Chapter

The Contrasting Effects between Caffeine and Theobromine on Crystallization: How the Non-fluoride Dentifrice Was Developed

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Abstract

Caffeine and theobromine are members of the xanthine family. Coffee and soft drinks contain caffeine, whereas, in cacao, theobromine is the main ingredient. The mineral contents of the tooth which sucked the caffeine-containing dam's milk were decreased. To determine if caffeine would affect enamel, dams were fed with a caffeine and pups were killed and first and second molars were extracted. Enamel was exposed to the acid solution and dissolved minerals from the enamel were measured. Calcium, phosphorus and magnesium from the first molars of the caffeine group were significantly dissolved. To determine why minerals were released, enamel was separated. The crystallite size of the enamel from the caffeine group showed decreased. If the pups with the same dietary regimen, but given a cariogenic diet, the caffeine group should show a higher incidence of dental caries. The caffeine group revealed higher caries scores. An in vitro experiment to grow apatite crystals was conducted, adding the various members of the xanthine. Theobromine produced larger crystal sizes than caffeine. Theobromine was added to the maternal diet. Dissolution experiments revealed that these minerals were far less dissolved. Comparative studies of the various parameters between theobromine and fluoride were conducted. Theobromine was superior to fluoride in every aspect.

Keywords: caffeine, crystallization, developing teeth, fluoride, growth and development, non-fluoride dentifrice, theobromine

1. Introduction

Caffeine (1,3,7-trimethylxanthine) is the substance most frequently consumed in our daily life. For example, coffee, most of the soft drinks and over-the-counter medications contain caffeine [1]. On the other hand, cocoa contains theobromine (3,7-dimethylxanthine). The main source of theobromine which is daily consumed

by humans from childhood to adult is chocolate. Cocoa produces no adverse effect, in normal dosage [2]. These two similar families of xanthine, but the opposite properties on the crystal formation of the hydroxylapatite (HAP) of the teeth were discovered accidentally. Fluoride is the only known chemical that affected the HAP of the enamel by converting it to fluorohydroxylapatite.

In the dental community, fluoride has been used not only as the main ingredient of dentifrice but is also used in many others. However, recently fluoride was designated as one of the developmental neurotoxicants as more adverse effects of fluoride are revealed [3]. Although besides the developmental neurotoxicants, fluoride is also known to affect the pineal gland [4] and thyroid gland [5] and there is even some evidence between bone disease and fluoride exposure [6].

In addition, there are many reports [7–12] that infants, newborns and young children were exposed to fluoride and fluorosis among them is common. Unfortunately, in dentistry, fluorosis was mainly considered as an esthetic problem to which no more serious consideration is paid attention. Recently, it was proposed that fluoride exposure in early life may become a root cause of the disease in later life [13].

Pups' teeth were affected by the sucking milk of lactating dams which were given a caffeine-containing diet [14]. (In each animal experiment described, we obtained the permission to use the animals from the animal care committee at the LSU Health Sciences Center.) Then, eventually, non-fluoride dentifrices were developed.

2. The effect of caffeine on enamel

2.1 Dissolution studies

Further studies were conducted to determine whether the effects of caffeine come from either enamel or dentin, or both. If certain effects come from enamel, it would be an extremely interesting phenomenon, because the only chemical known to affect the HAP of the enamel is fluoride. In addition, any changes in the HAP on the enamel by caffeine might be linked to possible incidences of future dental caries.

Using the method described [15] on how to study the enamel surface of the teeth which were affected by the nutritional deficiency, the condition of the enamel was studied. The samples from the first or second molars were obtained at postnatal day 22 which is the end of lactation. The teeth were exposed to weak acid for 80 minutes and four fractions were collected at 20 minutes intervals. The experimental procedures were described in detail [16, 17]. The enamel surface of secondary electron photomicrographs are shown in the control (**Figure 1**) and caffeine group (**Figure 2**).

The apparent effects of crystallization by caffeine on the enamel of first molars where calcium, phosphorus and magnesium were released more of the teeth in the caffeine exposed offspring (**Table 1**). The first molars showed a statistically significant amount of dissolution in the caffeine group of the respective ions measured for 80 minutes compared to the non-caffeine control group [16].

On the other hand, there is no significant difference between caffeine and the control group in each mineral dissolved in the second molars.

This difference in the caffeine's effects between first and second molars can be explained as follows. The first stage of development is called the hyperplastic growth period which is primarily an increase of DNA of the organ. Synthesis of DNA and cell division at first take place rapidly [18], but thereafter, slow down. Further tissue growth can occur by cytoplasmic enlargement.

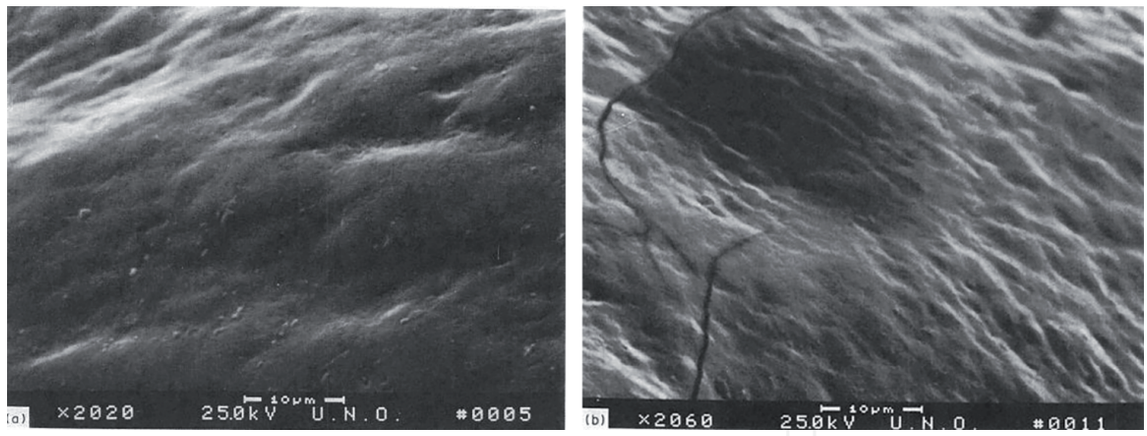

Figure 1.
Left: Before acid exposure in the control. Right: 80 minutes after the acid exposure.

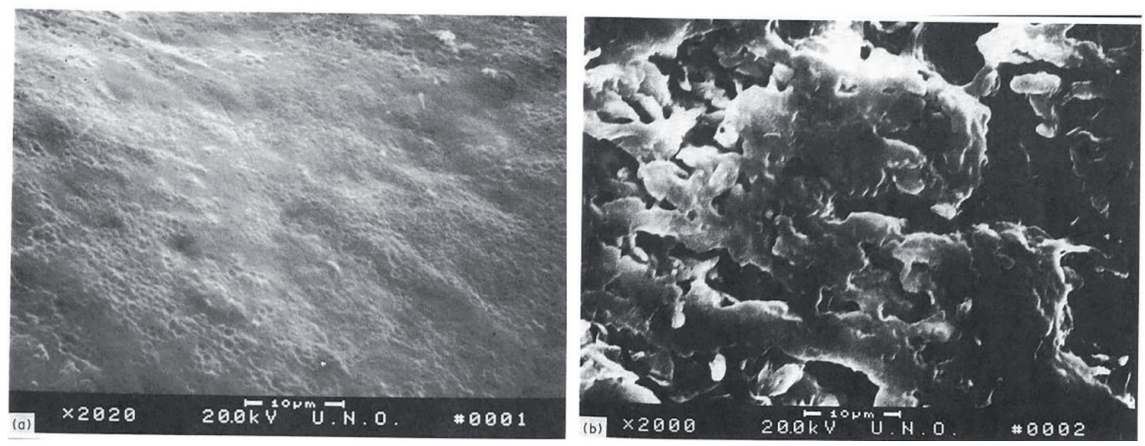

Figure 2.
Left: Before acid exposure of the caffeine group. Right: 80 minutes after the acid exposure.

The measured DNA content as an index of cell number distinguishes growth by an increase of cell number (hyperplasia). Cell division eventually ceases will be seen to determine when the organ is no longer vulnerable to nutritional stress. If nutritional stress, such as caffeine exposure in the early part of life were applied hyperplastic growth period, the organ or body would never recover to the original condition. Therefore, this period is called a critical growth period.

The second stage is a gradual decrease in cell number and a slow increase of cell size in the organ. The third stage is primarily an increase in cell size, which is called the hypertrophic growth period. If the nutritional stress were applied in this period, organ or animal grows back normally, provided that enough nutrition is given [19, 20].

During the period of growth and development, the critical period of growth is the most important concept. For example, the huge increase of DNA in the brain occurs primarily during gestation and early lactation period whereas an increase of the DNA of the heart continues until adulthood [21]. This indicates that the critical period of growth is different among the organs, therefore, the effects of the nutritional stress on each organ is different, depending upon when the stress is applied.

Likewise, caffeine exposures for the growing period were different between the first and second molars, indicating that the critical period of growth during caffeine exposure was different between the first and second molars. First molars are affected

	First molar			Second molar	
	Control	Caffeine		Control	Caffeine
			20 min		
Ca	5.23 ± 0.33	8.63 ± 1.07*		7.45 ± 1.29	7.36 ± 0.86
P	2.67 ± 0.36	5.66 ± 1.07*		4.09 ± 0.98	4.41 ± 0.89
Mg	0.20 ± 0.02	0.44 ± 0.07*		0.28 ± 0.04	0.31 ± 0.04
			40 min		
Ca	10.74 ± 1.17	16.83 ± 1.10*		13.59 ± 1.15	13.63 ± 0.92
P	7.34 ± 0.77	12.49 ± 1.40*		10.03 ± 1.45	8.68 ± 0.95
Mg	0.39 ± 0.06	0.72 ± 0.06*		0.45 ± 0.04	0.55 ± 0.05
			60 min		
Ca	12.36 ± 1.22	18.19 ± 1.12*		14.68 ± 1.15	14.85 ± 1.19
P	8.03 ± 0.78	14.72 ± 1.98*		10.47 ± 1.15	10.42 ± 0.97
Mg	0.43 ± 0.04	0.74 ± 0.06*		0.49 ± 0.03	0.55 ± 0.05
			80 min		
Ca	13.68 ± 1.30	19.64 ± 1.19*		15.21 ± 1.22	15.94 ± 0.91
P	9.39 ± 0.91	18.12 ± 2.62*		11.05 ± 1.10	12.88 ± 1.80
Mg	0.42 ± 0.04	0.71 ± 0.05*		043 ± 0.05	052 ± 005

Each value is an average of seven determinations. Significantly different from the control at P < 0.05.*

Table 1.
Mean amount of minerals dissolved from first or second molars during each time interval (μg/four first or second molars; mean ± SEM).

by caffeine intake by the offspring during this period. Therefore, the experiments were conducted only using the first molars.

2.2 Crystallization studies of HAP

To find out what is happening to the enamel of the first molars, samples that were not used in the studies were powered and enamel was separated from dentin [22]. Then a pure enamel sample was run for 4 hours on a Gandolfi X-ray powder camera. Also, various other aspects of the samples were studied in detail [17].

The filmstrip run on the Gandolfi camera recorded more diffuse lines for the samples of the caffeine group compared to the control (**Figure 3**).

X-ray diffraction analysis on enamel samples by the Gandolfi X-ray camera showed the caffeine supplementation in the maternal diet affected mineralization of enamel, as broader lines indicated smaller crystallites of enamel. Smaller crystallites increase susceptibility to dissolution.

This explains why the caffeine group of the teeth showed the higher dissolution of each ion from the surface of enamel throughout the experimental period [16].

2.3 Cariogenic studies

Because the teeth were affected by caffeine it is natural to make a simple assumption, that is, is it possible to produce in vivo dental caries in the caffeine group [16].

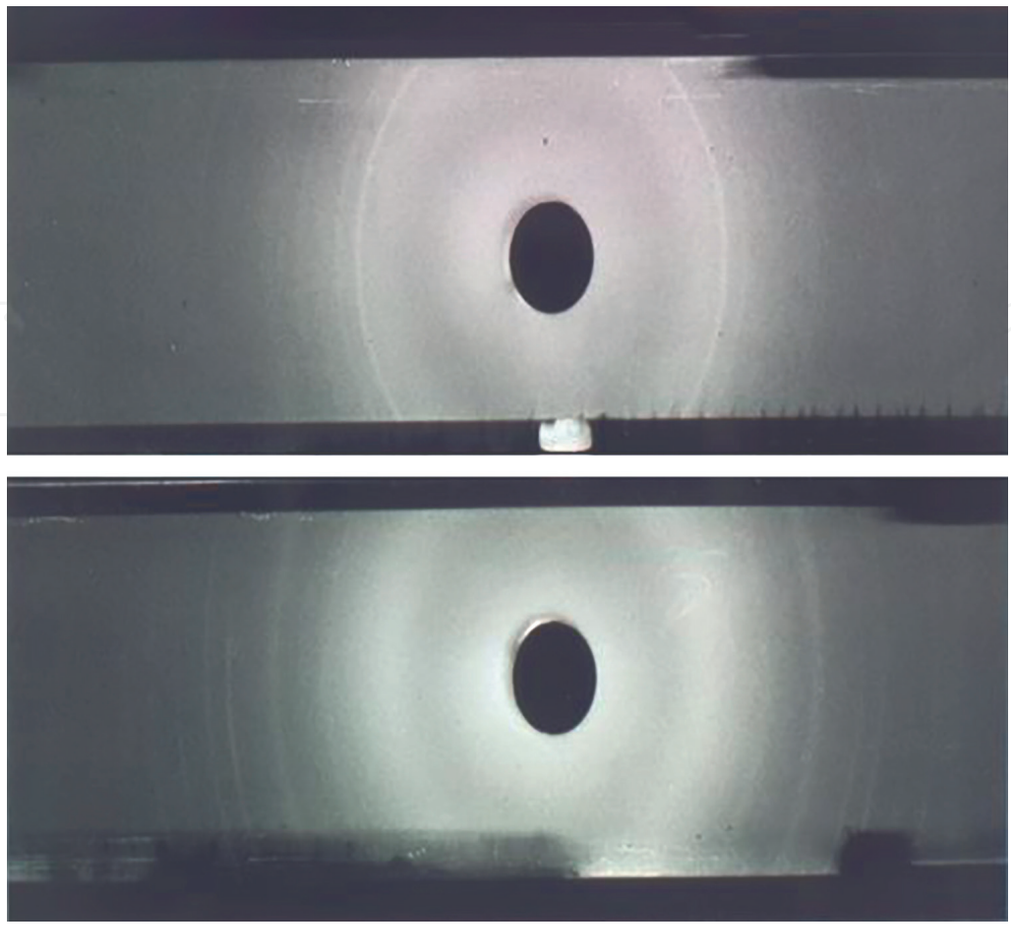

Figure 3.
Top: control. Bottom: caffeine group.

Thus, the experiment was conducted by raising the offspring the same way as above. At weanling on postnatal day 22, offspring were fed the cariogenic diet until day 50 (a total of 28 days) to see whether the caffeine group show a response in dental caries using the methods described [23]. The caffeine group showed significantly higher caries scores than that of the non-caffeine control ($P < 0.05$) (3.36 ± 0.33 versus 2.65 ± 0.22) (mean ± SEM) [24].

Therefore, the hypothesis turned to be true as is shown in **Figures 4** and **5**. The amount of caffeine that was added to the maternal diet was 2 mg/100 g bodyweight of the dam. The equivalent comparison between the caffeine in the rat and human is based on metabolic body weight ($kg^{0.75}$) [25]. (Metabolic rates are expressed in terms of metabolic body size—i.e., $kg^{0.75}$, the point at which the dependence on different body sizes disappears.) The human caffeine intake is comparable with slightly more than two cups of coffee daily.

This is the normal amount of caffeine consumed by humans. Although extrapolation from rat data to human dental caries incidence requires extreme caution, nevertheless present data indicate that caffeine exposure during the early growth period impairs the structure of the enamel crystal formation of the developing teeth. Particularly, it may require attention where the offspring born from a pregnant woman who habitually consumes caffeine-containing soft drinks and/or coffee drinkers may develop teeth that are prone to dental caries of the offspring in the future. It seems clear that caffeine exposure during the critical growth period affects the amelogenesis of the enamel, affecting the crystal size of the HAP during mineralization.

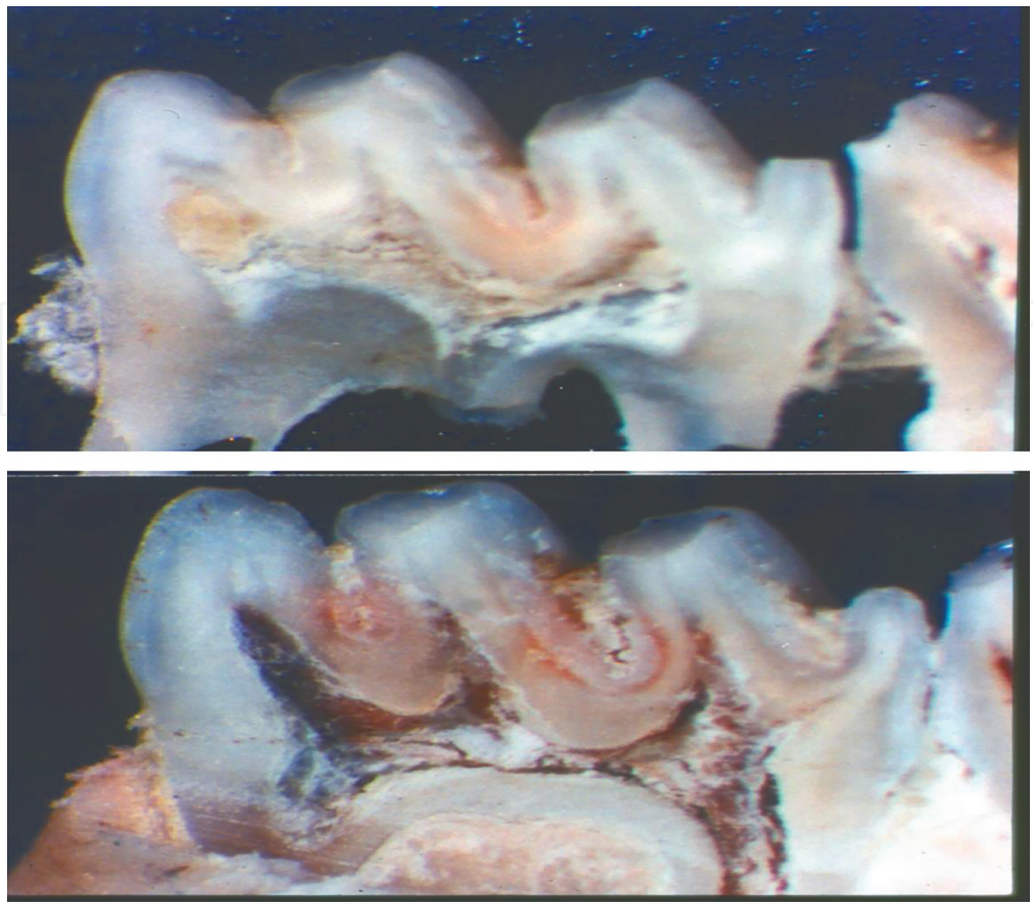

Figure 4.
Molar of the control at day 50 stained with 0.06% murexide in 70% ethanol for 16 hours (top), the caffeine group (bottom).

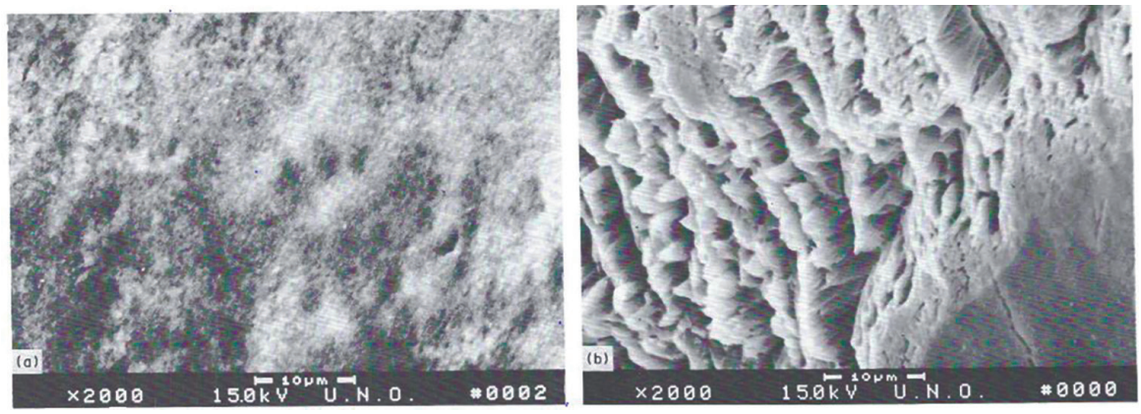

Figure 5.
Secondary electron image of control at day 50 (left) and the caffeine group (right).

3. In vitro study for other xanthine family members

3.1 HAP formation

A series of caffeine studies on crystallization research required more than several years. Thus, a simple in vitro study was conducted to see whether other xanthine family members could reveal if any, other different crystallization value(s) from caffeine.

All the solutions contained 0.01 molar $CaCl_2$ and Na_3PO_4. Several sets of experiments were conducted with the addition of each of methylxanthine at low concentrations, 50 mg, and 200 mg/L. The effect of the xanthine compounds was compared with a control solution containing $CaCl_2$ and Na_3PO_4 only. Solutions were mixed at 25°C, and pH adjusted to 9–9.5 with 0.1 molar NaOH and left to crystallize for 20 days. The crystalline was washed five times with distilled water and prepared for X-ray diffraction (**Figure 6**).

All the data from the various members of the xanthine family on the effects of crystallization were already reported [26, 27].

To our surprise, the value of theobromine was much lower than caffeine. Specifically, 1-methylxanthine values were lower than the caffeine group but not as low as the theobromine group.

Caffeine (small crystal size) and theobromine (large crystal size) with the lack of one position of the methyl group, crystal size was opposite. Therefore, it should be further investigated how the methyl position 1 alone can influence the crystallization.

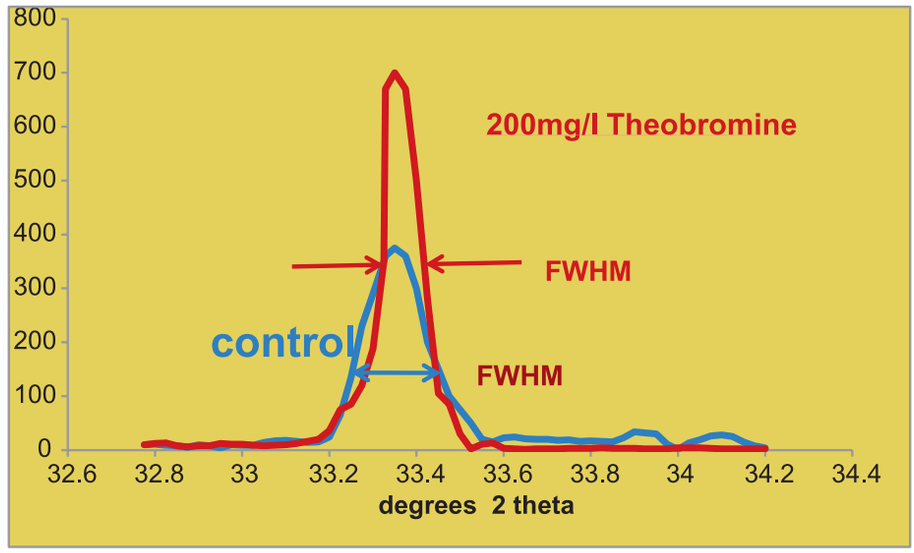

Figure 6.
The (300) reflection was scanned to investigate crystallinity. FWHM (full width-half maximum peak height) divided by maximum peak height (FWHM/M) and for the (300) reflection is given in **Table 2.**

FWHM/M of apatite grown in vitro		
Additive	**mg/L**	**FWHM/M**
Control	0	0.75
Caffeine	200	1.00
Caffeine	50	0.90
Theobromine	200	0.15
Theobromine	50	0.19
1-methylxanthine	200	0.60
1-methylxanthine	50	0.68
Lower values of the ratio indicate better crystal size.		

Table 2.
Hydroxylapatite which was grown in vitro of three xanthine families, caffeine, theobromine and 1-methylxanthine are shown.

The crystal size of position 1 alone did not have much effect on the crystal size. See the chemical formulae (**Figure** 7).

The crystal size of the theobromine group taken by an electron microscope was shown below and the size was four times bigger than that of the control group (**Figure** 8).

3.2 Theobromine and oral application

Theobromine and caffeine contents of the average commercial sweet chocolates are approximately 8:1 ratio [28]. The half-life of theobromine and caffeine is different and that of theobromine is longer than caffeine.

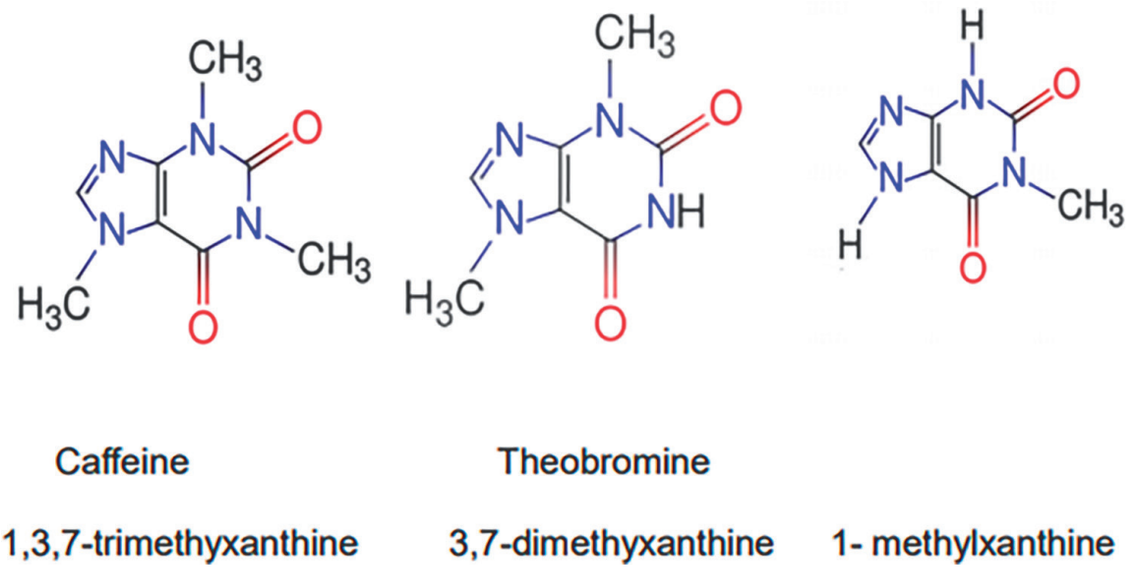

Figure 7.
The chemical formula of three xanthines.

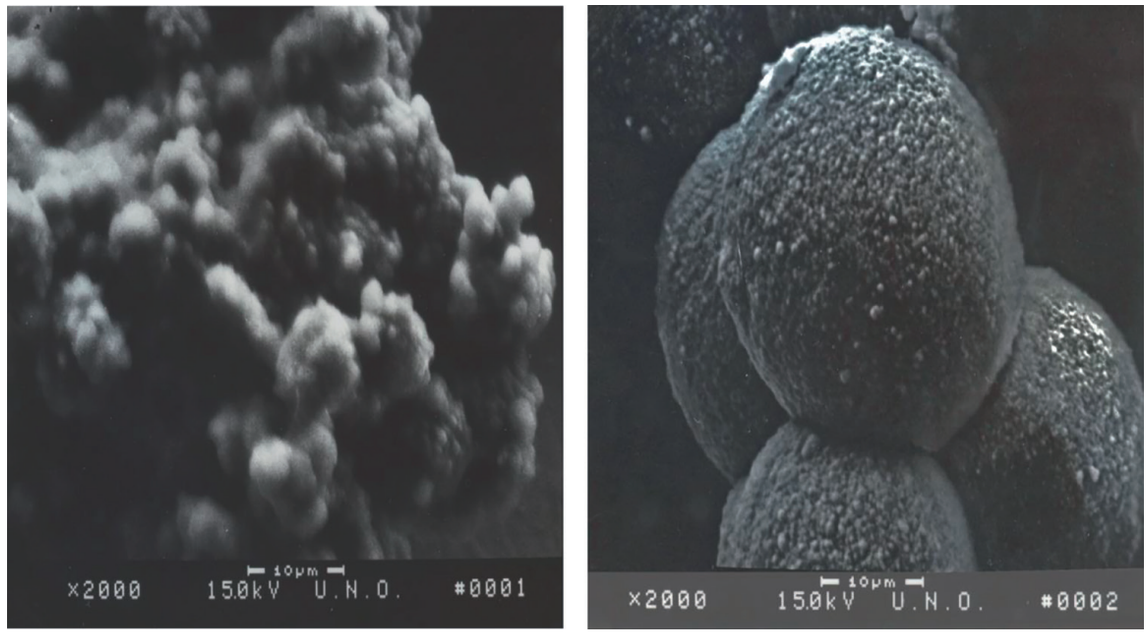

Figure 8.
(Left) Theobromine group: 200 mg/L, Crystal size: 2 μm. (Right) Control group: Crystal size: 0.5 μm.

The inhibitory effect of cocoa which contains theobromine on plaque accumulation and cariostatic activity has been suggested [29]. However, its anticaries activity is not strong enough to suppress significantly cariogenic activity [30]. On the other hand, the addition of a water-soluble extract of cacao powder significantly reduced caries scores in specific pathogen-free rats infected with *Streptococcus sobrinus* 6715 [31]. Theobromine-based dentifrice had the added benefits of increasing the salivary pH and decreasing the *S. mutans* levels [32].

4. In vivo study exposed theobromine

4.1 In vivo crystallization studies

The amount of theobromine added to the maternal diet in the study was 1 mg/100 g body weight. If this amount is converted by the metabolic body weight ($kg^{0.75}$) [25], this corresponds approximately to slightly more than one to three bars of 1 oz milk chocolate for a 65 kg human. After raising the offspring fed with a maternal diet containing theobromine, molars were extracted and studied the following aspects.

4.1.1 X-ray diffractometry

A consistent relationship of higher crystallinity, i.e., larger crystallites, in the whole molars from the rats exposed to theobromine, compared to the control and/or caffeine group was observed in the X-ray diffractometry [27].

4.1.2 Microprobe analysis

Calcium and phosphorus concentrations were determined in the enamel of molars extracted from theobromine exposed rats or control rats by an "ARL-SEMQ"™ electron

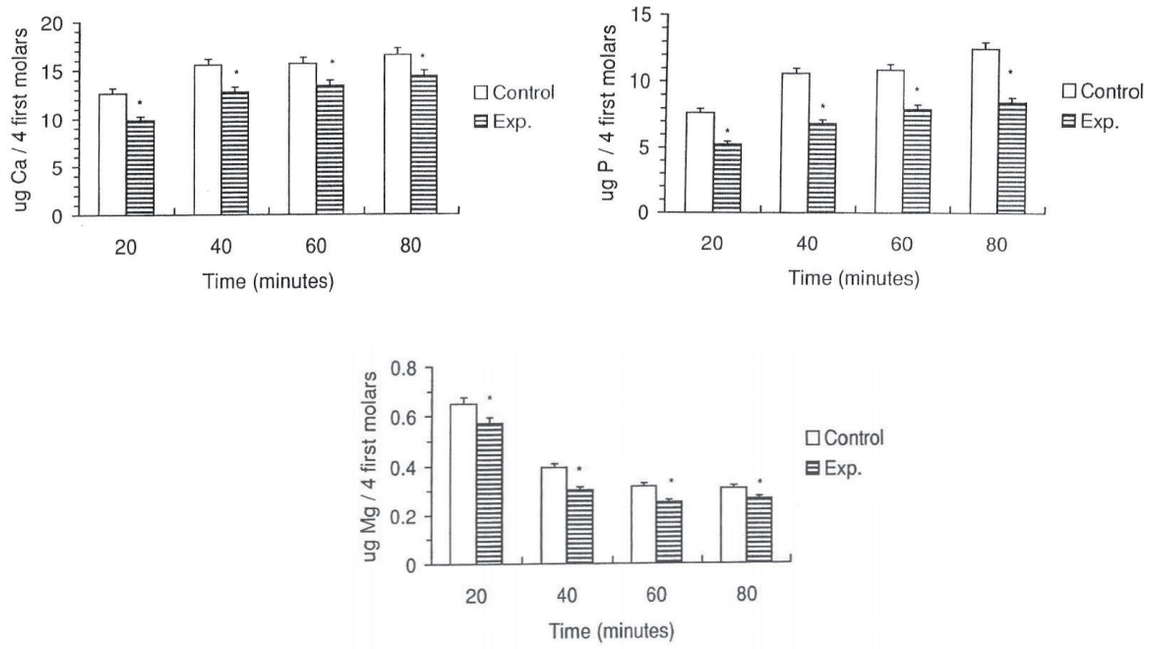

Figure 9.
Calcium (upper left), phosphorus (upper right) and magnesium (bottom) theobromine group were significantly ∗ ($P < 0.05$) less than the control.

microprobe analysis. The instrument was operated at an acceleration potential of 15 kV and a beam current of 1.0×10^{-7} A. Fluorapatite from Cerro de Mercado, Mexico, was used as a standard. The results obtained are shown in our previous report [26, 27].

This study was conducted to see whether the composition of crystallites of the enamel formed by theobromine is different from the control group. The overall content of CaO and P_2O_5 appears to be comparable within the expected margin of error. This suggests that the composition of crystallites of the enamel between control and experimental groups are the same.

4.2 Dissolution studies

The same experimental procedures, as was in the caffeine study [16], were conducted. The molars from the offspring whose dams were fed with the diet supplemented with theobromine were extracted. The dissolution studies were done. The result showed decreased dissolution (Ca, P, and Mg) of the ions from this group due to the larger crystal size (**Figure 9**) [27].

5. Comparison of theobromine and fluoride

5.1 Microhardness test

The unique roles of theobromine were accidentally discovered during the caffeine study. Because previously only fluoride has been known to affect the enamel, the next steps were to investigate the comparative studies between theobromine and fluoride on the effects of the enamel in a similar environment using the in vitro system.

If remineralization occurs, then the increased enamel is associated with increased microhardness [33]. The microhardness test was conducted using theobromine [34]. An in vitro study confirmed that theobromine increased the microhardness of the enamel [35–37].

The enamel of human teeth with varying concentrations of theobromine vs. sodium fluoride vs. control groups (distilled water) was performed. Scanning electron microscopy (SEM) and Knoop Microhardness Testing (NMT) were conducted. A Knoop microhardness instrument was used. Knoop Microhardness tests were performed every day for a period of 8 days on each sample and the data were recorded. Day 1 represents the baseline hardness.

On day 8, it was not tested to avoid interfering with the surface of the tooth before taking the scanning electron photomicrographs. The hardness data are shown at the end of the 8th day and the results were published [26]. **Figure 10** shows SEM photomicrographs of the enamel of control, fluoride and theobromine.

In general, the teeth that were coated with theobromine appeared to be cleaner, smooth and excellent mineralization was observed under the electron microscope especially when compared with control group teeth.

5.2 Acid dissolution study

Samples were covered with small uniformly cut circles of double-stick tape.

The tooth was covered in nail polish and the tape was removed to expose a fairly uniform enamel surface on each tooth.

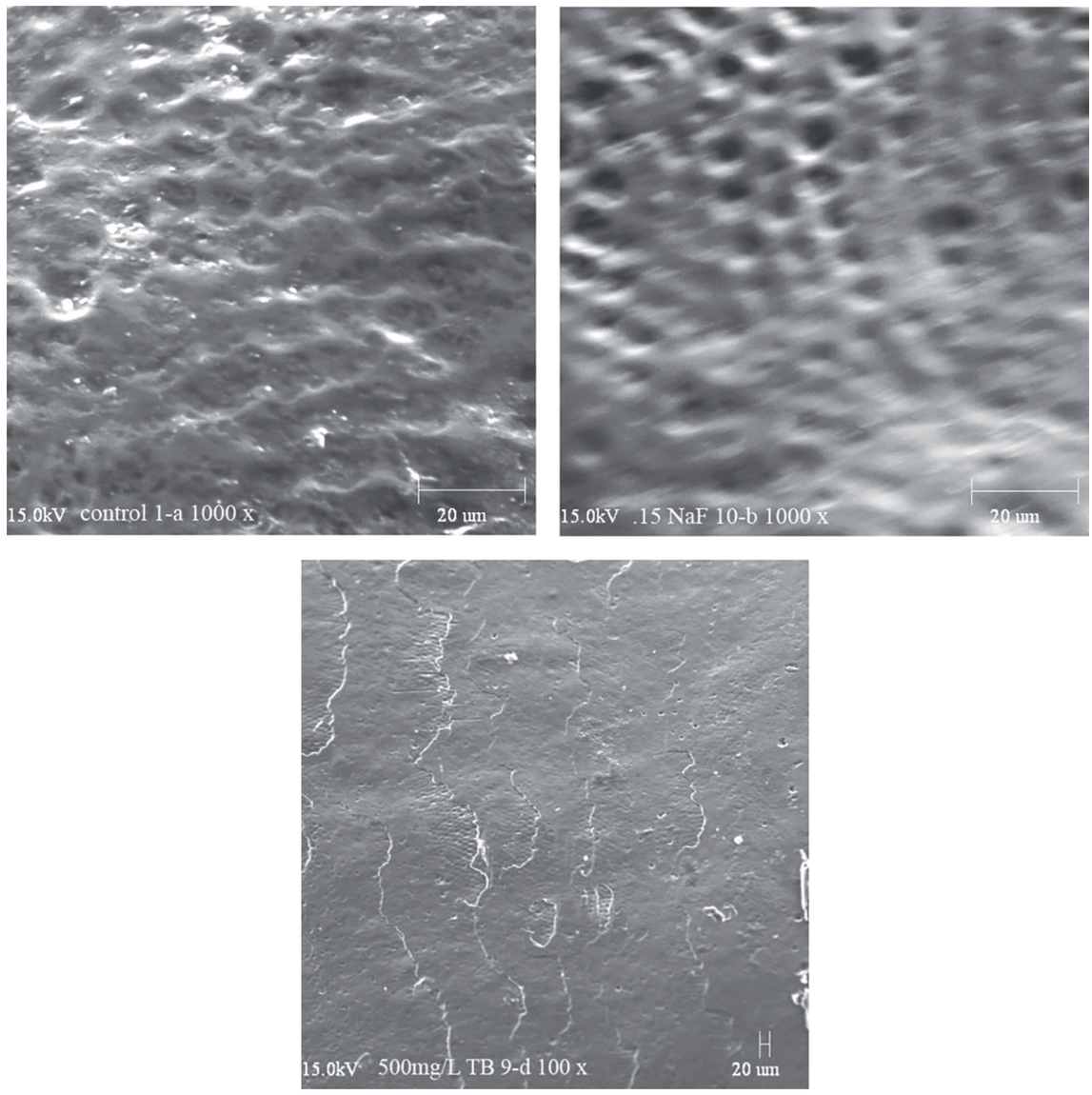

Figure 10.
Upper left: control group. Upper right: 0.15% w/v fluoride ion. Bottom: theobromine group: 500 mg/L.

Each tooth was immersed in either 0.15% w/v fluoride ion solution or theobromine 100 mg/L solution for 30 minutes. Then, each tooth was exposed to a 0.001 N HCl acid solution for 10 minutes to determine how much calcium is released from the enamel. The amount calcium (ppm) released from the surface of the enamel in the F-0.15% was an average of 0.930 whereas theobromine-100 mg was 0.848. The fluoride group of teeth released 9.66% more calcium compared to the theobromine group, suggesting that the pre-exposures by theobromine solution is more effective to make the enamel surface of teeth more resistant than fluoride solution. Theobromine is reported as an effective remineralizing agent [38].

5.3 Clinical hypersensitivity studies with either theobromine or fluoride-containing toothpastes

Hypersensitivity is a short and sharp pain arising from exposed dentin by an external stimulus [39]. The prevalence of hypersensitivity varies [40]. The therapeutic approach is to occlude dentin tubules [41].

Clinical studies were conducted in four different samples. The detail of the experimental design has been already described [26, 42]. Importantly theobromine-based toothpaste has shown that the hypersensitivity disappeared within a week [42]. On the other hand, fluoride-based toothpastes indicated practically no effects to alleviate sensitivity without much occlusion on the dentinal tubes during this experimental period of 1 week.

5.4 Preventive effect of dental caries by theobromine

A study using an established in vitro caries pH cycling model [43] was conducted [44]. Treatment with theobromine results in resistance to acid attack. A recent study has shown that theobromine gel had more effective remineralizing potential than fluoride gel [45].

In the oral environment, remineralization and demineralization are happening constantly. The hardness test conducted on the enamel surface [26] clearly showed that theobromine was much more effective than fluoride. However, in another study using pH cycling method, theobromine does not appear to offer any anti-caries benefits [46]. Applied theobromine to the demineralized enamel surface caused recrystallization and increased surface microhardness.

Crystallite size is the main factor that controls the dissolution of HAP. Small crystallites have a much higher surface area/volume ratio compared to larger crystallites. Dissolution is more rapid on smaller crystallites than on larger ones. Thus, larger crystallites of HAP in the enamel resist dissolution under cariogenic conditions better than smaller ones.

From the comparative studies between theobromine and fluoride each parameter measured indicated that theobromine is superior to fluoride. Recently, a clinical study on the evaluation of the anticaries activity of either theobromine or fluoride-based toothpaste against *plaque S. mutans* in children of age group from 6 to 9 years was conducted [47]. It was concluded that theobromine-based toothpaste is beneficial as a safe anti-cariogenic agent. Furthermore, theobromine showed more antimicrobial effects against *S. mutans*, *Lactobacillus acidophilus*, and *Enterococcus faecalis* in the in vitro study. Theobromine showed greater zones of inhibition than other commercially available fluoridated children's toothpastes [48].

In addition, a recent in vitro study indicated that theobromine is an effective cariostatic agent and a safe alternative to fluoride in preventive dental care [36, 49]. The theobromine group is superior compared to the fluoride group in each parameter studied.

6. How did Ancient Mayans embed round jade inlays?

In ancient times in the Mayan culture, cocoa was used only among the wealthy and even until recently, in certain countries, cocoa was used to exchange wealth. What is surprising about the skull is that jade was embedded as an inlay into each tooth (**Figure 11**), which required drilling the enamel surface of the tooth.

However, drilling the precise hole to embed the jade would have been difficult if not impossible. It seems that somehow after placing the jade into each tooth, they must have had the knowledge to fix the jade within the hole.

A simple experiment was conducted by us [26] to determine how ancient Mayan knew the unique role of cocoa 1100 years ago. We have experimented with the knowledge obtained in the past years with theobromine. One can see the study presented in the previous report [26]. Thus, the mystery of how ancient Mayan placed jade in the front teeth was solved. We know now that theobromine which was extracted from cocoa was used to fill the marginal space around the jade and initiate mineralization to fix the jade

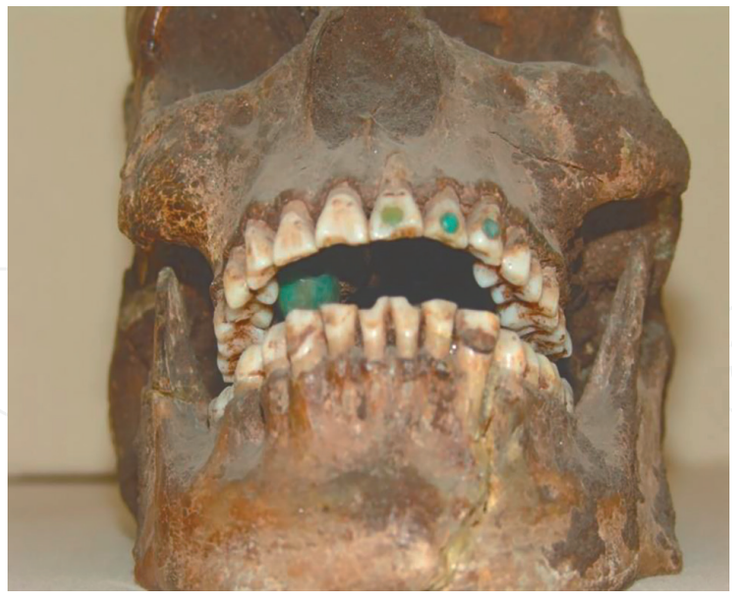

Figure 11.
Mayan skull was reported to be 1100 years old. Note that Jades were embedded.

insert on the teeth. These results are strong evidence that ancient Mayans knew the role of theobromine in cocoa on the mineralization of hard tissue over 1100 years ago.

It is also interesting to note that the strong and unusually heavy-looking mandible of this skull supports the finding that theobromine also plays a role in the growth and development of bones [27]:

7. Why the present finding of the role of theobromine is so revolutionary?

7.1 Adverse effects of fluoride

In the past, fluoride is the one that was solely used in the dental profession to prevent dental caries and added to most of the toothpaste. In addition, fluoride has been used a high amount of varnish solution [50] and glass-ionomer cement [51].

Fluoride has been described as the same category of alcohol, nicotine, and lead and advised to avoid them during pregnancy [52]. Fluorosis [53, 54] is very common.

Maternal exposure to fluoride during pregnancy was associated with lower IQ scores in children aged from 3 to 4 years [55]. This is the first report describing the possible effects of lower IQ scores of offspring as a result of maternal fluoride intake. Previously, numerous studies associated with fluoride exposures and lower IQ scores of children were already reported. This phenomenon was observed despite the parents' education and family income in China [56], India [57] and Taiwan [58]. Cognitive alterations in children born from exposed mothers to fluoride could start in early prenatal stages of life and appear later at school age; and likely continue into adulthood [59].

One of the neurodevelopmental disabilities is autism [60] and the decreased secretion of melatonin from the pineal gland alters circadian rhythms and sleep patterns [61].

Another interesting aspect is the relationship between caffeine-containing drinks and fluoride. The higher fluorosis severity was associated with soft drinks and coffee consumption, as most soft drinks contain caffeine [62]. This was explained that the presence of fluoride would remain longer due to the ingestion of caffeine-containing beverages [63].

7.2 Fluoride exposures in early life

A 6 oz. container of 1500 ppm fluoride toothpastes contains 254.7 mg of fluoride. A one-year-old (8.14 kg) child ingesting less than one-fifth of the contents of the container would possibly exceed a toxic dose [9]. It has been well known that coronary heart disease and related disorders such as stokes, diabetes and hypertension may originate during fetal development [64]. As further examples, overfeeding of newborns tend to lead to obesity in later adult life. Caffeine exposures during the gestation and lactation periods appeared reduced locomotive activity in the later ages of animals [65]. By the undernourishment in utero, intrauterine programming during this period could contribute to the risk of osteoporotic fractures in later life [66]. "Programming phenomena" in the body of early life [67] is explained as nutritional stress during the critical period of growth that causes permanent or long-term changes in the structure or function of the organ.

Unfortunately, certain areas of the U.S. have an incidence of fluorosis of about 70% [54] to 80% [53] in children. These examples stipulate very high incidences of possible fluoride's effects on growing children.

Consider each step. Fluorosis of teeth is a result of the effect of fluoride on ameloblasts cells. The other parts of the organs as well could have been affected by this chemical. Excess fluoride exposures have already been known to cause various diseases described above.

The exposure of fluoride must be not only the excess dose of fluoride but also the duration of exposure and timing [52] such as the critical growth period as explained. Fluorosis has been reported to be associated with the lower performance of neuropsychological tests [68]. Developmental enamel defect is twice as frequently with mental retardation [52]. However, there are not many studies that investigated the relationship between fluorosis in teeth and systemic diseases at the same time. For example, is the incidence of fluorosis related to specific diseases? To answer this question, there is a need for close clinical cooperation between dentistry and medicine in future studies of fluoride.

Patients with fluorosis may develop certain diseases in later life. The growth and development of organs or bodies on the surface could have been minor. However, genetic influences could have already occurred, possibly resulting in a slight alteration of structure or function at the cellular levels [67].

Despite little difference in average levels of tooth decay between fluoridated and unfluoridated water of the same country [69], there are still areas in the US where water is fluoridated and water fluoridation may add a small amount of fluoride into the bodies.

In the body, continuous formation and breakdown have been happening throughout the life cycle. If the periods of the formation far exceed breakdown such as the rapid growth period, minor changes of cellular levels might not appear readily. When the period of breakdown is exceeded in the later stages of life, diseases associated with fluoride exposures in early life could become a root cause of disease in later life [13].

We do not know at this time what kind of diseases, if any, might appear in later life by fluoride exposures in early life. However, early nutritional stresses on the developing fetus cause various diseases [64–66]. The current concept on the number of fluoride exposures in early life and root causes of disease in the future is not an unrealistic hypothesis. If this were proven to be true by future epidemiological studies and/or even one could argue "do we need to wait until such time". The time might have come now to reexamine the routine use of fluoride in dental practice from a fundamental aspect.

7.3 Development of non-fluoride dentifrice

During the study of caffeine on the effects of developing teeth, we have accidentally discovered that one of the xanthine family, theobromine, showed the opposite effects from caffeine on the crystallization of hydroxylapatite (HAP). Theobromine combined with calcium and phosphorus which is called "rennou" was added to accelerate the crystal formation of non-fluoride dentifrice.

Cacao contains theobromine. Chocolate which comes from cacao has been consumed without any ill effects by humans in the past. Cacao has an interesting history. In 1753, Swedish taxonomer Carl Linnaeus named the cacao plant "theo-broma" which translates to "food of gods". He was a believer in the power of cacao. Ancient Mayans at least 1100 years ago already knew the unique roles of cacao on crystallization and possibly used it to fix jade inside of the enamel. The specific characteristics of theobromine led to the formation of fluoride-free toothpastes.

Theobromine-based dentifrice is revolutionary. This is because space travel in our future, military or even camping where the water supply is most often limited, it is not needed to rinse the mouth with water or just spit after brushing teeth. However, most importantly, even if one swallows it, there are no adverse effects. Theobromine

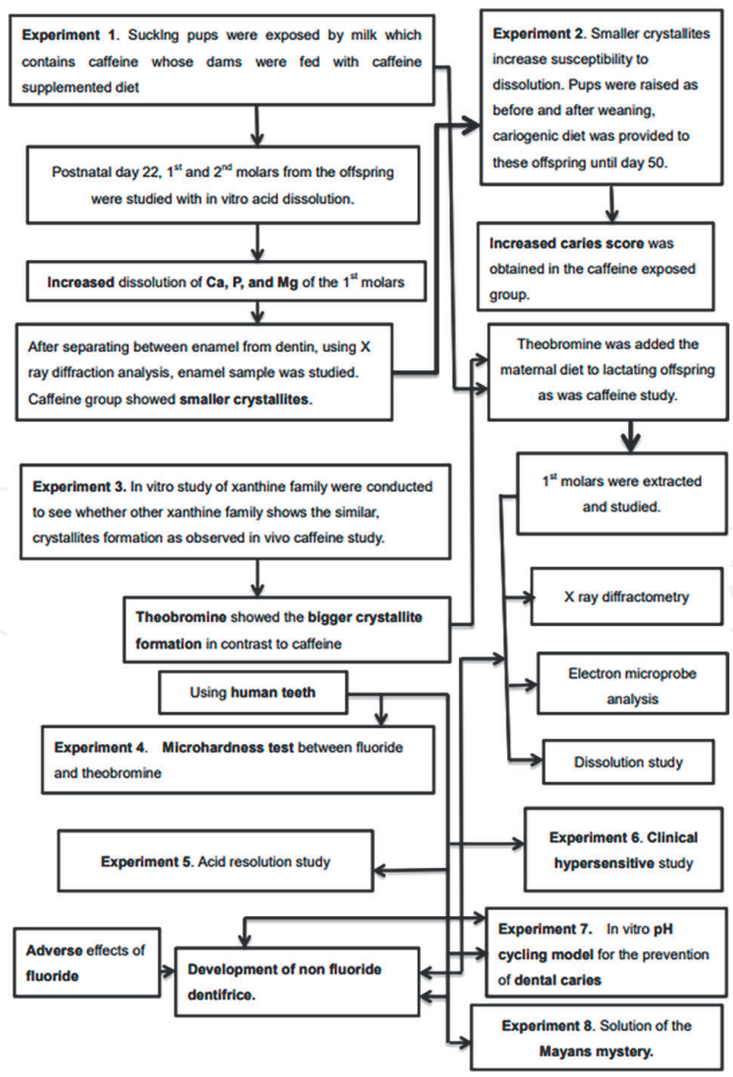

Figure 12.
The flow chart shows how non-fluoride dentifrice was developed.

changes the crystallization dynamics of apatite group species, resulting in fewer and larger crystallites. It likely interacts with ions being deposited at the growing HAP crystal surface. Thus, the ratio of surface area versus volume of the crystals is lowered and dissolution is not as rapid or pronounced as in smaller crystals.

In conclusion, non-fluoride-based dentifrice was introduced for the replacement of fluoride-based toothpastes. Further studies of theobromine-based dentifrice have to be examined as a reliable alternative of the safe replacement of fluoride-based dentifrice. This requires vigorous basic and clinical studies by scientists and clinicians.

So far, all the evidence presented shows that the theobromine is superior to that of fluoride and most importantly, the use of theobromine is safe. The current development of non-fluoride dentifrice is most timely. The flow chart in **Figure 12** was added to summarize the development of this non-fluoride dentifrice.

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