The Effect of Fluoride on the Physiology of the Pineal Gland


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Excerpts from pages: 1-9; 51-53; 167-177

Abstract:

The purpose was to discover whether fluoride (F) accumulates in the pineal gland and thereby affects pineal physiology during early development. The [F] of 11 aged human pineals and corresponding muscle were determined using the F-electrode following HMDS/acid diffusion. The mean [F] of pineal gland was significantly higher (p < 0.001) than muscle: 296 ± 257 vs 0.5 ± 0.4 mg/kg respectively. Secondly, a controlled longitudinal experimental study was carried out to discover whether F affects the biosynthesis of melatonin, (MT), during pubertal development using the excretion rate of urinary 6-sulphatoxymelatonin, (aMT6s), as the index of pineal MT synthesis. Urine was collected at 3-hourly intervals over 48 hours from two groups of gerbils, (Meriones unguiculatus), low-F (LF) and high-F (HF) (12 f, 12 m/group): under LD: 12 12, from prepubescence to reproductive maturity (at 9-12 weeks) to adulthood, i.e., at 7, 9, 11 1/2 and 16 weeks. The HF pups received 2.3 ug F/g BW/day from birth until 24 days whereafter HF and LF groups received food containing 37 and 7 mg F/kg respectively and distilled water. Urinary aMT6s levels were measured by radioimmunoassay. The HF group excreted significantly less aMT6s than the LF group until the age of sexual maturation. At 11 1/2 weeks, the circadian profile of aMT6s by the HF males was significantly diminished but, by 16 weeks, was equivalent to the LF males. In conclusion, F inhibits pineal MT synthesis in gerbils up until the time of sexual maturation. Finally, F was associated with a significant acceleration of pubertal development in female gerbils using body weights, age of vaginal opening and accelerated development of the ventral gland. At 16 weeks, the mean testes weight of HF males was significantly less (p < 0.002) than that of the LF males. The results suggest that F is associated with low circulating levels of MT and this leads to an accelerated sexual maturation in female gerbils. The results strengthen the hypothesis that the pineal has a role in pubertal development.
Chapter 1 Background Information

1.1 Introduction

In this study I attempted to discover whether fluoride (F) has pathophysiological effects on the pineal gland: a feasible proposition if F accumulates in the pineal and can thereby influence its physiology. The pineal gland, or seat of the soul as it is colloquially called, is situated near the anatomical centre of the brain. It is an integral part of the central nervous system (CNS). Fluoride metabolism in the CNS has not been systematically studied. It is generally believed that F has no effect on the CNS because it is excluded from brain by the blood-brain barrier (Whitford et al, 1979). Whole brain has a low F-content like normal soft tissues elsewhere in the body.

It is remarkable that the pineal gland has never been analysed separately for F because it has several features which suggest that it could accumulate F. It has the highest calcium concentration of any normal soft tissue in the body because it calcifies physiologically in the form of hydroxyapatite (HA). It has a high metabolic activity coupled with a very profuse blood supply: two factors favouring the deposition of F in mineralizing tissues. The fact that the pineal is outside the blood-brain barrier suggests that pineal HA could sequester F from the bloodstream if it has the same strong affinity for F as HA in the other mineralizing tissues.

The intensity of the toxic effects of most drugs depends upon their concentration at the site of action. The mineralizing tissues (bone and teeth) accumulate high concentrations of F and are the first to show toxic reactions to F. Hence, their reactions to F have been especially well studied. If F accumulates in the pineal gland, then this points to a gap in our knowledge about whether or not F affects pineal physiology. It was the lack of knowledge in this area that prompted my study.

Children are now exposed to more F than ever before. Fluorides are the cornerstone of all caries preventative programs. The substantial reduction in the incidence of dental caries in the western world over the past fifty years has been largely attributed to the access to fluoridated water supplies and the increased exposure to F in dental products. The fluoridation of water supplies is an important public health measure. It is endorsed by the WHO, the European Union directives, the Royal College of Physicians, the Royal College of General Practitioners, the BMA, and the medical and dental professions (Samuels, 1993).

Despite the endorsements, the prophylactic use of F in dentistry has been a controversial subject
for decades. One recent study reported an increase in osteosarcomas in male F3441N rats which had received drinking water containing 100-175 mg NaF/L (45-79 mg F/L) for two years (NTP, 1990). Following this report, three critical bodies analysed the public health benefits and risks from chronic F-exposure by reviewing the evidence from human epidemiological studies of the relationship between cancer and water fluoridation and also carcinogenicity studies in rodents. They unanimously agreed that F is safe and effective if used appropriately. The use of F is not associated with an increased cancer risk in humans. Dental fluorosis is the only adverse effect associated with the chronic ingestion of relatively low F-levels (Kaminsky et al, 1990; USPHS, 1991; NRC, 1993). Further research is required on the effects of F on the reproductive system in animals and humans (USPHS, 1991).

Dental fluorosis (defective, hypomineralized enamel) occurs when excessive amounts of F reach the growing tooth during its developmental stages. The manifestations of fluorosis range from barely noticeable opacities to severely pitted teeth. The greater the F-exposure during tooth formation the greater is the likelihood of dental fluorosis developing and the more severe is the pathology. The F-concentration at which fluorosis becomes apparent in a population corresponds to a daily intake of about 0.1 mg F/kg body weight (BW) up to the age of 12 years although there is no firm consensus on this issue. In fact, a high prevalence and severity of dental fluorosis was reported in populations with an estimated daily F-intake of less than 0.03 mg F/kg BW (Blum et al, 1987).

The so-called 'optimal' concentration of F in community water is defined as the concentration of F which gives maximum caries reduction and causes minimum dental fluorosis, i.e., between 0.7 and 1.2 mg/L depending on the mean ambient temperature. At the time of Dean's original studies, there was a 10-12 percent prevalence of mild dental fluorosis in children in the 1 mg F/L areas (Dean et al, 1941, 1942). This was 'accepted' in return for the benefits in caries reduction: a classic public health trade-off. In the 1930s and 1940s, virtually the only source of F was in the drinking water. Today, F is ubiquitous in the environment which means that man's daily F-intake comes from several sources besides tap water.

Systemic F-exposure to children has increased (Leverett, 1991). Mild dental fluorosis is now more common than one would predict on the basis of Dean's findings in the late 1930s and early 1940s: in fluoridated and non-fluoridated communities (Leverett 1986; Pendrys and Stamm, 1990; USPHS, 1991). Several recent studies report prevalence rates in the 20 and 80 percent range in areas with fluoridated water (Levy, 1994). The prevalence of 0.9 percent (recorded in the pre-fluoride days) in areas containing less than 0.4 mg F/L in the water has increased to 6.6 percent
The prevalence of moderate to severe dental fluorosis has increased USPHS (1991; Lalumandier and Rozier, 1995). The increased prevalence of dental fluorosis is causing concern within the scientific community because it is an early sign of F-toxicity and evidence that some children are now getting more F than is good for them. The issue has the potential to become a significant dental health problem.

Of all the tissues, the developing enamel organ is assumed to be most sensitive to the toxic effects of F. It contains significantly higher concentrations of F and calcium than other soft tissues. The enamel organs of 9-day-old rats contained significantly higher F levels than corresponding soft tissue (0.14 vs. 0.015 mg/kg). Following oral administration of F to the rat pups (0.5 mg/kg BW), the [F] of the enamel organ reached peak values (0.19 mg/kg) in 30 minutes. The enamel organ may be relatively sensitive to increased systemic F-intake because it accumulates F (Bawden et al, 1992).

Although the exact mechanism responsible for enamel fluorosis is not known, F may have specific effects on the normal activity of ameloblasts, developing enamel matrix and proteolytic activity in the maturing enamel (DenBesten and Thariani, 1992). The transition/early-maturation stage of amelogenesis is most susceptible to the effects of an increased plasma F-concentration. The aesthetically important maxillary central incisors are most vulnerable to F at 22-26 months (Evans and Stamm, 1991).

Alongside the calcification in the developing enamel organ, calcification is also occurring in the child's pineal. It is a normal physiological process. A complex series of enzymatic reactions within the pinealocytes converts the essential amino acid, tryptophan, to a whole family of indoles. The main pineal hormone is melatonin (MT). For some reason, young children have the highest levels of plasma MT. They also have higher plasma F levels (recommended from a dental perspective) than they did 50 years ago. An increasing number of children suffer from mild dental fluorosis: evidence that they received too much F during the first few years of life. If F accumulates in the pineal gland during early childhood, it could affect pineal indole metabolism. In much the same way that high local concentrations of F in enamel organ and bone affect the metabolism of ameloblasts and osteoblasts.

If F influences the high pineal MT output during early development, then the functions of the pineal may also be compromised (given that MT is the main mediator of pineal function). One putative function of the pineal is its involvement in the onset of puberty. If F compromises pineal function by altering the high rate of synthesis of MT during childhood, does this manifest as an alteration in the timing of puberty?
Although the extrapolation of results from animal studies to the human situation is difficult, this project may identify a potential health risk to humans. Therefore, the results will either affirm the safety of the extensive use of F in dentistry or suggest that harmful effects on human health have already occurred: either way, this investigation is worthwhile.

1.2 Review of the Literature

To the best of my knowledge, the Newburgh-Kingston study is the only reference on the effect of F on the timing of puberty in humans. It is the largest, most ambitious paediatric survey carried out to demonstrate the safety of water fluoridation. The New York State Department of Health initiated the study in 1944 because they realized that there would ultimately be a need for a long-term evaluation of any possible systemic effects as well as the dental changes from drinking fluoridated water over a long period of time.

Similar groups of children were selected for long-term observation from Newburgh (fluoridated to 1.0 to 1.2 mg/L in 1945) and Kingston (essentially F-free for the duration of the study). Newburgh and Kingston were chosen because they were well-matched: both were situated on the Hudson River about 35 miles apart with similar upland reservoir water supplies; both had populations of about 30,000 with similar demographic characteristics, social and economic conditions, levels of dental care, etc. In Newburgh, out of 817 children (aged from birth to nine years) who were selected in 1945, 500 were examined in 1954-1955; in Kingston, out of 711 children who were selected in 1945, 405 were examined in 1954-1955.

The medical and dental examinations began in 1944, and were repeated periodically until 1955. An assessment of any possible systemic effects arising from the consumption of fluoridated water was made by comparing the growth, development and the prevalence of specific conditions in the two groups of children as disclosed by their medical histories, physical examinations, and laboratory and radiological evidence. The age of onset of menstruation in girls was used as an index of the rate of sexual maturation.

At the end of ten years, the investigators reported no adverse systemic effects from drinking fluoridated water because no significant differences were found between the results from the two groups. The average age of first menarche was earlier among girls in Newburgh than those in Kingston: 12 years vs. 12 years and 5 months respectively (Schlesinger et al, 1956). Although this difference was not considered important, it does suggest an association between the use of fluoridated drinking water and an earlier onset of sexual maturation in girls. The Newburgh girls had not had a lifelong use of fluoridated water. For the first two years or so, they received unfluoridated
water. Furthermore, their only source of F was from the drinking water.

### 1.3 Sources of Fluoride

#### 1.3.1 Food

The normal daily F-intake is negligible (less than 0.01 mg) during the first few months of human life, because human breast milk contains merely a trace of F (6 to 12 ng/ml): regardless of the F-intake to the nursing mother. Ekstrand and co-workers (1981) analysed plasma and milk samples from five nursing mothers after they had taken an oral dose of 1.5 mg F. There was an immediate ten-fold increase in the [F] of plasma (70-86 ng/ml) within 30 minutes of dosing but the [F] of breast milk remained constant throughout the day (2-8 ng/ml). The mean F-concentrations of human breast milk were 8.9 and 5.0 ng/ml from nursing mothers living in 1.7 and 0.2 mg Fit areas respectively (Esala et al, 1982); 6.8 ± 0.4 and 5.3 ± 0.4 ng/ml (± SEM) from nursing mothers living in 1.0 and 0.2 mg Fit areas respectively (Spak et al, 1983).

The reason for the limited transfer of F from plasma to breast milk is unknown. It has been suggested that the physiological plasma-milk barrier actively protects the newborn from the toxic effects of F (Ekstrand et al, 1981). Cow's milk, like human milk, contains low levels of F (0.017 mg/L) even when F is added to the cow's food or drinking water (McClure, 1949). Breast-fed infants (or infants bottle-fed with cow's milk) are in negative F-balance: more F is excreted in the urine than is ingested in the diet. During the period of breast feeding, F (deposited in foetal bone during pregnancy) is mobilized and released into the extracellular fluids and subsequently excreted into urine. Therefore, early human development has always occurred in a virtually F-free milieu even in the high-F areas: a phenomenon which lasts until the age of weaning and the introduction of solid foods.

In contrast, the F-intake to bottle-fed infants living in fluoridated areas depends upon the [F] of. a) the water used to reconstitute the feed; b) the powdered formula-feed itself. Bottle-fed infants in fluoridated areas can receive 1.1 mg F from day 1: 150-200 times more F per day than breast-fed infants, i.e., 1100 vs. 5-10 j.tg/day (Ekstrand, 1989). The normal pharmacokinetics of F during infancy is reversed. Bottle-fed infants in fluoridated areas retain more than 50% of the ingested F-dose in the mineralizing tissues (Ekstrand et al, 1984; 1994).

Man's daily intake of F from food is low. Fresh, unprepared vegetables, fruits, pulses, roots, nuts, etc., rarely contain more than 0.2 to 0.3 mg F/kg (WHO, 1984). Most plant species have a limited capacity to absorb F from the soil even when F-containing fertilisers are applied (Davison, 1984). The flesh of meat, poultry and fish, (free from bone), contains low levels of F because virtually all F
in animals occurs in their bones and teeth. The skin and bones of tinned salmon and sardines contain 8 and 500 mg F/kg respectively because the fish are exposed to relatively high levels of F (1.2-1.4 mg/L) in sea-water (Jenkins, 1990).

Chapter 2 - Aims and Objectives

1. The purpose of the first experiment was to discover whether F accumulates in the human pineal gland. The objectives were to determine:

   a) The [F] of human pineal gland and corresponding muscle and bone so that the pineal [F] could be compared to that of muscle and bone.

   b) The [Ca] of human pineal gland so that pineal [Ca] could be correlated with pineal [F], and an estimation made of the amount of hydroxyapatite (HA) in the pineal.

2. The purpose of the second experiment was to discover whether F affects pineal physiology: specifically, its ability to synthesize melatonin (MT). The aim was to set up a controlled longitudinal study of the effects of F on the pineal output of MT during the transition from prepubescence through puberty into young adulthood using the Mongolian gerbil (Meriones unguiculatus) as the experimental animal model. The levels of urinary 6-suiphatoxymelatonin, aMT6s, were used as an index of pineal MT synthesis.

   The objectives were:

   a) To collect urine from two groups of gerbils, high-F (HF) and low-F (LF), at 7, 9, 11Y2 and 16 weeks of age at 3-h intervals over 48-h for the subsequent measurement of the levels of urinary aMT6s. The levels of urinary aMT6s were determined using radioimmunoassay (RIA).

   b) To validate the RIA for urinary aMT6s currently in use in the laboratory for use with gerbil urine.

   c) To demonstrate that the amount of MT synthesized by the gerbil pineal reflects the excretion rate of aMT6s in gerbil urine in 16-week-old gerbils. The aims were to determine the gerbil pineal MT contents at 6-h intervals over 24-h and the excretion rates of urinary aMT6s at 3-h intervals over 24-h using RIAs. The pineal MT/urinary aMT6s relationship was assessed: (i) qualitatively, by comparing the circadian profiles of
pineal MT content and urinary aMT6s excretion by 16-week-old gerbils; (ii) quantitatively, by correlating peak nocturnal pineal MT content with total urinary aMT6s pg/g BW/24-h.

d) To compare the rate and pattern of urinary aMT6s excreted by the BF and LF groups during sexual maturation.

e) To compare the circadian profiles of urinary aMT6s by the HF and LF groups at 11 Y2 weeks (sexual maturity) and at 16 weeks (adulthood) in order to discover whether F affects the rhythmicity of urinary aMT6s excretion, e.g., the amplitude, the time of appearance and decline of urinary aMT6s excretion, and the total amount of urinary aMT6s excreted during the daytime and night-time.

3. The purpose of the third experiment was to discover whether F affects the timing of the onset of sexual maturation in gerbils. The objective was to compare several physiological markers for the onset of puberty in the two groups, i.e., the areas of the ventral glands, age of vaginal opening, body weights and weights of testes.

4. The purpose of the fourth experiment was to demonstrate that F was the only variable between the two groups. The aim was to compare the [F] of gerbil bone ash from the HF and LF groups at various ages.

The project will provide basic information on the rate and circadian profiles of urinary aMT6s excretion during the development of the gerbil, a common species in pineal research. Such basic knowledge is a prerequisite for further studies using urinary aMT6s measurements as an alternative to pineal or plasma MT determinations in the gerbil. It was hoped that the results would contribute new knowledge on pineal MT output during puberty in gerbils and contribute towards knowledge about the pineal's function during sexual development.

The results will add new knowledge about the fate and distribution of F in the human body. Although it is difficult to evaluate the relevance of gerbil data to the human situation, the results may suggest a relationship between F and the timing of the onset of human puberty. In this way, the work may help to evaluate the propriety of the current extensive use of F in dentistry, i.e., affirm its safety or intimate that F has physiopathological effects on the pineal gland.

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Chapter 10 - Discussion
After half a century of the prophylactic use of fluorides in dentistry, we now know that fluoride readily accumulates in the human pineal gland. In fact, the aged pineal contains more fluoride than any other normal soft tissue. The concentration of fluoride in the pineal was significantly higher (p < 0.001) than in corresponding muscle, i.e., 296 ± 257 vs. 0.5± 0.4 mg/kg (wet weight) respectively. The low fluoride content found in muscle in the current study was in agreement with the low fluoride content in soft tissues - less than 1 mg F/kg (WHO, 1984). This indicates that the method used in the present study had been properly executed; that fluoride in the pineal gland was endogenous and had not been introduced to the cadavers since the time of death, e.g., via the preserving formalin fluid. However, the pineal gland is unique in that it can be classified as a soft or as a mineralizing tissue. In terms of mineralized tissue, the mean fluoride concentration in the pineal calcification was equivalent to that in severely fluorosed bone and more than four times higher than in corresponding bone ash, i.e., 8,900 ± 7,700 vs. 2,040 ± 1,100 mg/kg, respectively. The calcification in two of the 11 pineals analysed in this study contained extremely high levels of fluoride: 21,800 and 20,500 mg/kg.

There is increasing interest in the determination of essential and toxic elements in neurological tissues. Fluoride metabolism in CNS has not been systematically studied. It is generally agreed that the CNS is impervious to the effects of fluoride by virtue of the blood-brain barrier (Whitford et al, 1979). The human pineal is outside the blood-brain barrier. The significance of this is not clear but it may be that the pineal needs to 'sample' the circulating blood. The results from this study are important because the pineal gland is obviously a hitherto unrealized target for chronic fluoride-toxicity.

The pineal fluoride content varied considerably between subjects (14-875 mg/kg) although it was directly correlated to pineal calcium content: r = 0.73, p < 0.02. Large amounts of calcium have been demonstrated in the pineals from young children. Indeed, the prevalence of pineal calcification in young children is higher than one may have been led to believe from radiological evidence alone (Tapp and Huxley, 1971; Reyes, 1982). In addition to its high calcium content, the pineal contains intracellular colloids, a high magnesium content (Krstic, 1976; Michotte et al, 1977; Allen et al, 1981); and a very profuse blood supply. These are all factors encouraging the acquisition of fluoride by soft tissues (WHO, 1970). High levels of magnesium, manganese, zinc and copper have been demonstrated in pineals which appear 'uncalcified' (Michotte et al, 1977). Therefore, it is likely that the child's pineal also accumulates fluoride although this needs verification. The deposition of fluoride within the child's pineal must be a recent phenomenon. The plasma-fluoride levels in young children are normally very low and what little there is is rapidly
sequestered by the growing skeleton. The extensive use of fluorides in dentistry has caused an unprecedented increase in plasma-fluoride levels in infants and young children.

Any adverse physiological effects of fluoride depend upon the concentration at various tissue sites. Can pinealocytes function normally in close proximity to high concentrations of fluoride? One would predict that a high local fluoride concentration would affect pinealocyte function in an analogous way that a high local fluoride concentration affects: i) bone cells, since histological changes have been observed in bone with 2,000 mg F/kg (Baud et al, 1978); ii) ameloblasts, since dental fluorosis develops following fluoride concentrations of 0.2 mg F/kg in the developing enamel organ (Bawden et al, 1992). The consequences are disturbances in the functions of bone and enamel, i.e., changes in structure (poorly mineralized bone and enamel). If the pineal accumulates fluoride at an earlier age than in previous decades, one would anticipate that a high local concentration of fluoride within the pineal would affect the functions of the pineal, i.e., the synthesis of hormonal products, specifically melatonin. The highest levels of pineal melatonin are produced during early childhood.

The controlled animal study carried out in this study produce compelling evidence that fluoride inhibits pineal melatonin output during pubertal development in the gerbil. The LF males and LF females excreted similar amounts of the melatonin metabolite, aMT6s, in urine from prepubescence (7 weeks), throughout puberty to young adulthood (16 weeks). For example, at 7 weeks, the LF males and LF females excreted 30.7 ± 7.9 and 26.8 ± 6.8 ng aMT6s/24-h, respectively; at 16 weeks, 31.6 ± 10.9 and 29.8 ± 8.2 ng aMT6s/24-h. There was no sex difference. These results agree with previous reports that the rates of urinary aMT6s excretion remain constant during human puberty with no sex difference (Young et al, 1988; Bojkowski and Arendt, 1990; Tetsuo et al, 1982).

When the data were corrected for body weight, the LF group excreted progressively less urinary aMT6s from 7 to 16 weeks (p <0.01). The LF males and LF females excreted significantly more aMT6s at 7 weeks: 569 ± 148 and 602 ± 168 pg/g BW/24-h than at 16 weeks: 397 ± 148 and 443 ± 126 pg/g BW/24-h, respectively. This unique pattern of urinary aMT6s excretion has also been demonstrated in human pubertal studies (Young et al, 1988; Rager et al, 1989; Bojkowski and Arendt, 1990). The LF males and LF females excreted similar total aMT6s from 7 to 16 weeks and their circadian profiles of urinary aMT6s were strikingly similar at 11 1/2 weeks and 16 weeks. This is in agreement with previous human studies which found no sex difference between the relative rates of aMT6s excretion during puberty.

The results of urinary aMT6s excreted by LF gerbils during pubertal development are ‘classical' in
the sense that they are similar to those reported in several human pubertal studies. Therefore, the LF group represent 'normal' gerbils with respect to urinary aMT6s levels excreted during sexual maturation. That the results from the LF group were foreseen indicates that the experiment had been properly executed. Therefore, this project has produced useful baseline data on the rates of urinary aMT6s excretion by the gerbil which can be used in future investigations using measurements of urinary aMT6s as an alternative to pineal melatonin measurements. However, the exactitude of the results of the LF group accentuates the divergent results from the HF group.

At 7 weeks, the prepubescent HF males excreted almost half as much urinary aMT6s as the LF males: 16.4 ± 4.2 vs. 30.7 ± 7.9 ng/24-h; p < 1.5E-05; in relative terms, 308 ± 76 vs 569 ± 148 pg/g BW/24-h, respectively: p < 0.00002. The HF males continued to excrete significantly less aMT6s than the LF males throughout puberty: at 9 weeks, 19.6 ± 4.7 vs 27.9 ± 7.7 ng/24-h (p < 0.004); in relative terms, 320 ± 75 vs. 425 ± 113 pg/g BW/24-h, respectively (p <0.01); at 11 1/2 weeks, 21.9 ± 5.7 vs. 33.0 ± 9.8 ng/24-h (p< 0.003); in relative terms, 299 ± 74 vs. 449 ± 111 pg/g BW/24-h, respectively, (p <0.001). By 16 weeks, the HF males excreted normal levels of aMT6s. Indeed, young adult gerbils excreted similar total aMT6s and exhibited similar circadian profiles of aMT6s, irrespective of gender or treatment.

At 7 weeks, the I-IF females also excreted significantly less aMT6s than the LF females, 18.1 ± 5.5 vs. 26.8 ± 6.8 ng/24-h, (p <0.002); in relative terms, 359 ± 109 vs. 602 ± 168 pg/g BW/24-h, respectively, (p <0.0004). Thereafter, the level of statistical significance between the rate of aMT6s excretion by the HF females and LF females progressively declined: at 9 weeks, p <0.02; and at 11 1/2 and 16 weeks, the FT females and LF females excreted similar total aMT6s with similar circadian profiles.

The HF group not only excreted significantly less urinary aMT6s than the LF group but the patterns of excretion were different from the LF group. After correction for body weight, the HF group had a uniform, constant rate of aMT6s excretion during sexual maturation: unlike the LF group which excreted progressively less aMT6s during puberty. At 7 weeks, the HF group (unlike the LF group) did not excrete their highest relative levels of urinary aMT6s. At 11 1/2 weeks, the FM males produced a dampened circadian profile of urinary aMT6s with a diminished amplitude of nocturnal peak values, reduced duration of nocturnal elevated values and a shift in the temporal pattern. These changes would not be distinguishable from those observed following photoperiod manipulation. At 11 1/2 weeks, the I-IF males excreted significantly less urinary aMT6s than the FM females: 21.9 ± 5.7 vs. 26.1 ± 9.5 ng/124-h, (p< 0.05); in relative terms, 299 ± 74 vs. 407 ± 134 pg/g BW/24-h, respectively, (p < 0.02).
The project also demonstrated that urinary aMT6s levels reflect pineal melatonin output in the gerbil. By inference, from prepubescence to young adulthood, the gerbil pineal (male and female) normally secretes a constant output of melatonin although, after correction for body weight, there is a significant progressive decline in melatonin output with age. Fluoride inhibited the pineal synthesis of melatonin in prepubescent male and female gerbils. The inhibitory effects of fluoride on pineal melatonin output lasted longer in males than females. A 'normal' pineal melatonin output was produced by the FT females at 11 1/2 weeks; by the HF males at 16 weeks.

Female gerbils reach functional sexual maturity earlier than male gerbils. Female gerbils can give birth as early as 72 days (Cheal, 1983) whereas male gerbils can be 130 to 140-days-old before they sire their first litters (Norris and Adams, 1972). The results suggest that fluoride inhibited pineal melatonin synthesis up until the time of sexual maturation in the gerbil. Fluoride did not inhibit pineal melatonin synthesis in female gerbils once they were sexually mature (at 11 1/2 weeks) and allowed the pineal melatonin output to reach 'normal' values. Fluoride continued to inhibit pineal melatonin synthesis in male gerbils at 11 1/2 weeks because male gerbils take longer to reach sexual maturity. Their pineal melatonin output only reached 'normal' values at 16 weeks of age.

The most plausible hypothesis for the observed significant decrease in the rate of urinary aMT6s excretion by the HF group is that fluoride affects the pineal's ability to synthesize melatonin during pubertal development in the gerbil. Fluoride may affect the enzymatic conversion of tryptophan to melatonin. Although melatonin was the hormone investigated in this project, fluoride may also affect the synthesis of melatonin precursors, (e.g., serotonin), or other pineal products, (e.g., 5-methoxytryptamine). This would depend on the position(s) of the susceptible enzyme(s). For some unknown reason, pineal calcification starts intracellularly. Calcium has been demonstrated in pinealocyte mitochondria. Therefore, it may be a mitochondrial enzyme that is sensitive to the effects of fluoride, e.g., tryptophan-5-hydroxylase. Alternatively, fluoride may affect pinealocyte enzymes which require a divalent co-enzyme because such enzymes are particularly sensitive to fluoride.

Puberty is a developmental stage related to an increase in the hypothalamic-pituitary axis and is triggered by mechanisms which have not yet been fully worked out. Melatonin is a putative neuromodulator involved in the complex process. One well-known hypothesis is that depressed plasma melatonin levels accelerate the onset of puberty. This project offered a unique opportunity to explore this hypothesis because the HF group had depressed plasma melatonin levels during puberty.
The section on the effects of fluoride on the physiological signs of sexual maturity in the gerbil was a preliminary, pilot study. There were not enough subjects to make any firm conclusions so an interpretation of the data is conjectural. However, the results do suggest that the HF females had an accelerated onset of puberty as judged by several indices of pubertal development in rodents. At 7 weeks, the HF females were significantly heavier than the LF females (p < 0.004); as heavy as the HF males and LF males. The ventral gland in the HF female developed significantly earlier than in the LF female (p < 0.004). Vaginal opening occurred earlier in the HF female than in the LF female (p <0.03). If there was a difference in male pubertal development between the groups, the elementary methods used in this study were not able to make that distinction.

At 16 weeks, the HF males had a significantly lower mean testes weight than the LF males: 1.10 ± 0.11 vs. 1.32 ± 0.18 g, respectively (p <0.002). The reason for this is not clear. At 11 1/2 weeks, the HF males produced significantly less melatonin than at 16 weeks (when their pineal melatonin output reached 'normal' values). Therefore, between 11 1/2 and 16 weeks, the HF males had progressively increasing levels of circulating plasma melatonin. This is unlike the LF males whose circulating plasma melatonin levels were progressively decreasing during the same period of development. (The LF male pineal secreted a constant melatonin output with a uniform rhythm from 11 1/2 to 16 weeks and the increase in body weight, from 73 g at 11 1/2 weeks to 80 g at 16 weeks, would dilute the levels of circulating melatonin). The amplitude, duration and timing of pineal melatonin release, and the phase angle between melatonin rhythms and other reproductive hormones are known to be important in determining the reproductive effects of melatonin. Therefore, the pineals in the FT males relayed an unusual melatonin message to the tissues and organs between 11 1/2 and 16 weeks which may have affected the male reproductive system.

Alternatively, the reason for the reduced rate of urinary aMT6s affected the clearance rate of aMT6s by the kidneys or the rate of melatonin metabolism in the liver. A recent study (Dunipace et al, 1995) investigated the effects of fluoride on kidney and liver function using four groups of rats fed 0, 5, 15 or 50 mg F/L in their drinking water for 18 months. They concluded that fluoride had no adverse physiological or genotoxic effects; did not alter the levels of blood 'wellness' markers of tissue integrity and function; and similar histopathologies in kidney and liver specimens were present across the groups. The rats maintained on water with 50 mg F/L had significantly higher urine urea and creatinine (p <0.05) than the other groups.

However, bone ash from rats maintained on water with 50 mg F/L for 12 weeks contained 5,764 ± 142 mg F/kg (Dunipace et al, 1995) which is significantly higher than the fluoride concentration in
bone ash from gerbils in the current study which were maintained on food with 37 mg F/kg for 28 weeks, i.e., 2,781 ± 95 mg/kg. The fluoride-concentration in bone is a good index of previous fluoride exposure. Therefore, the fluoride-dose used in this study was not excessive and it is unlikely that the reduced pineal melatonin output by the FT group is due to the effects of fluoride on liver or kidney function.

The daily fluoride-intake by the gerbils in the current study was well tolerated. There was only one mortality (a HF female at 14 weeks). The rationale behind the administration of fluoride to the gerbil pups was to simulate infants bottle-fed on powdered milk formula reconstituted with fluoridated water. These infants receive up to 200 times more fluoride from day 1 than breast-fed infants (Ekstrand et al, 1988). The neonatal fluoride-dose to the gerbils (2.3 μg F/g BW/day) was 23 times greater than the estimated threshold fluoride-dose to infants for the development of dental fluorosis. The rat has to receive a 4-5 times higher fluoride dose in order to achieve a plasma-fluoride level comparable to humans. Dental fluorosis occurred in rats ingesting 25-60 mg F/L in the water (Angmar-Månsson and Whitford, 1984).

The best protection against dental caries is achieved if fluoridation is available from birth (Ripa, 1993). The current view of how fluoride works to prevent the development of dental caries is the maintenance of high fluoride levels in the oral environment (Burt, 1995). Therefore, in order to obtain the maximum reduction of dental caries, the plasma-fluoride levels are increased during infancy and early childhood. Fluoride is now introduced at a much earlier stage of human development than ever before and consequently alters the normal fluoride-pharmacokinetics in infants.

But can one dramatically increase the normal fluoride-intake to infants and get away with it? The safety of the use of fluorides ultimately rests on the assumption that the developing enamel organ is most sensitive to the toxic effects of fluoride. The results from this study suggest that-the pinealocytes may be as susceptible to fluoride as the developing enamel organ. The possibility of a species difference between humans and gerbils does not allow the extrapolation of the gerbil data to humans. However, if increased plasma-fluoride levels cause a decline in the levels of circulating melatonin during early human development, significant physiological consequences may have already occurred. Changes in plasma melatonin concentrations are serious functional disturbances because melatonin has many functions in the organism. The pinealogists have not completely unravelled the mechanisms by which the pineal gland performs its tasks in the brain. The neurochemical phenomenon elicited by melatonin in CNS are unclear.

The first step in assessing a health risk by a substance to humans is the identification of its harmful
effects on animals. A health risk to humans is assessed using results from human epidemiological studies in conjunction with results from animal studies. The Newburgh-Kingston Study (Schlesinger et al., 1956) showed an earlier age of first menarche in girls living in the fluoridated Newburgh than in unfluoridated Kingston. The current animal study indicates that fluoride is associated with an earlier onset of puberty in female gerbils. Furthermore, more research was recommended on the effects of fluoride on animal and human reproduction (USPHS, 1991). This project has contributed new knowledge in this area.

I do not intend to discuss the relative merits of the claims made by the anti-fluoridationists that chronic ingestion of low levels of fluoride has harmful effects on human health, i.e., increases the risk of cancer, affects the immune system, and hastens the aging process. These claims could be associated with the effects of fluoride on the pineal because the gland has been linked to oncogenesis, immunocompetence, and, in recent years, to the process of aging.

In conclusion, the human pineal gland contains the highest concentration of fluoride in the body. Fluoride is associated with depressed pineal melatonin synthesis by prepubertal gerbils and an accelerated onset of sexual maturation in the female gerbil. The results strengthen the hypothesis that the pineal has a role in the timing of the onset of puberty. Whether or not fluoride interferes with pineal function in humans requires further investigation.