOD), which catalyzes superoxide radical dismutation: $\cdot\text{O}_2^- + \cdot\text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$. The resulting hydrogen peroxide in turn is decomposed by the enzyme glutathione peroxidase (GSH-Px) and catalase (CAT).

The basic activity of neutrophilic granulocytes - polymorphonuclear (PMN) leukocytes - is to defend the organism against infections. Fluoride is known to stimulate the so-called respiratory burst and the production of superoxide radicals of human and rabbit neutrophils as well as those of guinea pig.³⁻⁵ This process is associated with the activation of NADPH-dependent membranous oxidase appearing in these cells. Analogous effects are evoked by fluoride ions in leukemic cells (HL-60).⁶

Similar effects are exerted by fluoride stimulation in human PMN leukocytes, whereas $\text{OH}\cdot$ is also produced alongside $\cdot\text{O}_2^-$, while added exogenous SOD and/or CAT do not overcome these effects. Moreover, high fluoride concentrations are likely to inhibit SOD. Production of $\text{OH}\cdot$ and $\cdot\text{O}_2^-$ radicals is dependent on fluoride concentration; superoxide radicals prevail at high fluoride concentrations, whereas at low ones there is dominance of hydroxyl radicals generated in the Haber-Weiss reaction with the participation of Fe^{2+} as well as H_2O_2 and $\cdot\text{O}_2^-$.^{7,8} Decrease in the activity of free radical-scavenging enzymes (SOD, GSH-Px) also occurs in people living in areas of endemic fluorosis.⁹ A similar inhibitory effect of fluoride on seed germination has been observed by Wilde and Yu. They are of the opinion that the toxicity of free radicals and H_2O_2 is greater if fluoride can impair the production of free radical scavengers such as ascorbic acid and glutathione, or the functioning of protective enzymes such as ascorbate peroxidase and SOD.¹⁰

Isolated rat mast cells incubated with fluoride release histamine and produce oxygen radicals.¹¹ Stimulation of superoxide radical production by fluoride occurs to such an extent that it is not inhibited even by added adenosine, abolishing the influence on neutrophils by the well-known inductor of peroxide

production, which is N-formylmethionylleucylphenylalanine (fMLP).¹² In a study by Bell *et al* the anti-inflammatory drug diclofenac inhibited superoxide generation induced by serum-treated zymosan (STZ) and fluoride anion, but not by phorbol myristate acetate (PMA) *in vitro*. Following treatment of patients with rheumatoid arthritis, inhibition of superoxide production occurred when STZ and PMA, but not fluoride, were used as stimuli.¹³

In rat liver macrophages fluoride ions elicit a release of arachidonic acid and prostaglandins but not formation of inositol phosphates or superoxide. The effects of fluoride require extracellular calcium and are inhibited by staurosporine and by phorbol ester treatment of the cells.¹⁴ Similar results have been observed with respect to leukotrienes in human neutrophils.¹⁵ The cascade of arachidonic acid begins by lipoxygenation, while when the resulting endoperoxides of fatty acids in the reaction with H_2O_2 produce hydroxyl radicals.¹⁶ Oxygen radicals can damage living cells by intensifying lipid peroxidation, leading to the formation of malondialdehyde which is inhibited by fluoride.¹⁷

Escherichia coli mutants deprived of superoxide dismutase are unable to grow in aerobic environment, because there is a fall in the activity of 6-phosphogluconate dehydratase. Incubation of these mutants with fluoride protects this enzyme against the action of superoxide radicals. Practically 100% protection was achieved at a fluoride concentration of 10 mM; at a concentration of 0.2 mM fluoride protection was close to 100% at the beginning and around 75% after one hour. Fluoride ions also protect this enzyme against the action of such chemical species as ferricyanide, diamide, and nitrite, as well as hydrogen peroxide.¹⁸

The process of photosynthesis together with the accumulation of energy in the form of ATP (adenosine triphosphate) proceeds with the participation of systems transporting electrons that produce radicals formed during monoelectron passages. The simultaneous production of oxygen occurs by photolysis of water. Substitution of chloride ions, appearing in the photosystem II, by fluoride ions inhibits the photooxidation of water and increases production of new radical forms in protein chains of this system, unable to commence the process of photolysis.¹⁹

Farhangrazi *et al* are of the opinion that oxidation of horseradish peroxidase by $IrCl_6^{2-}$ is significantly accelerated in the presence of fluoride. This acceleration results in the formation of a new compound which is a ferric-fluoride complex containing a porphyrin pi-cation radical.²⁰

Radicals may also form with the participation of ionizing radiation. Irradiating crystals of fluoroapatite containing various amounts of fluoride produces different radical signals in ESR spectroscopy. Doublets and singlets were observable up to 1.82% fluoride content. At higher fluoride levels, both doublets and singlets were barely discernible until, at 3.69% fluoride (essentially pure fluoroapatite), a weak but clear singlet was again observed.²¹

Despite numerous reports, the relationship between fluoride in free radical reactions remains unclear and requires further investigations.

REFERENCES

- 1 Cross CE, Halliwell B, Borish ET *et al.* Oxygen radicals and human disease. *Annals of Internal Medicine* 107 526-545 1987.
- 2 Ciechanowski K. On free radicals in medicine. *Polski Tygodnik Lekarski* 42 939-941 1987.
- 3 Della Bianca V, Grzeskowiak M, Dusi S, Rosi F. Fluoride can activate respiratory burst independently of Ca^{2+} stimulation of phosphoinositide turnover, and protein kinase C translocation in primed human neutrophils. *Biochemical and Biophysical Research Communications* 150 955-964 1988.
- 4 Bober J. *Effect of fluoride ion on oxygen metabolism and function of human and rabbit neutrophil granulocytes*. PhD thesis, Pomeranian Medical Academy, 1992.
- 5 Toper R, Aviram A, Aviram I. Fluoride mediated activation of guinea pig neutrophils. *Biochimica et Biophysica Acta* 931 262-266 1987.
- 6 Klein JB, Scherzer JA, McLeish KR. Interferon-gamma enhances superoxide productions by HL-60 cells stimulated with multiple agonists. *Journal of Interferon Research* 11 69-74 1991.
- 7 Wang YY, Zhao BL, Li XJ *et al.* Spin trapping technique studies on active oxygen radicals from human polymorphonuclear leukocytes during fluoride-stimulated respiratory burst. *Fluoride* 30 (1) 5-15 1997.
- 8 Zhao BL, Li XJ, Xin WJ. ESR studies on active oxygen radicals produced in the respiratory burst of human polymorphonuclear leukocytes. *Cell Biology International Reports* 13 529-536 1989.
- 9 Li J, Cao S. Recent studies on endemic fluorosis in China. *Fluoride* 27 (3) 125-128 1994.
- 10 Wilde LG, Yu MH. Effect of fluoride on superoxide dismutase (SOD) activity in germinating mung bean seedlings. *Fluoride* 31 (2) 1998 (in press).
- 11 Hong XJ, Francker A, Diamant B. Effects of N-acetylcysteine on histamine release by sodium fluoride and compound 48/80 from isolated rat mast cells. *International Archives of Allergy and Applied Immunology* 96 338-343 1991.
- 12 Burkey TH, Webster RO. Adenosine inhibits fMLP-stimulated adherence and superoxide anion generation by human neutrophils at an early step in signal transduction. *Biochimica et Biophysica Acta* 1175 312-318 1993.
- 13 Bell AL, Adamson H, Kirk F *et al.* Diclofenac inhibits monocyte superoxide production *ex vivo* in rheumatoid arthritis. *Rheumatology International* 11 27-30 1991.
- 14 Schulze-Specking A, Duyster J, Gebicke-Haerter PJ *et al.* Effect of fluoride, pertussis and cholera toxin on the release of arachidonic acid and the formation of prostaglandin E_2 , D_2 , superoxide and inositol phosphates in rat liver macrophages. *Cellular Signalling* 3 599-606 1991.
- 15 Brom C, Köller M, Brom J, König W. Effect of sodium fluoride on the generation of lipoxygenase products from human polymorphonuclear granulocytes, granulocytes, mononuclear cells and platelets - indication for the involvement of G proteins. *Immunology* 68 240-246 1989.
- 16 Nave JF, Jacobi D, Gaget C *et al.* Evaluation of 5- and 6-fluoro derivatives of arachidonic acid and 5,8,14-eicosatrienoic acid as substrates and inhibitors of 5 lipoxygenase. *Biochemical Journal* 278 549-555 1991.
- 17 Shayiq RM, Raza H, Kidwai AM. Fluoride and lipid peroxidation: a comparative study in different rat tissues. *Bulletin of Environmental Contamination and Toxicology* 37 70-76 1986.
- 18 Gardner PR, Fridovich I. Superoxide sensitivity of the Escherichia coli 6-phosphogluconate dehydratase. *Journal of Biological Chemistry* 266 1478-1483 1991.
- 19 Baumgarten M, Philo JS, Dismukes GC. Mechanism of photoinhibition of photosynthetic water oxidation by Cl^- depletion and F^- substitution: oxidation of a protein residue. *Biochemistry* 29 10814-10822 1990.
- 20 Farhangrazi ZS, Sinclair R, Powers L, Yamazaki I. Formation of a porphyrin pi-cation radical in the fluoride complex of horseradish peroxidase. *Biochemistry* 34 14970-14974 1995.
- 21 Peckauskas RA, Pullman I. Radiogenic free radicals in apatite: the influence of fluoride and hydroxide. *Calcified Tissue Research* 21 21-29 1976.

**XXIInd CONFERENCE OF THE INTERNATIONAL
SOCIETY FOR FLUORIDE RESEARCH**

August 24-27, 1998

Best Western Lakeway Inn

714 Lakeway Drive, Bellingham

Washington 98226, USA

Lodging for 1-2 persons: \$69.85 per night, plus 7.8% local tax

Bellingham International Airport is about 35 minutes from Seattle by air. Buses also run from Seattle and from Vancouver in Canada.

The Conference registration fee, if paid before June 1, is \$250 and includes lunches, coffee breaks, and guided bus tours.

Those who plan to present a paper, platform or poster must submit an abstract not exceeding 350 words by May 15, if possible also on a floppy disk.

Further information, and registration and abstract
submittal forms, can be supplied by

Professor Ming-Ho Yu

Huxley College of Environmental Studies

Western Washington University

Bellingham WA 98225-9181, USA

Phone (USA) 360 650 3676 Fax (USA) 360 650 7284

E-mail mhyu@titan.cc.wvu.edu

GASTRODUODENAL MANIFESTATIONS IN PATIENTS WITH SKELETAL FLUOROSIS.

S Dasarathy, T K Das, I P Gupta, A K Susheela and R K Tandon
New Delhi, India

Abstract from *Journal of Gastroenterology* 31 (3) 333-337 1996

A prospective case-controlled study was performed to evaluate the gastrointestinal symptoms and mucosal abnormalities occurring in patients with osteofluorosis. Ten patients with documented osteofluorosis and ten age- and sex-matched healthy volunteers were included in the study. Clinical evaluation, real-time ultrasound, and upper gastrointestinal endoscopy and biopsy from the gastric antrum and duodenum were performed in all subjects. The biopsies were subjected to a rapid urease test and light and electron microscopic examinations. Ionic fluoride levels were estimated in the drinking water, serum, and urine using an ION 85 ion analyzer. All patients with osteofluorosis had gastrointestinal symptoms, the most common being abdominal pain. Endoscopic abnormalities were found in seven patients with osteofluorosis. In all 7 of these patients, chronic atrophic gastritis was seen on histology. Electron microscopic abnormalities were observed in all 10 patients with osteofluorosis. These included loss of microvilli, cracked-clay appearance, and the presence of surface abrasions on the mucosal cells. None of the control subjects had any clinical symptoms or mucosal abnormalities. It was concluded that gastrointestinal symptoms as well as mucosal abnormalities are common in patients with osteofluorosis.

Key words: Electron microscopy; Gastrointestinal symptoms; Skeletal fluorosis.

Reprints: Dr R K Tandon, Department of Gastroenterology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India.

ACUTE FLUORIDE TOXICITY FROM INGESTING HOME-USE DENTAL PRODUCTS IN CHILDREN, BIRTH TO 6 YEARS OF AGE

J D Shulman and L M Wells
Dallas, Texas, USA

Abstract from *Journal of Public Health Dentistry* 57 (3) 150-158 1997

Objectives: This paper analyzes reports of The American Association of Poison Control Centers (AAPCC) of suspected overingestion of fluoride by children younger than 6 years of age between 1989 and 1994, and estimates the probably toxic amounts of various home-use fluoride products in children younger than 6 years of age.

Methods: Annual incidence rates of reported fluoride exposures attributed to dietary supplements, toothpaste, and rinses were calculated. Probably toxic amounts of each product were calculated using the frequently cited dose of 5 mg/kg.

Results: Children younger than 6 years of age accounted for more than 80 percent of reports of suspected overingestion. While the outcomes were generally not serious, several hundred children were treated at health care facilities each year. A 10 kg child who ingests 50 mg fluoride (10.1 g 1.1% NaF gel; 32.7 g 0.63% SnF₂ gel; 33.3 g 1,500 ppm F toothpaste; 50 g 1,000 ppm F toothpaste; and 221 mL 0.05% NaF rinse) will have ingested a probably toxic dose.

Conclusions: Overingestion of fluoride products in the home is preventable. Dentists and other health care providers should educate parents and child care providers about the importance of keeping fluoride products out of reach of children. Manufacturers should be encouraged by the ADA and the FDA to use child-resistant packaging for all fluoride products intended for use in the home.

Key words: Acute fluoride toxicity; Fluoride gels; Fluoride mouthrinse; Fluoride supplements; Fluoride toothpaste; Self-applied fluorides.

Reprints: J D Shulman, Texas A and M University, Baylor College of Dentistry, PO Box 660677, Dallas, TX 75266, USA.

OUTBREAK OF ACUTE FLUORIDE POISONING CAUSED BY A FLUORIDE OVERFEED, MISSISSIPPI, 1993

A D Penman, B T Brackin and R Embrey
Jackson, Mississippi, USA

Abstract from *Public Health Reports* 112 (5) 403-409 1997

Objective: To determine the extent and confirm the cause of an August 1993 outbreak of acute fluoride poisoning in a small Mississippi community, thought to result from excess fluoride in the public water supply.

Methods: State health department investigators interviewed patrons of a restaurant where the outbreak first became manifest and obtained blood and urine samples for measurement of fluoride levels. State health department staff conducted a random sample telephone survey of community households. Public health environmentalists obtained water and ice samples from the restaurant and tap water samples from a household close to one of the town's water treatment plants for analysis. Health department investigators and town water department officials inspected the fluoridation system at the town's main water treatment plant.

Results: Thirty-four of 62 restaurant patrons reported acute gastrointestinal illness over a 24-hour period. Twenty of 61 households that used the community water supply reported one or more residents with acute gastrointestinal illness over a four-day period, compared with 3 of 13 households that did not use the community water supply. Restaurant water and ice samples contained more than 40 milligrams of fluoride per liter (mg/L), more than 20 times the recommended limit, and a tap water sample from a house located near the main treatment plant contained 200 mg/L of fluoride.

An investigation determined that a faulty feed pump at one of the town's two treatment plants had allowed saturated fluoride solution to siphon from the saturator tank into the ground reservoir and that a large bolus of this over-fluoridated water had been pumped accidentally into the town system.

Conclusions: Correct installation and regular inspection and maintenance of fluoridation systems are needed to prevent such incidents.

Key words: Acute fluoride poisoning; Fluoride overdose; Fluoridation.

Reprints: A D Penman, Mississippi State Health Department, 2423 N St, Jackson, MS 39215, USA.

TESTING THE POTENTIAL OF SODIUM FLUORIDE TO AFFECT SPERMATOGENESIS IN THE RAT

R L Sprando, T F X Collins, T N Black, J Rorle, M J Ames and M O'Donnell
Beltsville, Maryland, USA

Abstract from Food and Chemical Toxicology 35 (9) 881-890 1997

The potential of sodium fluoride (NaF) to affect spermatogenesis and endocrine function was assessed in P and F-1 generation male rats. Male and female experimental rats received sodium fluoride in their drinking water at one of four concentrations (25, 100, 175, 250 ppm). P generation male and female rats were exposed to sodium fluoride in their drinking water for 10 wk and then males were mated to females within the same treatment groups. Reproductive tissues were collected from P generation male rats after approximately 14 wk of treatment. Pregnant females (P) were exposed to sodium fluoride via their drinking water through gestation and lactation. F-1 generation weanling male rats remained within the same treatment groups as their parents. F-1 generation male rats were exposed to sodium fluoride in their drinking water for 14 wk, at which time reproductive tissues were collected. Dose-related effects were not observed within the P and F-1 treatment groups in testis weights, prostate/seminal vesicle weights, non-reproductive organ weights, testicular spermatid counts, sperm production per gram of testis per day, sperm production per gram of testis, LH, FSH or serum testosterone concentrations. Histological changes were not observed in testicular tissues from either the P or F-1 generation. We conclude that prolonged exposure to sodium fluoride in drinking water at the doses administered in this study does not adversely affect spermatogenesis or endocrine function in the P and F-1 generation male rats.

Key words: Long-term fluoride exposure; Male rat; Reproductive toxicology; Spermatogenesis; Testis.

Reprints: R L Sprando, US Food and Drug Administration Center for Food Safety and Applied Nutrition, Division of Toxicology Research, 8301 Muirkirk Rd, Beltsville, MD 20708, USA.

CHROMOSOMAL ABERRATIONS AND MICRONUCLEI IN LYMPHOCYTES OF WORKERS AT A PHOSPHATE FERTILIZER FACTORY

Z Q Meng and B Zhang
Taiyuan, China

Abstract from *Mutation Research - Genetic Toxicology and Environmental Mutagenesis* 393 (3) 283-288 1997

The frequencies of chromosomal aberrations (CA) and micronuclei (MN) in peripheral blood lymphocytes of 40 workers at a phosphate fertilizer factory in North China, were studied. HF and SiF₄ are the main air pollutants and small amounts of dust containing fluoride, NH₃ and SO₂ were also present in the factory. It was shown that the chemicals caused an increase in both CA and MN. The mean frequencies per 100 metaphase of major CA type (chromosome rings, translocations, and dicentrics) of the workers and the non-exposed controls were 0.91 and 0.24 ($p < 0.01$), respectively. The average percentages of lymphocytes with MN of the workers and the controls were 1.55 ± 0.71 and 0.62 ± 0.54 ($p < 0.01$), respectively. Both CA frequency and MN frequency of the workers increased with length of the chemical exposure period up to 10 years.

Key words: Chromosomal aberration; Human lymphocyte; Micronuclei.

Reprints: Z Q Meng, Shanxi Agricultural University, Department of Life Sciences, Division of Environmental Biology and Toxicology, Taiyuan, 030006 PR China.

EFFECTS OF FLUORIDE ON STRUCTURE AND FUNCTION OF CANINE GASTRIC MUCOSA

G M Whitford, D H Pashley and R H Garman
Augusta, Georgia, USA

Abstract from *Digestive Diseases and Sciences* 42 (10) 2146-2155 1997

These studies were done to determine the effects of fluoride (F) on the structure and function of the canine gastric mucosa and the possible protective effects of 16,16-dimethyl-prostaglandin E-2 (dmPGE(2)). A portion of the stomach with its vascular supply intact was mounted in a two-compartment chamber, one side of which contained a control solution. Minor effects were caused by exposure to 1 mmol/liter F. Both 5 and 10 mmol/liter F caused marked increases in the fluxes of water and Na, K, and H ions; mucus secretion; and tissue swelling and redness. The extent of these changes did not increase appreciably upon exposure to 50 or 100 mmol/liter F. Histological findings included marked thinning of the surface cell layer, reduced uptake of PAS stain, localized exfoliation and necrosis of surface cells, acute gastritis, and edema. It was concluded that: (1) the threshold F concentration for effects on the structure and function of the gastric mucosa was approximately 1 mmol/liter; (2) the maximum or near-maximum effects were caused by 10 mmol/liter F; (3) the effects persisted for at least 6 hr after the exposure; and (4) dmPGE(2) (0.5 μ g/mL) did not attenuate the effects induced by F.

Key words: DmPGE(2); Gastric mucosa; Hydrogen fluoride; Stomach; Toxicity.

Reprints: G M Whitford, Medical College of Georgia, Department of Oral Biology, Augusta, GA 30912, USA.

ARTHROPODS AS INDICATORS OF THE EFFECTS OF FLUORIDE POLLUTION ON THE SUCCESSION FOLLOWING SAND MINING

K E Madden and B J Fox
Sydney, New South Wales, Australia

Abstract from *Journal of Applied Ecology* 34 (5) 1239-1256 1997

1. This study examines the effect of fluoride pollution on arthropod recolonization of sites after sand mining at Tomago, NSW, Australia. This represented a simplified ecological system with a known history, to study the effects of fluoride pollution on arthropod succession without the complicating factor of previous fire regimes.
2. Fluoride appears to have an indirect effect on arthropods, with fluoride-induced changes in the vegetation structure being reflected in the relative abundance of arthropod groups.
3. Additionally, fluoride pollution appears to have a number of direct effects on arthropod populations. Arthropod diversity, beetles, spiders and possibly flies appear to demonstrate the effects of direct toxicity at the sites of highest foliar fluoride levels ($> 8 \mu \text{mol g}^{-1}$). Ants decreased in abundance above low fluoride levels ($> 1.85 \mu \text{mol g}^{-1}$). Mites and cockroaches, however, were found to increase in abundance in direct relation to fluoride.
4. Arthropod succession is significantly altered when fluoride fallout is imposed on the existing disturbance of sand mining. The repercussions of changes in the abundance of elements of the arthropod community on ecosystem processes are potentially great. The sensitivity to fluoride demonstrated by ants may make them particularly good candidates as bio-indicators of fluoride stress.

Key words: Ants; Australia; Disturbance; Fluoride pollution; Sand mining; Vegetation.
Reprints: K E Madden, University of New South Wales, School of Biological Sciences, Sydney, NSW 2052, Australia.

UPTAKE OF FLUORIDE INTO DEVELOPING SHEEP TEETH, FOLLOWING THE 1995 VOLCANIC ERUPTION OF MOUNT RUAPEHU, NEW ZEALAND

G E Coote, T W Cutress and G W Suckling
Lower Hutt and Wellington, New Zealand

Abstract from *Nuclear Instruments and Methods in Physics Research. Section B-Beam Interactions with Materials and Atoms* 130 (1-4) 571-575 1997

In the southern Spring of 1995 (mid-October) the active volcano Mount Ruapehu in the central North Island of New Zealand erupted explosively, spreading up to 40 million m^3 of rhyolite tephra over thousands of km^2 of farmland during the lambing season. This ash contained a high concentration of soluble fluoride, and more than 2000 lactating ewes died of acute fluoride

poisoning. To investigate the effects of this brief but acute dose on the teeth of grazing animals we examined the distributions of fluorine and calcium in the permanent incisor teeth of sheep which were one year old at the time. Where part of an incisor had been in the first (secretory) stage of calcification the erupted tooth disclosed surface pitting, a thin layer of enriched mineral across the enamel with as much as 1000 ppm F w/w, and a separate layer with similar to 4000 ppm down the dentine. The part of an incisor which had attained the later (maturation) stage showed enriched layers only in the outer enamel and in the dentine. This study has demonstrated some important features of the calcification process, and the risk of fluoride toxicity to grazing animals

Key words: Fluoride uptake; New Zealand; Sheep teeth; Volcanic eruption.

Reprints: G E Coote, Institute of Geological and Nuclear Science, PO Box 31-312, Lower Hutt, New Zealand.

PREVENTION OF INDUSTRIALLY-INDUCED CATTLE AND SHEEP FLUOROSIS

G Milhaud and H J Van Weering
Maisons-Alfort, France

Abstract from *Recueil de Médecine Vétérinaire* 173 (1,2,3) 53-58 1997

Two industrial plants emitting fluorides, were built in an industrial area at Vlissingen, The Netherlands ; one produced phosphorus from natural phosphates and the other produced aluminium through electrolysis. Control systems were installed to reduce emissions. An agreement was made between the farmers, manufacturers and provincial authorities of Zeeland to examine possible claims and develop measures for prevention. A 2 to 3 km area around the plants, was designated unsuitable for agriculture and cattle-rearing. Beyond that area, contamination of pasture was measured every 15 days. About thirty, differently contaminated herds were visited at regular intervals to evaluate fluoride intake and to initiate prevention by distribution of rations containing 6 per cent aluminium sulfate to reduce fluoride bioavailability and replacing contaminated feed with fluoride-free feed. Pasture contamination was much higher in winter than in spring and summer ; at the highest contaminated site, the average for autumn-winter was up to 100 ppm in the period 1970-1980, whereas it generally remained below 50 ppm in spring and summer. The levels recently decreased to half those values. Prevention of fluorosis has proven entirely successful; no damage compensation has been granted since 1985.

Key-words : Cattle; Damage compensation; Fluorosis; Industrial emission; Prevention; Sheep.

Reprints: Prof G Milhaud, École Nationale Vétérinaire d'Alfort, 7 Avenue du Général de Gaulle, 94704 Maisons-Alfort Cedex, France.

SULFURYL FLUORIDE

Caroline Cox
Eugene, Oregon, USA

Abstract from *Journal of Pesticide Reform* 17 (2) 17-20 1997

The fumigant sulfuryl fluoride is widely used to kill termites and other unwanted insects in buildings, ships, railroad cars, and wood products. Sulfuryl fluoride is "an extremely hazardous gas". Typical symptoms of exposure include nose, eye, and throat irritation, weakness, nausea, difficult or painful breathing, seizures, and kidney injury. With repeated exposure or higher concentrations, breathing failure occurs. There is no known antidote for sulfuryl fluoride poisoning. Sulfuryl fluoride is also toxic to the nervous system. Neurological symptoms include muscle aching and fatigue, co-ordination problems, depression, slurred speech, dizziness, and stumbling, weaving, and staggering when walking. Sulfuryl fluoride has adversely affected reproduction in laboratory animals. When rats inhale sulfuryl fluoride for a period spanning two generations, the weight of the offspring was reduced in both the first and second generation. Sulfuryl fluoride was not tested for its ability to cause cancer as part of the registration process. All tests for effects on nontarget animals and plants, as well as all environmental fate tests were waived during the registration process. It is, however, clearly toxic to nontarget animals and plants. Regulatory agencies and the courts have found repeated violations of fumigation safety have occurred during sulfuryl fluoride treatments. According to one judge, the practices of a major extermination company was "nothing short of scary".

Key words: Fumigant; Sulfuryl fluoride; Toxicity.

Reprints: Northwest Coalition for Alternatives to Pesticides, PO Box 1393, Eugene, Oregon 97440, USA.

**EFFECT OF DRINKING WATER FLUORIDATION
ON THE PREVALENCE OF OTOSCLEROSIS**

E Vartiainen and T Vartiainen
Kuopio, Finland

Abstract from *Journal of Laryngology and Otology* 111 (1) 20-22 1997

The effect of drinking water fluoridation on the prevalence of clinical otosclerosis was investigated in an area where the natural waters have a very low fluoride content. The methods included a retrospective chart review and a residential history questionnaire. Only subjects born between 1948 and 1962 were included. In this age group, the prevalence of clinical otosclerosis was found to be 0.35 per cent of persons exposed to fluoridated tap water and 0.32 per cent of those consuming fluoride-poor water. It seems that a sodium fluoride intake of 1 to 3 mg daily cannot prevent the development of clinical otosclerosis in a low-fluoride area.

Key words: Deafness; Fluoridation; Otosclerosis; Sodium fluoride.

Reprints: E Vartiainen, Kuopio University Hospital, Department of Otolaryngology, Fin-70210, Kuopio, Finland.

RISE AND FALL OF CARIES PREVALENCE IN GERMAN TOWNS WITH DIFFERENT F CONCENTRATIONS IN DRINKING WATER

W Kunzel and T Fischer
Erfurt, Germany

Abstract from *Caries Research* 31 (3) 166-173 1997

The rise and fall of caries prevalence (DMFT) and its relation to changing F concentration of drinking water and other health-related factors is analysed based on dental findings of more than 286,000 subjects of either sex (6-15 years old) from the two industrial towns Chemnitz and Plauen. Water fluoridation (1.0 ± 0.1 ppm F) was implemented in Chemnitz (formerly Karl-Marx-Stadt) in 1959. It was in operation until autumn 1990 with an interruption lasting 22 months around the year 1971. In the F-poor town of comparison, Plauen, 55% of the citizens were supplied with F-enriched drinking water (0.9 ppm F) during the years 1972-1984. Another 20% received F-containing mixed water (0.4-0.7 ppm F). During the first three decades of the study the level of caries prevalence was strictly correlated with the availability of an optimal caries preventive F concentration in the drinking water. Water fluoridation was followed by a decrease of caries, and interruptions in fluoridation were followed by increasing caries levels. A different caries trend was observed in the years from 1987 to 1995. There was a significant caries decrease down to the lowest DMFT (2.0) since 1959 in spite of the fact that only F-poor water was available over years in both towns. This improvement of oral health is explained by changes in caries-preventive and environmental conditions.

Key words: Caries prevalence; Fluoride concentrations; Germany; Water fluoridation.

Reprints: W Kunzel, School of Dentistry, Nordhäuser Str 78, D-99089 Erfurt, Germany.

DENTAL CARIES AND DENTAL FLUOROSIS AT VARYING WATER FLUORIDE CONCENTRATIONS

K E Heller, S A Eklund and B A Burt
Ann Arbor, Michigan, USA

Abstract from *Journal of Public Health Dentistry* 57 (3) 136-143 1997

Objectives: The purpose of this study was to investigate the relationships between caries experience and dental fluorosis at different fluoride concentrations in drinking water. The impact of other fluoride products also was assessed. **Methods:** This study used data from the 1986-87 National Survey of US School-children. Fluoride levels of school water were used as an indicator of the children's water fluoride exposure. The use of fluoride drops, tablets, professional fluoride treatments, and school fluoride rinses were ascertained from caregiver questionnaires. Only children with a single continuous residence ($n = 18,755$) were included in this analysis. **Results:** The sharpest declines in dfs and DMFS were associated with increases in water fluoride levels between 0 and 0.7 ppm F, with little additional decline between 0.7 and 1.2 ppm F. Fluorosis prevalence was 13.5 percent, 21.7 percent, 29.9 percent, and 41.4 percent for children who consumed < 0.3 , 0.3 to < 0.7 , 0.7 to 1.2, and > 1.2 ppm F water. In addition to fluoridated water, the use of fluoride supplements was associated with both fewer caries and increased fluorosis. **Conclusions:** A suitable trade-off between caries and fluorosis appears to occur around 0.7 ppm F. Data from this study suggest that a reconsideration of the policies concerning the most appropriate concentrations for water fluoridation might be appropriate for the United States.

Key words: Caries prevention; Dental caries; Dental fluorosis; Water fluoridation.

Reprints: K E Heller, University of Michigan, School of Public Health, Program of Dental Public Health, Ann Arbor, MI 48109, USA.

DOSE FOR INCIPIENT ACUTE FLUORIDE INTOXICATION: TRUE SCIENCE AND FALSE SCIENCE

In Japan it is widely believed that the minimum dose of fluoride ion that causes slight or mild, early-stage acute intoxication in humans is 2 mg/kg of body weight. This hypothesis, which may be called the "2-mg theory," was originally proposed by Dr Kinichi Horii, former Professor of Preventive Dentistry at Niigata University School of Dentistry.

At one time Dr Horii played a major role in advocating fluoridation of drinking water in Niigata Prefecture. Later he promoted a fluoride mouthrinsing program in primary and middle schools after failing to achieve fluoridation of the communal water system in Niigata City, where there was strong public opposition.

His 2-mg theory has been quoted uncritically by many influential health authorities with whom he has close ties. They have not only imposed his program, but they have thereby created the miserable situations by ignoring the symptoms headaches, abdominal pains, nausea and the like of which some children complained after mouthrinsing with fluoride solutions in their schools. But I could hardly ignore what was actually happening, and I spent many years examining the supposed scientific basis of the theory.

For this theory, Dr Horii cited a report by Herbert B Baldwin titled "The Toxic Action of Sodium Fluoride."¹ What dumbfounded me when I looked it up was that it was published nearly a century ago in 1899. Such archaic material is not usually found in medical or dental school libraries in Japan. I therefore had to go to the National Diet Library to obtain a copy and was surprised again to learn that it did not provide an acceptable basis for the 2-mg theory. In my report in Japanese,^{2,3} I gave a detailed discussion of this matter which may be summarized as follows:

As far as I am able to determine, the 2-mg theory at issue is based on Professor Horii stating: "Baldwin voluntarily tasted sodium fluoride and reported that no effect was produced with 30 mg, slight salivation with 90 mg, and, with 250 mg, nausea after two minutes which became most intense after twenty minutes From these facts it is assumed that the amount of fluoride which causes acute intoxication is 2 mg/kg."

I do not think any explanation is needed to recognize that this statement cannot be a scientifically reliable basis for the 2-mg theory. Moreover, Dr Horii has distorted the sense of what Baldwin actually wrote, which was: "Merely tasting small quantities of sodium fluoride produced a slight feeling of nausea with slight salivation. 0.03 gram swallowed with some bread produced no effect. Neither did 0.09 gram taken one hour later, except a little salivation. 0.25 gram, however, taken two days afterward on an empty stomach, produced nausea in two minutes. This gradually increased in severity for twenty minutes when the period of greatest intensity was reached. There was a largely increased flow of saliva and some retching but no vomiting occurred at that time although the desire was very great." It is puzzling how from this passage the conclusion can be reached that "the amount of fluoride which causes acute intoxication is 2 mg/kg."

If we assume Dr Horii took Baldwin's highest sodium fluoride intake figure of 0.25 g (250 mg) as the basis for his calculation, then this quantity of sodium fluoride contains 113 mg of fluoride ion. For a person weighing 56 kg, this amount corresponds to 2 mg F/kg body weight. But at this level of intake the acute toxicity symptoms are already more than slight or early mild.

Dr Horii is considered one of our greatest authorities on fluoride. When he declares fluoride is safe, obedient health officials echo him without question. Nevertheless, if the scientific ground on which he stands is not firm, then his claim for the safety of fluoride is not trustworthy.

In my search of the Japanese literature for information on the acute toxicity of fluoride, I found that the minimum dose reported to cause slight or incipient intoxication ranges from 0.08 to 0.2 mg per kg of body weight, or less than one-tenth of 2 mg/kg.

Dr Kenji Akiniwa has recently extended my work, consulting a very large number of reports, to determine the minimum dose of fluoride that causes slight acute intoxication in humans.⁴ The result was far below the 2-mg figure proposed by Dr Horii and was very close to what I found above.

As Dr Akiniwa states, fluoride mouthrinsing is a "drug treatment that should be strictly controlled." However, when it is beyond our control, who in fact can control it when it is applied to small children? It is a medical treatment that could be a health risk and should therefore be discontinued.

According to science writers Joel Griffiths and Chris Bryson, recently declassified U.S. government documents shed new light on the beginnings of the still-controversial public health measure, water fluoridation.⁵ Their research has revealed a surprising connection between public exposure to fluoride and the dawning of the nuclear age in the famous Manhattan Project to build atomic bombs during World War II. Much of the original proof that fluoride is safe for humans in low doses was generated by scientists in that project who had been secretly ordered to provide "evidence useful in litigation" to assist defense contractors facing claims for fluoride injuries caused by their operations in producing atomic weapons.

I just shudder to think how many cases of fluoride poisoning have been covered up by false science.

Tohru Murakami, Vice President
Japanese Society for Fluoride Research
1-5-16 Kamikoide-Machi
Maebashi-Shi, Gunma-Ken
Japan 371

REFERENCES

- 1 Baldwin HB. The toxic action of sodium fluoride. *Journal of the American Chemical Society* 21 (6) 517-525 1899.
- 2 Murakami T. Sinwa no hokai suru toki. *Shikaitenbo* (Dental Outlook) 73 (4) 893-911 1989. [In Japanese, no English title or English summary]
- 3 Murakami T. Shiryō (Material). *Fluoride Research* (Journal of the Japanese Society for Fluoride Research) 10 49-52 1989 [In Japanese, no English title or English summary].
- 4 Akiniwa K. Re-examination of acute toxicity of fluoride. *Fluoride* 30 (2) 89-109 1997.
- 5 Griffiths J, Bryson C. Fluoride, teeth and the atomic bomb. *Waste Not* (82 Judson, Canton, NY 13617, USA) #414 1-8 September 1997 [Abstracted in *Fluoride* 30 (4) 261-266 1997].

REPLY TO CRITIQUE

Thank you for this opportunity to respond to the critique of our article by Bill Wilson (*Fluoride* 30 (4) 268-269 1997).

Firstly the referee states that "the full study does not support the conclusions in the abstract." The conclusion(s) in the abstract were "that fluoride exposure during the first 12 years of life, which reduced caries in this population, may also protect teeth from wear to some extent". The referee accuses us of publishing the DMFS data of the entire group presumably to enhance (our) pro-F views. These data were objectively included to illustrate that caries in non-fluoridated subjects does increase with age. However owing to lack of availability of water fluoridation or fluoride supplements to subjects over 50 years of age in this region, comparative groups of subjects, over 50 with F were not available for valid tooth wear comparisons. As the DMFS rates of fluoridated subjects, less than 50 years of age, were consistently lower than the non-fluoridated subjects it seems reasonable to conclude that our subjects were experiencing the reduction in decay, as found in other much larger surveys. Likewise our decision to record DMFS for subjects in the age range of 10-14 is entirely defensible as these subjects did or did not have F within the first 12 years of life, presumably as did subjects of older age ranges.

This was not a study of the incidence of dental fluorosis and we do not claim "total absence of dental fluorosis." Overt caries or restored carious lesions and tooth wear were measured grossly by sextant. Any attempt to discriminate between white lesions of enamel due either to fluorosis, demineralisation by plaque acids or demineralisation due to extrinsic or intrinsic acid erosion would have been fraught with logistical and reliability problems.

Paradoxically we agree with the "prevailing view that topical rather than systemic fluoride may confer benefit". We did not state we believed topical fluoride from toothpaste had little if any effect. However this is the only study to date that has attempted to address what, if any, effect systemic fluoride has on tooth wear. We conclude it may have an effect and may empirically support the use of fluoride mouthwashes in the prevention of dental erosion. However we also recorded, for the first time, that fluoride does not appear to protect against severe erosion of lower molar occlusal surfaces which is of real concern to practising dentists. This paper was not intended to be a contribution to the fluoride debate but as a contribution to understanding dental erosion and its management.

W G Young
The University of Queensland
Oral Biology and Pathology
Brisbane, Qld 4072, Australia

REJOINDER

Professor Young has not addressed the main point that the age discrepancies, between fluoride and non-fluoride exposed subjects, made the study¹ of little value. The other points in his response are easily answered.

1. He implies that there was only one conclusion in the abstract, yet his study title,¹ and his above quotation, clearly imply two: reduced caries and reduced wear.
2. My statement about pro-F views is confirmed by my quotes from the study.

3. The comment about the non-availability of water and supplement F-exposed subjects over the age of 50 years of age seems to have also largely applied to subjects between 35 and 50 years old.
4. The study conclusion states "Prior fluoride exposure in the first 12 years of life appears to confer some resistance to excessive tooth wear . . . in adulthood . . .". It is invalid to use tooth wear data of the under 12 year olds or even under 16s in this study, for the obvious reason that they had not yet experienced tooth wear as adults.
5. Young states "we do not claim total absence of dental fluorosis". I quote from the full study: "None of the patients was identified as having mottling or dental fluorosis."
6. My critique quoted from the study that the subjects were well aware "of the protective effects of fluoride." Since fluoridated toothpaste dominates the market, it is reasonable to assume that most of the subjects used it. The study claims that subjects obtaining fluoride from water or supplements had less caries and tooth wear. It follows that topical fluoride from toothpaste appears to have had little effect.
7. It is indeed paradoxical that Young agrees "with the prevailing view that topical rather than systemic fluoride may confer benefit." His study introduction stated "There is considerable evidence that fluoride ingestion in the early years of life, when the teeth are developing, results in reduced risk of dental caries ... by incorporation in the apatite lattice".
8. Young's reply above also concludes that fluoride may have a systemic effect on tooth wear and hence "may empirically support the use of fluoridated mouthwashes in the prevention of dental erosion". Young appears to be very confused, since mouthwash effects, if any, will be topical (unless the mouthwashes are going to be drunk, injected or used in suppositories).
9. Albeit not intended, the study in question, because of its subject, is an interesting contribution to the fluoride debate.
10. Young concludes above that "...our subjects were experiencing the reduction in decay, as found in other much larger surveys." The assumption of the efficacy and safety of fluoride is mainly based on the four North American studies of half a century ago, and 128 later studies quoted by Murray and Rugg-Gunn.² The late Dr Sutton of Melbourne University is one of the few scholars who reviewed every one of these studies which were still available. His book,³ with the critiques, responses from authors, and refutations of the responses, should be required reading for all who still so unquestioningly claim that fluoride benefits are proven.

Bill Wilson
118 Forrest Hill Road
North Shore City
Auckland, New Zealand

REFERENCES

- 1 Teo C, Young WG, Daley TJ, Sauer H. Prior fluoridation in childhood affects dental caries and tooth wear in a South East Queensland population. *Australian Dental Journal* 42 (2) 92-102 1997.
- 2 Murray JJ, Rugg-Gunn AJ. *Fluorides in Caries Prevention*. Wright PSG, Bristol 1982. Table 21 pp 58-63.
- 3 Sutton PRN. *The greatest fraud: Fluoridation*. Kurunda Pty, Lorne (PO Box 22, Victoria 3232, Australia) 1996.

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 in British £ or Japan ¥) per year in advance. Send to Treasurer, ISFR, 81A Landscape Road, Mount Eden, Auckland 1004, New Zealand.

COPIES of articles in *Fluoride* are available from:

University Microfilms International, Box 91, Ann Arbor, MI 48106, USA.

Institute for Scientific Information, 3501 Market St., Philadelphia, PA 19104, USA.

BICOSIS, c/- Advanced Information Consultants, Box 87127, Canton, MI 48187, USA.

The UnCover Company, 3801 E. Florida, Suite 200, Denver, CO 80210, USA.

MANUSCRIPTS, including papers presented at ISFR conferences, are accepted for publication after appropriate evaluation and recommendation by qualified reviewers. Send to Prof A W Burgstahler, Scientific Editor, *Fluoride*, 1620 Massachusetts Street, Lawrence, KS 66044-4254, USA. Fax (USA) 785 843 0736. The following instructions apply to original research reports. Research reviews and discussion papers, with appropriate variations in format, may also be accepted.

INSTRUCTIONS TO AUTHORS

The submitted paper, with a copy, should be written concisely in English. Either American or British spelling is accepted. Measures should be in metric system. Double space with generous margins. A computer disk containing the text is much appreciated.

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

Summary: Begin with a brief factual summary.

Key words: List the major themes or subjects.

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

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

Results: List the direct conclusions of the work.

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

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

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

References: See current issues of the journal for usual style, which identifies references by superscripted numbers in the order in which 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. The membership fee is US\$40 a year, which includes subscription to the journal. This reduced subscription rate is also available, on application to the Treasurer, to individuals who support the Society's aims.