SUCROSE LOAD, CALCIUM-DEFICIENCY AND DENTAL CARIES ON MOLARS OF GROWING RATS

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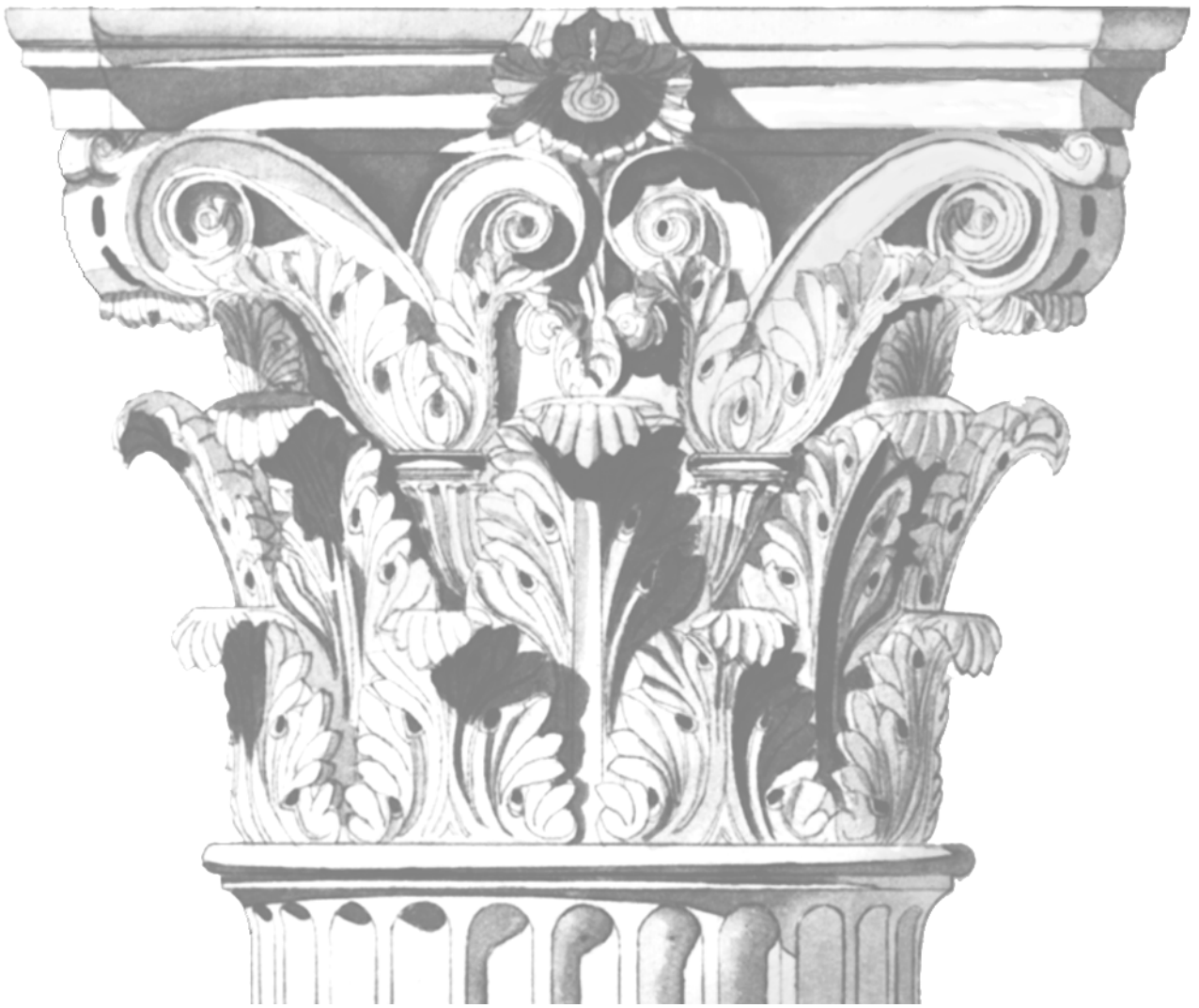
OULU 2003



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Academic Dissertation to be presented with the assent of the Faculty of Medicine, University of Oulu, for public discussion in the Auditorium 1 of the Institute of Dentistry, on June 14th, 2003, at 12 noon.

OULUN YLIOPISTO, OULU 2003

Reviewed by
Professor John Leonora
Docent Juha Tuukkanen

ISBN 951-42-7035-5 (URL: http://herkules.oulu.fi/isbn9514270355/)

ALSO AVAILABLE IN PRINTED FORMAT
Acta Univ. Oul. D 729, 2003
ISBN 951-42-7034-7
ISSN 0355-3221 (URL: http://herkules.oulu.fi/issn03553221/)

OULU UNIVERSITY PRESS
OULU 2003

Pekkala, Esa, Sucrose load, calcium-deficiency and dental caries on molars of growing rats

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Oulu, Finland
2003

Abstract

The effects of dietary sucrose feeding, intragastric sucrose feeding and dietary calcium-deficiency on primary dentinogenesis of the pulp-dentin organ and caries progression were examined in an experimental rat model. The possible role of calcium balance and reduced mineralization of dentin organic matrix as a cause of reduced dentin formation in young, fast growing, rats were also studied. During 3-6 weeks of feeding, immediately after weaning at 3 weeks the urinary calcium, phosphorus, potassium and sodium levels and excretion rates were determined. The areas of dentin formed, the width of the predentin layer, serum mineral and insulin levels, and the areas of dentinal caries lesions were quantified at the end of the experiment. Also the occurrence and progression level of caries lesions were measured. In rat pups, dietary sucrose reduced dentin formation both during the lactation and experimental periods, increased urinary Ca^{2+} excretions, reduced urinary P, K and Na excretions, and enhanced dental caries occurrence and progression, but it did not affect the width of the predentin layer or the serum mineral and insulin levels. Intragastric sucrose reduced dentin formation and increased Ca^{2+} excretion, but did not affect the width of the predentin layer, serum mineral and insulin levels in the blood or induce dental caries. Dietary calcium-deficiency reduced dentin formation, increased the width of the predentin layer, caused hypocalcemia, and reduced urinary Ca^{2+} excretion. These results show that sucrose and calcium-deficiency reduce the rate of primary dentinogenesis through different mechanisms. Calcium imbalance or reduced mineralization of the dentin organic matrix does not explain the reduced dentinogenesis in sucrose fed rats. In conclusion, the present findings indicate that a sucrose load reduces dentinogenesis by impairing the synthesis rate of the dentin organic matrix, but also points out the importance of the local sucrose challenge in initiating dental caries.

Keywords: calcium-deficiency, caries, dentinogenesis, sucrose

Acknowledgements

This thesis was carried out in 1997-2003 in the Department of Pedodontics, Cariology and Endodontics at the Institute of Dentistry, University of Oulu, Oulu, Finland.

I wish to express my deepest gratitude to my supervisor, Professor Markku Larmas, D.D.S., Ph.D., who gave the opportunity to work under his constructive guidance during my undergraduate years. With his understanding and supportive attitude, it was possible to carry out this study.

I am also grateful to my other mentor, Docent Eeva-Liisa Hietala, D.D.S., Ph.D., for enjoyable co-work and for introducing me to this particular field of dentistry. Her valuable comments and encouragement helped me especially during setbacks.

I wish to thank the official referees of this thesis Professor John Leonora, Ph.D., and Docent Juha Tuukkanen, D.D.S., Ph.D., for their constructive and fruitful discussion and good advice. Especially I am grateful to Professor John Leonora for correcting the English language and for the necessary scientific criticism.

I owe thanks to Chief Chemist Matti Puukka, University Hospital of Oulu, for professional and efficient collaboration. I also warmly thank Päivi Laukkanen, M.Sc., for valuable comments and guidance concerning biostatistics and Docent Leo Tjäderhane, D.D.S., Ph.D., whose personal attitude to scientific work could serve as an example for anyone.

I thank all the former and present faculty and staff at the Institute of Dentistry, who helped me during the preparation of this thesis, especially Dr. Liisa Välikangas, D.D.S.. I thank for the help I was given by the staff of the Laboratory Animal Centre of University of Oulu, the Laboratory of the Institute of Dentistry and the Technical Department of the Institute of Dentistry. Special thanks to Mrs. Liisa Asikkala and Mr. Pekka Risuaho for taking care of welfare of the animals and to Mrs. Eeva-Maija Kiljander for technical assistance in laboratory work. Thanks also to my present principal Dr. Riitta Autio, D.D.S., Jämsä Municipal Health Centre, for her positive attitude towards my thesis.

I am deeply indebted to my parents for their encouragement and support during the past years full of studying and work.

This work was supported by the Finnish Dental Society Apollonia.

Jämsä June 2003 Esa Pekkala

Abbreviations

ATP-ase	adenosine triphosphatase
EDJ	enamelo-dentin junction
Ca-ATPase	calcium adenosine thiphosphatase
Calcium def.	Calcium deficient group
Cnt dams	standard-dieted dams
CNT, Cnt.	control group, Standard diet group
Cnt-Cnt pups	standard diet during lactation and experiment
Cnt-Suc pups	standard diet during lactation and sucrose diet during experiment
DIS	dietary sucrose group
IGS	intragastric sucrose group
RDA	Recommended Daily Allowance
Suc.	Sucrose diet group
Suc dams	sucrose-dieted dams
Suc-Cnt pups	sucrose diet during lactation and standard diet during experiment
Suc-Suc pups	sucrose diet during lactation and experiment

List of original publications

This thesis is based on the following original articles, which are referred to in the text by Roman numerals:

I Pekkala E, Hietala E-L & Larmas M (1998) Comparison of dentin apposition and dentinal caries progression in the mandibular and maxillary molars of the rat. Acta Odontol. Scand. 56:293-298.

II Pekkala E, Hietala E-L, Puukka M & Larmas M (2000) The effect of sucrose diet of rat dams on the dentine apposition and dental caries of their pups. Archs. Oral Biol. 45:193-200.

III Pekkala E, Hietala E-L, Puukka M & Larmas M (2000) The reducing effects of calcium deficient diet and high sucrose diet on dentin apposition of rat molars. Calcif. Tissue Int. 66:383-387.

IV Pekkala E, Hietala E-L, Puukka M & Larmas M (2000) The effects of a high sucrose diet and intragastric sucrose feeding on the dentinogenesis, dental caries and mineral excretion of the young rat. Acta Odontol. Scand. 58:155-159.

Contents

1 Introduction

Dentinogenesis serves as a good model to study the formation process of calcified tissues because, contrary to bone, resorption and remodelling normally do not occur in dentin. Consequently, the disturbance on the formation and mineralization process is more permanent in dentin than in bone. The function of dentin-forming cells, odontoblasts, is modulated by the properties of the pulp-dentin organ and, in general, they act under the influence of biological factors in the host, which includes the effect of diet (Larmas *et al.* 1992).

Dentinogenesis starts with differentiation of the odontoblasts during embryonic development and continues as primary dentinogenesis. This is an active secretory phase of odontoblasts during tooth formation. Once tooth formation is complete and the tooth is in its functional position, the secretory activity of odontoblasts decreases. Dentin formation continues slowly as does the regulation of dentinal response proceeding secondary dentinogenesis. A close secretory inter-relationship between the original odontoblasts and the pulpo-dentinal tissues is noticed at the site of injury, especially in the local environmental response of dentinogenesis, which is classified as a reactionary dentinogenesis. Greater intensity of injury can lead to cell death of the original odontoblasts. Differentiation of a new generation of odontoblasts-like cells derived from pulpal progenitor cells can lead to reparative dentinogenesis (Björndal & Mjör 2001, Mjör *et al.* 2001, Smith & Lesot 2001). Many similarities between dentin development and dentinal repair, including matrix-mediation of the cellular processes and the involvement of growth factors as signaling molecules exist. However, the appearence and regulation of molecular mediators during primary dentinogenesis are not necessarily similar during the cellular response to secondary and tertiary dentinogenesis (Smith & Lesot 2001).

Dietary sucrose affects odontoblast metabolism by reducing the rate of dentin formation during primary dentinogenesis (Larmas *et al.* 1992, Tjäderhane *et al.* 1994, Hietala & Larmas 1995). The inhitory effect is dose-dependent so that the higher the dietary sucrose concentration is, the more inhibitory is the effect on dentinogenesis (Huumonen *et al.*1997). Also, the early weaning of rat pups, and thereby the earlier exposure to sucrose, enhances the reduction of dentin formation and the caries progression in dentin (Hietala *et al.* 1997). A high sucrose diet also has a deterioratory

effect on bone metabolism (Tjäderhane *et al.* 2000). The high-sucrose diet decreases the mechanical strength of bones in rats (Tjäderhane & Larmas 1998).

The effect of a high sucrose diet on dental caries has been primarily studied from the microbial aspect. Increasing the amount of sucrose in the diet increases dental caries gradually (Huxley 1977, Hefti & Schmid 1979), which shows the importance of fermentable carbohydrates in organic acid formation by oral micro-organisms. Most studies on the etiology of caries have concentrated on its initiation on the enamel surface of the teeth and on its microbiological backround. An expanded approach to the background of the carious process introduces the role of the host on the dentinal response to caries. A high level of dietary sucrose down-regulates dentinal fluid flow in rats (Leonora *et al.* 1992), which appears to be mediated, at least in part, via the hypothalamic-parotid gland endocrine axis (Leonora *et al.* 1992, 1993, 2002a). Dietary carbamyl phosphate has been reported to reduce the dietary sucrose effect on dentinogenesis (Leonora *et al.* 2002b). Huumonen *et al.* (2001) found that high dietary sucrose impairs the defensive reactions of the pulp-dentin organ (pulpo-dentinal complex) against dentinal caries, which further shows the importance of the local endogenous factors affecting caries progression (Huumonen 1999).

In this study, I have studied the systemic effects of sucrose exposure (dietary and intragastric), and calcium-deficiency on dentin formation, predentin mineralization and mineral homeostasis in young growing rats. Dentin formation and predentin mineralization was considered to reflect odontoblast function during active dentinogenesis under dietary sucrose exposure or calcium-deficiency. To clarify the systemic effect of sucrose in dentition, the occurence of dental caries was estimated and the number of carious vs. intact teeth counted. Also the rate of dentin formation was measured in both mandibular and maxillary molars. Since a shortage of necessary mineral elements in the pulpal environment might explain the reduced dentinogenesis and possibly illuminate the mechanism for reduced dentinogenesis caused by sucrose diet, the importance of mineral homeostasis during sucrose feeding was estimated by measuring serum and urinary levels of calcium, phosphorus, sodium and potassium, and urinary mineral excretions in both rat pups and their dams during lactation. To exclude the direct oral effect of sucrose on dentinogenesis and caries initiation via the immature enamel of erupting molars, intragastric sucrose feeding of the pups was performed.

2 Review of the literature

2.1 Dentin and bone as calcifying tissues

2.1.1 Tooth

The tooth is a hard tissue consisting of mineralized and unmineralized components. Mineralized components are several type of calcified tissues which are synthesized by separate lines of cells. Enamel is synthesized by ameloblasts and it forms the outer surface of the tooth crown in the oral cavity. Cementum is synthesized by cementoblasts covering the anatomic root of the tooth. Dentin is synthesized by odontoblasts and it forms the main part of tooth under the enamel of the crown and the cementum of the root. Soft tissues, the unmineralized part of the tooth consists of periodontal tissue (cementoblasts, fibroblasts, blood vessels, lymphatics, sensory nerve fibers) (Carranza 1990) and dental pulp tissue (odontoblasts, blood vessels, undifferentiated mesenchymal cells, fibroblasts, blood and lymph vessels, nerve fibers) (Mjör *et. al*. 2001).

2.1.2 Pulp-dentin organ

The dental pulp is integrally connected to dentin in the sense that physiologic and pathologic reactions in one of the tissues will also affect the other. Anything that affects dentin will affect the pulp and vice versa (Mjör *et al.* 2001). The components of the dental pulp, the cells, blood and lymph vessels, nerves, and the interstitial fluid, have importance in the response to carious or restorative procedures. In newly erupted teeth, the predominant cells in pulp tissue are undifferianted mesenchymal cells and fibroblasts. The fiber–poor and cell-rich state is characteristic of young pulp. Most cells are considered to to be undifferentiated or immature but they have the potential to develop into specialized cells, eg. odontoblast-like cells. Age-related changes are important

because progenitor cells can deferentiate into the other cell types or take part in reparative processes of the pulp-dentin complex in young persons. In older individuals, a large fibrous component is present and the number of cells is low. Also age-related changes in pulp nerves and blood vessels accompany the cellular changes (Fried 1992, Mjör *et al.* 2001).

It is difficult to base knowledge of pulpal reactions to dental caries on the histopathology of stained pulp tissue, because no exact clinical criteria have been established for evaluation. The onset of pulpal reactions to caries is difficult to examine because the enamel is lost when the histopatological sections of the tooth are prepared. However, some conclusions can be drawn: caries lesions may cause an inflammatory reaction in pulp tissue. The presence of chronic inflammatory exudate, including lymphocytes, macrophages and plasma cells has been found. An increased number of macrophages is associated with pulpal injury and polymorphonuclear leukocytes may also be found in normal pulp (Björndal & Mjör 2001).

Dentin is a composition of inorganic minerals (75%), organic material (20%) and water (Linde & Goldberg 1993). Dentin is a connective dental tissue that includes dentin-forming cells (original odontoblasts or odontoblast-like cells), predentin (collagenous matrix), mineralized solid dentin and dentinal fluid inside dentinal tubules. Dentin and predentin are traversed by tubules 1 to 2 µm in diameter. The dentinal tubules extend from the enamel to the pulp in the coronal part of the tooth (Mjör *et al.* 2001). Due to permeability, dentin may act as a port of entry to the pulp for bacteria, bacterial antigens, toxic, and allergenic components (Björndal & Mjör 2001). There are also observations in the literature that, compared to a physiologically dynamic tooth, the inward permeability of dentin in the compromized tooth increases significantly (Steinman *et al.* 1980). Also growth factors present in dentin, which are released during the carious process, may be important in initiating defensive mechanisms in the pulp-dentin organ (Magloire *et al.* 1992, Cassidy *et al.* 1997, Björndal & Mjör 2001).

Odontoblasts also have an intimate relationship with pulpal nerves, and blood and lymphatic vessels. The structure of pulpal blood vessels is basically similar to that in other organs, but it is recognizable that many of the capillarities are nonfunctional in normal pulp. If necessary, blood flow to specific area can be increased quickly without the ingrowth of new capillaries (Mjör *et al.* 2001). During the life cycle of teeth, the pulp-dentin organ is capable of responding to various intrinsic and extrinsic factors that are related to teeth development and function.

2.1.3 Bone

Bone consists of bone marrow (resembling pulpal tissue in the tooth) and mineralized or mineralizing hard tissue (resembling dentin in the tooth). Bone is modelled by osteoblasts and removed, and hence remodelled, by osteoclasts. In bone there is an unmineralized layer of organic matrix called osteoid (corresponding to predentin in dentin), which is formed and organized prior to mineralization. Similarly as in dentin, the organic matrix of the bone is mainly constituted of type I collagen. Osteoblasts are also responsible for the

mineralization of bone organic matrix. Bones of mammals are formed either by direct intramembranous or indirect endochondral bone formation. The bones of the appendicular skeleton, vertebral column and the base of the skull are classified as endochondral and the bones of the face and clavicle are typical membranous bones. Macroscopically two types of bone can be distinguished: cancellous (or spongy) and cortical (or compact). No sharp boundary can be drawn between the two types of osseous tissues, cancellous turns into compact bone gradually. Both bone types contain the same histologic elements and the most characteristic feature is lamellar structure, bone matrix being organized into layers or a lamellae arranged in various ways. Between birth and maturity, the skeleton increases in size and strength and adapts its parts to mechanical and other demands.

The osteoblast function during cancellous bone modelling has been considered to resemble the function of original odontoblasts during primary dentinogenesis during tooth development. The physiological osteoclast function during bone resorption appears to parallel proton production by cariogenic bacteria during the pathological demineralization of dentin. Thereby, the remodelling of cortical bone and secondary dentinogenesis appear to functionally resemble each other, as a biological phenomenon; particularly as it related to reparative dentinogenesis formed by odontoblast-like cells derived from pulpal cells (Larmas 2001).

2.2 Dentinogenesis

2.2.1 Odontoblasts

Dentin-forming cells, odontoblasts, which originate from the ectomesenchyme, form a single layer of cells between the dentin and pulp. The cell body is located on the pulpal wall of dentin and the cellular process extends into the dentinal tubule within the mineralized dentin (Torneck 1994). The cell bodies are from 3 to 5 μm wide and 20 to 40 μm long depending on the age of the tooth. The odontoblastic process fills the lumen of the dentin tubule and it is composed of a main trunk, with a diameter of 0.5 to 1 μm, and lateral branches. Contrary to the cell body, cell organelles (Golgi apparatus, rough endoblastic reticulum or mitochondria) usually do not appear in the odontoblastic process (Ten Cate 1994); however, microtubules, filaments and coated vesicles are present (Linde & Goldberg 1993). Odontoblasts are connected to each other with interodontoblastic collagen, the so-called von Korff fibers. Frequent bundles of collagen fibrils enter the odontoblast layer from predentin and are present between odontoblast cell bodies. Ultimately they pass through the odontoblast layer into pulp (Bishop *et al.* 1991). Histologically, secretory odontoblasts are columnar in shape. A large number of cytoplasmic organelles are identifiable in young odontoblasts, whereas, aged odontoblasts lose their columnar shape and contain a small number of Golgi apparatus and a small-sized rough endoblasmic reticulum (Couve 1986).

2.2.2 Formation and mineralization of dentin extracellular matrix

Odontoblasts synthesize dentin matrix during their entire lifetime. The unmineralized zone between the odontoblasts and mineralized dentin is called predentin Young odontoblasts are secretorily active and produce predentin at a faster rate than the older cells. During the phase of collagen aggregation, the odontoblasts regulate the order of collagen fibers and modify the composition of the extracellular matrix. Dentin is formed by two simultaneous processes, the formation of collagenous matrix (predentin) and the formation of mineral crystals on this matrix (Linde 1995). Dentin formation starts with the synthesis of the extracellular matrix which is mainly formed by the fibrous web of type I collagen. In addition, type V collagen, proteoglycans and other non-collagenous proteins (serum proteins, phosphoproteins and Gla-proteins) are also secreted (Butler & Richie 1995, Linde & Goldberg 1993). The non-collagenous matrix proteins may act as mediators of cell-matrix interactions, matrix maturation and mineralization (Boskey 1991). As a result of odontoblastic activity, some non-collagenous proteins are secreted and the density of the collagenous network increases before the dentin mineralization front (Linde 1989, Linde & Goldberg 1993).

The mineralization process of the extracellular matrix is controlled by the function of various inductive and reductive non-collagenous proteins, which initiate and control the formation and the growth of hydroxyapatite crystals (Boskey *et al.* 1989, Linde & Goldberg 1993). The initiation of mineralization is believed to be constituted by an ion nucleation phenomenon mediated by odontoblasts. Calcium is essentially the only ion for which data are available. A major portion of the Ca^{2+} ions are transported through a cellular route under the control of odontoblasts. Odontoblasts maintain the Ca^{2+} ion balance by transmembranic transport mechanisms, including Ca-ATPase, Na^{+} exchangers and calcium channels (Linde 1995). The odontoblastic plasma membrane Ca^{2+} pumps, present in mineralizing dentin, may play a role in calcium transport and mineralization (Borke *et al.* 1993).

Non-collagenous proteins could be involved in the nucleation of calcium- and phosphate crystals (hydroxyapatite) and may also control the initial growth and orientation of the ionic nucleus (Houllé *et al.* 1997). Phosphophorin, at a low concentration, acts as a nucleator, but at higher concentration acts as an inhibitor of ionic nucleation (Boskey *et al.* 1990). The low affinity of dentin sialoprotein (DSP) seed crystals and its limited effect on hydroxyapatite formation and growth suggests that DSP is not a primary regulator in dentin mineralization (Boskey *et al.* 2000). Collagen is thought to control mineral orientation and organization (Linde & Goldberg 1993). The hydroxyapatite crystals formed are oriented with the long axes, parallel to the long axes of the collagen fibrils.

2.2.3 Dentin types

The primary dentin is formed rapidly during tooth formation. It outlines the pulp chamber and constitutes the main part of the dentin mass. The outer layer of primary dentin, which

is synthesised at the onset of dentinogenesis, is called mantle dentin. Mantle dentin is slightly less mineralized than other layers of the primary dentin *i.e.* circumpulpal dentin. The formation of primary dentin continues until the tooth becomes functional (Linde & Goldberg 1993) or until the root apex is closed (Torneck 1994). Thereafter dentin formation proceeds as secondary dentinogenesis, which continues at a slower rate than the primary dentinogenesis during the life-time of the individual.

The secondary dentin is considered to be more irregular in structure (Torneck 1994) and sometimes less mineralized than the primary dentin (Ten Cate 1994). In rat molars, dentin formation slows down without apparent transition from primary to secondary dentinogenesis (Johannessen 1961, Hietala & Larmas 1992, Kortelainen & Larmas 1994). Romagnoli *et al.* (1990) have shown some evidence that the odontoblasts of rat molars may atrophy after the formation of primary dentin.

Tertiary dentin (reactionary or reparative or irregular secondary dentin) is the outcome of odontoblastic response to irritation occuring mainly during secondary dentinogenesis and is caused by dental abrasion, attrition, cavity preparation, erosion or dental caries (Torneck 1994). Lesot *et al.* (1993) defines reactionary dentin to be the result of irritation of post-mitotic odontoblasts, whereas reparative dentin is formed by odontoblasts or odontoblast-like cells which differentiate from pulp cells after the cell death of primary odontoblasts (Magloire *et al.* 1992, Magloire *et al.* 1996). Reactionary dentinogenesis during dental caries may result from the solubilization of growth factors, transforming growth factor-beta (TGF-beta), from the dentin matrix which initiate the stimulation of odontoblasts (Smith *et al.* 1995, Sloan *et al.* 2000a). It has been demonstrated that TGF-beta 1 and beta 3 can stimulate secretion of extracellular matrix by odontoblasts, are mitogenic to pulp cells, and that TGF-beta 3 may have inductive effects on pulpal cells (Sloan & Smith 1999). Recent studies show that dentin and bone matrix contain various angiogenic growth factors (Roberts-Clark & Smith 2000), bone morphogenic proteins (Sloan *et al.* 2000b), bone sialoproteins and osteopontin (Qin *et al.* 2001), which may be beneficial to the reparative response of the dentin-pulp complex. The form and the regularity of reparative dentin appears to be dependent on the intensity of the external stimulus (Linde & Goldberg 1993, Torneck 1994).

Mineralized dentin can also be divided into intertubular and peritubular (intratubular) dentin. The intertubular dentin is formed by odontoblasts through predentin mineralization, whereas peritubular dentin is formed in peripheral parts of the mineralized dentin inside the walls of dentin tubules. The formation of peritubular dentin can be accelerated by environmental stimulus or irritation (Linde & Goldberg 1993, Torneck 1994). The peritubular dentin is highly mineralized and it also contains little collagen, while the intertubular matrix has a dense collagen matrix (Mjör *et al.* 2001).

2.3 Extrinsic modulation of calcifying tissues

2.3.1 Carbohydrate load

A carbohydrate-rich diet has been reported to decrease trabecular bone volume and trabecular thickness in rodents after ingesting the diet for six weeks (Saffar *et al.* 1981). After exposure to the diet for two months a reduction in bone volume was found, but the decrease in trabecular thickness was absent (Saffar & Makris 1982). Dietary high fat-sucrose exposure reduced the biomechanical strength of tibial and femoral bones in rats (Li *et al.*1990, Zernicke *et al.* 1995). A sucrose-rich diet, alone, decreased the mechanical strength of bones (Tamura *et al.* 1983, Tjäderhane & Larmas 1998, Smith *et al.* 2000) and reduced the rate of primary dentin formation in post-weaning rats (Kortelainen & Larmas 1990, Tjäderhane *et al.* 1994, Huumonen *et al.* 2001). The reductive effect of sucrose on dentinogenesis is concentration-dependent. The minimum sucrose concentration is between 30 to 40% of the diet (Huumonen *et al.* 1997). Early weaning of rat pups accelerates the reduction rate of dentin formation in sucrose fed animals (Hietala *et al.* 1997).

Dietary sucrose in rats induces insulinemia (Hallfrisch *et al.* 1979,Vallerand *et al.* 1986, Kergoat *et al.* 1987) and glucose intolerance (Hallfrisch et al. 1979, Wright et al. 1983, Gutman et al. 1987, Grimditch et al. 1988). A glucose-rich diet fed to lactating rats increases the amounts of glucose and lipid in milk (Matsuno *et al.* 1999). A moderate amount of glucose in the maternal diet, along with a higher fat intake, promotes greater pup weights at weaning, suggesting a role of dietary macronutrients in optimizing lactational performance (Matsuno *et al.* 1999). A severe glucose-restricted diet reduces the concentration of milk carbohydrate and fat, which reduces growth and increases the mortality of rat pups (Koski *et al.* 1990, Lanoue & Koski 1994). Similarly, a dietary protein restriction of rat dams decreases their milk production, without affecting the concentration of milk lipids, protein and lactose and the result is a decrease in the body weight of the litter (Grigor *et al.* 1987). Fructose exposure during gestation can cause hyperglycemia, hyperinsulinemia and greater weaning weight gain in rat dams and hyperinsulinemia in their offsprings (Rawana *et al.* 1993). Sucrose feeding during late gestation decreases the response of fat cells to insulin and causes hypertriglyceridaemia in rat dams (Jen *et al.* 1991). A dietary sucrose load fed to rat dams during the suckling period increases their plasma insulin concentration and causes hypertrophy of pancreatic β-cells (Vadlamudi *et al.* 1993). Some evidence suggests that high carbohydrate-induced hyperinsulinemia may also pass to the next generation (Laychock *et al.* 1995, Vadlamudi *et al.* 1995).

It has been shown that insulin may be a mediator of glucose-induced hypercalciuria (Wood & Allen 1983) by reducing renal tubular reabsorption of calcium (Roy & Seely 1981). Insulin administration and thyroparathyroidectomy promote antiphosphaturia (Webster & Haramati 1985). Part of the antiphosphaturic effect of insulin may be an outcome from the antagonism of parathyroid hormone at the cellular level of the renal cortex (Northrup *et al.* 1979). Despite occuring calciuria, a high protein diet does not

significantly affect calcium balance, change in bone composition or promote bone loss in adult rats (Whiting & Draper 1981).

Similarly in humans, as in rats, a high amount of carbohydrate intake elevates serum insulin levels (Holl & Allen 1987, 1988), may inhibit tubular reabsorption of calcium (De Frozo *et al.* 1975, Lemann *et al.* 1970) and induces urinary calcium (Ericsson *et al.* 1990, Nguyen *et al.* 1993). A high protein diet does not affect serum parathyroid hormone concentration in humans (Kim & Linkswiler 1979).

2.3.2 Calcium deficiency

A diet containing 0.5% calcium has found to be optimal for bone development (Persson *et al.* 1993). In rats, a low calcium diet (0.03%) raises the level of blood $1,25(OH)_2D_3$ and parathyroid hormone. It also lowers the blood level of $25(OH)D_3$ and the ionic calcium levels. The end result is reduced bone density. Low calcium intake does not impair the maturation or synthesis of bone collagen matrix (Shoshan & Pisanti 1971). Dietary calcium deficiency, without vitamin D deficiency, causes experimental osteopenia, a condition more closely related to osteoporosis than to osteomalacia. Calcium and vitamin D deficiencies together induce bone changes which are closer to osteomalacia than osteoporosis (Ferguson & Hartles 1963). In dentition, calcium deficiency alone delays mineralization of dentin in the constantly growing incisors of rats (Ferguson & Hartles 1964, Lozupone & Favia 1989, Rasmussen 1972) leaving a broad layer of unmineralized predentin (Rasmussen 1977). Defective mineralization, associated with lowered serum calcium values and increased predentin width, only partially explains the reduction in dentinogenesis (Engström *et al.* 1977).

Enhanced odontoblast activity, following low calcium diet, can restore the rate of dentin formation to normal. Odontoblastic activity returns to normal by using calcium supplementation during the dietary recovery period (Lozupone & Favia 1994, Tjäderhane *et al.* 1995a). In calcium deficient animals, dietary xylitol has been reported to improve the bioavalability of calcium both in bone (Hämäläinen 1994, Svanberg *et al.* 1993) and dentin (Tjäderhane *et al.* 1995a).

Calcium deficiency also affects enamel development. Diet-induced chronic hypocalcemia interferes with both cellular and extracellular events during enamel development. Morphological analysis shows an increased number of ameloblasts and cyst-like structures. Also the basal lamina, that normally separate ameloblasts from enamel during the maturation, can be missing in some areas of maturing enamel (Nanci *et al.* 2000).

2.3.3 Other extrinsic factors

Ovariectomy of female rats impairs their calcium absorption (Kalu & Orhii 1999) and decreases cancellous bone mineral content and density (Kalu & Orhii 1999, Hietala 1993,

Hietala & Larmas 1997). Dietary xylitol has a protective effect against ovariectomy-induced bone mineral loss (Svanberg & Knuuttila 1994), bone resorption (Mattila *et al.* 1996), weakening of bone biomechanical properties (Mattila *et al.* 1998), and age-related osteoporotic changes (Mattila *et al.* 2001). By itself, xylitol reduces dentin formation (Tjäderhane *et al.* 1996), attenuates enhanced dentin formation following ovariectomy (Svanberg *et al.* 1994) and further induces the reduction of dentin formation caused by a high sucrose diet (Tjäderhane *et al.* 1995b). Enhanced dentin formation in ovariectomized rats was suggested to be mediated via estrogen receptors found in odontoblasts (Hietala *et al.* 1998).

Fluoridated drinking water (1 or 19 parts/10^6) may further enhance the reduced dentin formation caused by high sucrose feeding in rats (Kortelainen & Larmas 1990). Metabolic acidosis, caused by drinking acidified water, reduced dentin formation and a high-sucrose diet enhanced the reducing effect of acidic drinking water on dentin growth (Bäckman *et al.* 1996), whereas the reductive effect of chronic metabolic alkalosis on the dentinogenesis of the rat was slight (Bäckman & Larmas 1997). Glucocorticoid supplementation reduces dentin formation, but does not suppress dentinogenesis as much as the high sucrose diet. Reduction in dentin mineralization, during the experiment, was found to be greater in sucrose-fed animals, when compared to the effect obtained with administrative glucocorticoid (Huumonen *et al.*1996).

2.4 Dental caries

2.4.1 Enamel caries

Acids formed by bacterial fermentation from dietary sugars leads to a pH fall in the plaque which dissolve tooth enamel, initiating the development of carious lesions. The progression of demineralization in enamel continues to the point where dissolution of hydroxyapatite exeeds remineralization, i.e. regrowth of hydroxyapatite crystals. In enamel, the progression of caries lesion is not only dependent on the remineralization potential of saliva (phosphate and calcium ions derived from plaque), but also dietary factors, such as xylitol (Arends *et al.* 1984, Havenaar *et al.* 1984, Tanzer 1995) and fluoride (Weaver 1950, Shellis & Duckworth 1994, Seppä *et al.* 1996). The microbiological evironment in the oral cavity (De Soet *et al.* 1991, Tappuni & Challacombe 1993) and even the eruption stage of the tooth (Carvalho *et al.* 1989, Ekstrand & Björndal 1997) may also affect the rate of dental caries.

Different types of caries lesions have been described: primary, secondary and remaining lesions. A progressing lesion is referred to as an active lesion and when the progression is permanently stalled, under favorable conditions, as an arrested lesion. The primary lesion starts with so called white spot lesion. As the demineralization deepens, the greatest degree of tissue porosity follows the direction of enamel rods. The central part of enamel lesion is typically thicker than the peripheral part of the same lesion, and thereby the oldest part of lesion is central and the youngest part is peripheral. In this way,

the conical shape of enamel lesion is formed. If enamel lesion is allowed to progress, the lesion will reach the enamelo-dentin junction and penetrate into the dentin (Björndal & Mjör 2001).

2.4.2 Pulpo-dental response to carious process

When caries in the enamel advances and finally reaches the enamelo-dentin junction (EDJ), the response of the odontoblasts and pulp tissue to caries takes place (Björndal & Mjör 2001). In enamel lesions, the cytoplasm/nucleus ratio of odontoblasts may decrease, as well as the width of the predentin zone at the lesion site (Björndal & Darvann 1999). Under initial active lesions, the bacteria have not invaded the dentinal tissue, a reduction in the number and size of intracellular organelles, as well as the enlargement of the intercellular spaces between odontoblasts, is noticed (Magloire *et al.* 1992). In lesions with EDJ contact, enhanced peritubular dentin mineralization, seen as increased radiodensity, and extradentinal or tetriary dentin formation, is visible in the central part of the lesion area (Björndal *et al.* 1997). The conventional concept is that the carious lesions spread laterally at the EDJ. However, some evidence has shown that the demineralization front does not necessarily spread in a lateral direction in non-cavitated occlusal lesions (Björndal *et al.* 1999). Ekstrand *et al.* (1998) suggested that lateral spread of caries at the EDJ is related to advanced lesions with cavity formation. The response of dentin to the carious process is also closely related to the progression speed of the lesion. Beneath active lesions, the structure of tertiary dentin is more atubular and irregular than under the slow-progressing lesions (Björndal & Darvann 1999).

Pulpal tissue subjacent to deep caries lesions often shows the presence of chronic inflammation, including lymphocytes, macrophages and plasma cells. Formation of tertiary dentin is usually visible on the pulpal aspect and the increase in dentin thickness, accompanied by a reduced odontoblastic layer in the affected area, can also be seen (Björndal & Mjör 2001). In the case of advanced caries development in dentin and the destruction of original odontoblasts, pulpal cells are capable of differentiating into dentin-forming cells (Lesot *et al.* 1993). D'Souza *et al.* (1995) found that odontoblast-like cells, differentiated after the cell death of original odontoblasts, synthesize type I but not type III collagen and form reparative dentin. Similarly, damaged pulp beneath a drilled cavity shows evidence of cell proliferation, neovascularization and the presence of functional cuboidal cells close to the injured area (Magloire *et al.* 1996). The presence of odontoblast-like cell isolates on the pulpal cell population has been reported. These postnatal human dental pulp stem cells (DPSCs) have the ability to proliferate and the potential to generate a dentin/pulp-like complex (Gronthos *et al.* 2000). The above phenomenon, appears to be clearly different from the formation of reactionary dentin resulting from the original odontoblasts (Lesot *et al.* 1993) as a response to operative procedures (Smith *et al.* 1994, Mjör *et al.* 2001).

2.5 The rat as an experimental animal

After birth, the average length of the lactation period and, thereby, also the weaning time of the pups is considered to be 21 days when the difference in body weight between male and female rats is marginal. Rats are considered to be in a continual state of growth during their lifetime, but the growth rate of young rats is higher in males than in females. The difference in body weights becomes noticeable postweaning. The female rat becomes reproductively functional between 40 and 60 days of age, but the most successful reproductive period is between 100 and 300 days. The average lifetime for male rats is about 700-800 days. Females live about 10 percent longer (National Researchs Council 1972). Rats provide a fairly good experimental model for research on calcified tissues because similar physiological mechanisms control bone and dentin metabolism both in rats and humans (Frost & Jee 1992).

2.5.1 Rat dentition

Rats are widely used as experimental animals in dental research. The growth of incisors is continuous and rat dentition is monophyodontic. In contrast, humans have both deciduous and permanent dentition.

Because of the fast, continuous growth of incisors during the entire life-time of the rat, clinical dental caries does not occurs in incisors. Rat molars, however, resemble human molars in many ways, although the tips of rat molars lack enamel. The crown of the rat molar is multi-cuspal and multi-fissural, and the morphological development stages are very similar to humans (Lange & Hammarström 1984), although the development time-table is characteristic for the rat. Dentinogenesis in the first molars begins around the 22nd day post-fertilization, at the time of birth (Paynter & Hunt 1964). In the Sprague-Dawley strain, the masticatory function develops simultaneously with the eruption of the first mandibular and maxillary molars into the oral cavity around the age of 16 days (Navia & Narkates 1980). The second molars erupt into the mouth by the age of 20 days (Hunt *et al.* 1970a).

There are morphological differences between the mandibular and maxillary molars of the rat (König *et al.* 1958, Hunt *et al.* 1970a), which may lead to a different cariogenic challenge. In the mandibular first molar, there are two transverse fissures dividing the teeth into three lobes and these lobes are bisected by a one deep longitudinal sulcus. In the mandibular second molar, there is only one transversal fissure, which is bisected by a longitudinal sulcus similarly to the first molar. The maxillary molars lack longitudinal sulci and transverse fissures (Hunt *et al.* 1970a), thereby the fissural system is shorter and less deep in the maxillary than in mandibular molars.

2.5.2 The rat as a caries model

The rat has been commonly used to study the cariogenity of various nutritional components and diets to ascertain the microbiological background of caries and the effectiveness of anticarious agents.

In rat molars, caries lesions on the buccal and lingual surfaces are more extensive and progress more rapidly when sucrose is present in the diet. Inoculation of *Streptococcus mutans* into the oral cavity usually increases the lesions on the smooth surfaces and in the occlusal sulci (Shaw 1981). A high sucrose diet changes the indigenous oral bacterial population, enhancing particularly *Actinomyces naeslundii* and *Streptococcus rattus* bacteria, which is reflected in the increased level of dental caries (Zhu *et al.* 1997). Hefti & Schmid (1979) found that increasing the dietary sucrose concentration also increases the incidence of smooth surface and fissure caries, so that the higher the sucrose concetration is, the more caries occurs. In some cases an almost linear response between the dietary sucrose amount and caries has been found (Huxley 1977a). Tjäderhane *et al.* (1995b) reported a negative correlation between the dentin formation rate under the caries lesions and dentinal caries progression in sucrose-fed rats.

Streptococcus mutans is a very important bacteria in the etiology of dental caries, but other streptococci species have also been found to be cariogenic. *Streptococcus gordonii* colonizes the oral cavity and has cariological significance in the presence of sucrose (Tanzer *et al.* 2001). *Streptococcus sobrinus* has the capability to adhere to enamel and it also produces acid in large amounts and more rapidly than *Streptococcus mutans*, which may cause a larger number of advanced dentinal lesions in the fissures of the rat molars (De Soet *et al.* 1991). Guggenheim *et al.* (1999) observed that micellar casein may prevent oral colonisation of *Streptococcus sobrinus* and thereby inhibit the formation of advanced dentinal fissure and smooth surface caries.

A positive correlation between increased feeding frequency and increased caries incidence has been reported (König *et al.* 1968, Huxley 1977b). More advanced fissure lesions occur in the maximum feeding frequency (34 times daily) group than in lower feeding frequency groups (17 or 26 times daily) (Huxley 1977b) probably because increasing frequency also increases the total time for acid formation of cariogenic bacteria (König *et al.* 1968). Eating frequency of 24 or 30 times daily results in the same amount of fissural lesions as feeding *ad libitum* (König *et al.* 1968). Accumulation of food debris has been suspected to be a critical factor in the initiation and progress of dentinal caries. Stomach-tube feeding excludes food accumulation and reduces the numbers of carious lesions and carious teeth, whereas desalivation (removal of the principal salivary glands) increases caries incidence in molars of normally fed rats (Kite *et al.* 1950). Similarly, parotidectomy reduces dentinogenesis in young rats (Leonora *et al.* 2002ab), presumably via the hypothalamus-parotid gland endocrinal axis (Leonora *et al.* 1993).

Some minerals and trace elements have a profound effect on rat caries. Sodium, calcium and magnesium trimetaphosphates are equally capable of inhibiting carious lesions in the occlusal sulci (Shaw 1980). Calcium addition given alone does not significantly affect occlusal caries, whereas it decreases smooth surface caries (Reussner *et al.* 1977). Calcium given together with lactose by tube feeding resulted in a decrease in

caries development, which was considered to be an outcome from the modification of mineral metabolism in bone and teeth because tube feeding excluded a direct oral effect from the minerals tested (Toyry and Mechin 1976). Watson *et al.* (1997) reported that pre- and perinatal exposure to lead results in almost a 40 % increase in the prevalence of caries in rats. They suggested that maternal lead ,stored in bones, was mobilized during lactation and transported to suckling rat pups via the maternal milk.

The incidence of dental caries varies among different rat strains. Larson *et al.* (1968) compared Hunt-Hoppert caries-susceptible and caries-resistant strains and showed a clear difference in caries activity among these two specific rat lines.

3 Purpose of the present studies

Calcium balance has a central role in the mineralization process in bone and dentin. Calcium deficiency may act as a supressing factor on dentinogenesis similar to that manifested in bone. A sucrose diet reduces dentinogenesis and induces urinary calcium excretion, which appears to be mediated via an insulin effect on the renal handling of calcium. Due to of the sucrose effect on calcium balance, the reductive effect of sucrose on dentinogenesis was presumed to be the consequence of the reduced mineralization of predentin caused by the disturbed calcium homeostasis.

The purpose of maternal sucrose feeding was to test the predisposing effects of sucrose on dentinogenesis and dental caries in the second generation. The maternal sucrose diet was presumed to induce the reduction of dentinogenesis and to enhance dental caries progression in their offspring during the postweaning period.

The purpose of the intragastic sucrose feeding procedure was to assure that the reduction in dentinogenesis was the outcome of the sucrose effect on the host. Intragastric feeding was presumed to reduce both primary dentinogenesis and intraoral caries initiation.

The following questions were selected as the aims of the present study. (the number in parenthesis refers to the original papers):

1. Is the rate of primary dentinogenesis similar in maxillary and mandibular molars, and does the reduced dentinogenesis, caused by dietary sucrose, lead to the acceleration of dentinal caries progression in both jaws (I)?
2. Does the maternal sucrose diet predispose the reduction of dentinogenesis of the offspring as much as their direct experimental exposure to sucrose, and does reduced dentinogenesis enhance the progression of dental caries ? Do the maternal and experimental sucrose exposures reduce the rate dentinogenesis and induce caries progression cumulatively during the postweaning time ? Does dietary sucrose affect mineral homeostasis in the dams and their pups (II)?
3. Does the high sucrose diet reduce the rate of primary dentinogenesis as much as dietary calcium-deficiency by decreasing the mineralization of predentin ? Could the sucrose effect on dentinogenesis be mediated via calcium homeostasis (III)?
4. Does intragastric and dietary sucrose feeding reduce the rate of primary dentinogenesis, and does intragastric sucrose feeding exclude the local cariogenic challenge in the oral cavity (IV)?

4 Materials and methods

4.1 Animals

The experiments were performed using Sprague-Dawley rats (Møllegård Ltd, Ejby, Denmark). The experimental procedures on the pups were started after birth (II) or after weaning, at the ages of 19 (IV) or 21 days (I, III). The experimental procedures on the rat dams were performed during their lactation period (II). Altogether six rat dams (II) and 99 pups (51 males and 48 females) (I-IV) were used in the experiments. The rats were born and raised in the Laboratory Animal Centre, University of Oulu.

The animals were subjected to normal atmospheric conditions at 21°C, the same regimen of lightning (12 h of light and 12 h of dark) and the same feeding times, handling and noise level. Food and drinking water were freely available.

4.2 Ethical approbation

All the experiments were performed by a person licenced to perform animal experiments and the protocols were approved by the Experimental Animal Committee of the Medical Faculty, University of Oulu, Oulu, Finland.

4.3 Experimental procedures

On the day of birth, the litters were grouped to be as similar as possible in relation to sex, delivering dam, and the number of pups. At weaning the animals were randomly divided into the experimental groups and given an intraperitoneal injection of oxytetracycline hydrochloride (30 mg/kg, Terramycin®, Pfizer, Brussels, Belgium). To mark the areas of dentin apposition during the test period, a second injection was given one (IV) or two

days (I-III) before the termination of the experiment. In experiment I all animals were inoculated with a fresh suspension of *Streptococcus sobrinus* (ATCC 27531 K 1 Fitzgerald) to ensure a cariogenic challenge. Since the main interest of the other experiments (II-IV) was the systemic effect of sucrose or a low calcium diet on dentinogenesis, instead of caries, no contamination with specific cariogenic bacteria was performed.

All dams in experiment II were individually housed in metabolic cages for four six hour periods during lactation and the urine excreted during that time was collected in Erlenmeyer bottles to minimize evaporation. The urine samples of the pups (II-IV) were collected using a similar procedure to the dams except that the nocturnal collection time was 12 hours. Feces were not collected because of the coprophagy among rats (Barnes *et al.* 1957) and preventing coprophagy decreases the growth rate of the animals (Geyer *et al.* 1947) Increasing sucrose load (0.2 - 12 ml, 0.65 g/ml) in experiment IV (one group) was performed using intragastric sucrose feeding, which was begun one day after weaning and ended on the day of termination.

The duration of the test period in dams was three weeks (II) and in pups from three (II, III) to four (IV) and five weeks (I). At the end of the test periods, the animals were anaesthetized with a combination of midazolam (Dormicum®, Rache, Basel, Switzerland), fentanyl-fluanisone (Hypnorm®, Jansen Pharmaceutica, Brussels, Belgium) and aqua (1:1:2, at 0.3 ml/ 100g, intraperitoneally). The blood samples were collected under anaesthesia by cardiac puncture (II-IV) before the animals were decapitated. After a 10 minute incubation, the blood was centrifuged and serum was stored by freezing.

4.4 Diets

The control (standard) diet was specially manufactured for growing rats and mice (Lactamin R 36, Ewos AB, Södertälje, Sweden) (I-IV). The sucrose diet (I-IV) contained 41 % sucrose , 10.1 % barley flour, 10.3 % wheat flour and 5.8 % casein, which was added to compensate for the loss of protein when the wheat and barley flour content was partly replaced with sucrose (R 642, Ewos AB, Södertälje, Sweden). The other ingredients of the sucrose diet were the same as in the standard diet. The low calcium diet (III) contained 0.5 % calcium (R 414, Ewos AB, Södertälje, Sweden). All diets were commercially available and in a form of fine powder because small food particles are pressed into the sulci of molar teeth during mastication, and are available there for cariogenic bacteria (Shaw 1981). A detailed compositions and nutrient requirements of the diets are presented in the Tables 1 and 2.

Table 1. The compositions of the experimental diets. Roman numerals refer to the experiment in which the diet was utilized.

Diet	Ingredient	Amount %
Control/Standard diet(I-IV)	Barley flour	34.0
	Wheat flour	43.0
	Wheat grains	5.0
	Soya	5.0
	Fish powder	4.0
	Vitamins and trace elements	4.5
	Other ingredients	4.5
Sucrose diet(I-IV)	Sucrose	41.0
	Barley flour	10.1
	Wheat flour	10.3
	Casein	5.8
	Otherwise as Control/Standard diet	
Calcium-deficient diet (III)	Casein	20.0
	Corn starch	62.6
	Cellulose meal	5.0
	Mineral mixture	4.0
	Soybean oil	2.0
	Fat	5.0
	Vitamin-mineral mixture	

Table 2. Micro and macro components of the diets. (The variations between dietary components and between feed quantities is presented in parenthesis as the number of the experiment in which it has been used)

	Control (III/I,II,IV)	Sucrose (III/I,II,IV)	Calcium-deficient (III)	RDA
Energy, kJ/g	12.60	13.53	13.40	18.42
Protein, mg/g	185.0	158.0	176.0	133.0
Fat, mg/g	40.0	31.0	70.0	55.0
Linoleic acid, mg/g	10.0	10.0	15.0	2.4
Calcium, mg/g	9.8	9.8	0.5	5.6
Phosphorus, mg/g	7.5	7.5/7.2	5.8	4.4
Potassium, mg/g	6.0	6.0/5.5	4.0	2.0
Sodium, mg/g	3.5	3.5	2.5	6.0
Ferric, mg/g	0.19	0.19	0.27	0.04
Manganese, mg/g	0.10	0.06	0.04	0.06
Zinc, mg/g	0.11	0.11	0.07	0.01
Copper, mg/g	0.03	0.03	0.12	0.01
Vitamin D, IU/g	1.50	1.50	1.50	1.11
Retinol, µg/g	0.36	0.36	0.36	0.67
dl-α tocopherol acetate, mg/g	0.063/0.081	0.063/0.068	0.042	0.039
Thiamin hydrochloride, mg/g	0.003/0.013	0.003/0.006	0.004	0.001
Riboflavin, mg/g	0.012/0.014	0.012/0.013	0.012	0.003
Pyridoxine hydrochloride, mg/g	0.004/0.01	0.004/0.006	0.005	0.008
Cobalamin, µg/g	0.020	0.020	0.020	0.006
Calcium pantothenate, mg/g	0.011	0.010/0.006	0.011	0.002

4.5 Analytical methods

4.5.1 Analyses of dentin and predentin formation

Both mandibular and maxillary molars were analysed in experiment I. In experiments II-IV, only the mandibular molars were used. The jaws were defleshed and right hemimandibles (I-IV), and hemimaxillae (I), were preserved in absolute ethanol. The first and second molars were sectioned sagittally along the midline under water cooling by the modified method of Keyes (1958) using a diamond disc (Horico, Diaflex, Berlin, Germany) with a diameter of 23 mm and a thickness of 0.1 mm. The disc was mounded on a technical handpiece (KaVo, EWL 9, Germany). Left hemimandibles (II-IV) were fixed in 10 % formalin before being decalcified in 5 % formic acid. After the decalcification, the jaws were processed with glycomethacrylate (GMA) they were

embedded in blocks and cut into histological sections. The sections were stained with toluidine blue (STB).

To measure the area of dentin formed during the test periods, a method based on the fluorescence reaction of tetracycline labeling of dentin was used as described by Larmas & Kortelainen (1989). In experiment I, the main central fissure of the first and the second molars was photographed under a microscope (magnification x 16; Ortoplan Ploemopack, Leitz, Westlar, Germany; subsidiary, Midland, ON, Canada) equipped with UV light using Kodak Ektachrome daylight film (400 ASA). The tetracycline-marked areas of dentin apposition were determined planimetrically from the video images of the films by circumscribing their respective areas on a monitor (Salora 445 A RGB, Salo, Finland; camera Hitachi VKM 96 E, Japan), using a serial ”mouse” connected to a PCVision Frame Grabber (Imaging Technology, Inc., Woburn, MA, U.S.A.) (Larmas & Kortelainen 1989). In experiments II-IV the above method was modified and the areas of dentin appositions were measured planimetrically directly from the specimens using a microscope (magnification x 5, Leica DMRB, Leica Mikroskopie und Systemic GmbH, D 35530 Wetzlar, Germany) equipped with a fluorescent light and a computer-connected video image analyzer (Leica Q 500 MC, Leica Cambridge Ltd, U.K.).

The width of the predentin layer was measured from histological sections (III,IV) under a microscope (magnification x 40) at six sites under the main fissures of the molars, and the mean was considered to represent the thickness of predentin for that particular tooth (Hietala & Larmas 1995).

4.5.2 Caries scoring

Two separate methods were used to analyze dental caries. The areas of dentinal caries, seen as spontaneous fluorescence of carious dentin under ultraviolet light (Larmas & Kortelainen 1989, Hietala *et al.* 1993, Banerjee & Boyde 1998), were measured using the same principles as with the measurements of the areas of dentin apposition (I, II, IV) (Fig. 1.).

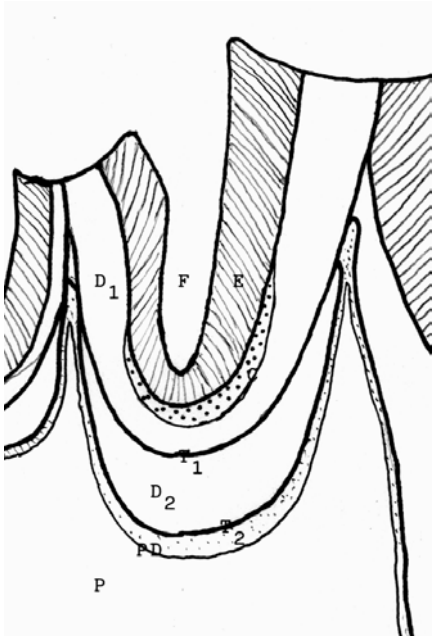

Fig. 1. A schematic presentation of the main central fissure of the rat mandibular second molar. Abbreviations: C = dentinal caries lesion, D_1 = dentin formed preweaningly, D_2 = dentin formed postweaningly, E = enamel, F = fissure, P = pulpal tissue, PD = predentin layer, T_1 = tetracycline stripe formed at the onset of the experiment period, T_2 = tetracycline stripe formed at the end of the experiment period.

To classify different types of caries lesions, modification of the classical Shiff's reaction was used to stain the caries lesions (Hietala *et al.* 1993). In this method, the reaction is based on detection of aldehyde groups from by-products of the proteolysis of the dentinal matrix (König *et al.* 1958). The occurrence and the progression of dental caries were determined under the main fissures of the mandibular first and second molars (II, IV). The fissures were classified as intact (sound) fissures, superficial enamel lesion, advanced enamel lesion or dentinal lesion.

4.5.3 Analyses of blood and urine samples

Serum and urine inorganic phosphorus was determined using a UV method (UV test for phosphate, SYS 1, Boehringer Mannheim/Hitachi 704/911, Cat No 1489348) modified for Cobas Fara II centrifugal analyzer (F Hofmann-La Roche Ltd, Diagnostic Division, Basel, Switzerland). Calcium, potassium and sodium levels of serum and urine were determined by flame photometry (Eppendorf Efox 5053, Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) (II-IV).

Serum insulin levels (IV) were determined using a radioimmunoassay (Rat Insulin RIA Kit, Linco Research Inc, St Charles, MO, U.S.A.), which utilizes an antibody made specifically against rat insulin.

4.6 Statistics

Means and standard deviations were calculated for the areas of dentin apposition, areas of dentinal caries lesions, volume of urine excretion, urinary mineral level, urinary mineral excretion, serum mineral concentration, serum insulin level, predentin width, mean weight, weight gain, daily sucrose load, daily food intake and daily growth rate of the animals (I-IV). Median values with 25 % and 75 % quartiles were calculated for the areas of dentinal caries (I).

Serial measurements of urinary mineral excretion (Ca, P, K and Na) were done during the test period (III). The excretion amounts of each mineral were determined by measuring the area under the curve for each animal. The atomic weight of each mineral was used for calculating the individual urinary excretions, where the arithmetic mean urine and mineral excretion values of the groups were counted (Matthews *et al.* 1990).

If the data was under normal distribution and two separate groups were analysed, the independent samples of T-test (I, II) were used to determine the differences among the groups. The differences among several groups were analysed using one way ANOVA with Tukey's honestly significant difference test (II-IV). When the data did not meet the assumption of homogeneity of variances, the nonparametric Kruskal-Wallis ANOVA was used to detect the differences between the groups (IV). If differences were detected, the analysis was done using the nonparametric Mann-Whitney U-test (II, IV).

The statistical analyses were performed using SPSS for Windows Release 7.5 (SPSS, Chigago, IL, USA). The level of statistical significance was set at $P < 0.05$ (Potter 1994).

5 Results

5.1 The growth of the animals and food intake

No difference in the mean weight of the experimental animals was noticed between dietary sucrose and standard diet groups (I-IV). However, the higher mean growth rate of male pups increased the mean weights of male pups significantly in both sucrose and standard diet groups by the end of the experiment I. Maternal sucrose diet had no affect on the growth rates of the pups probably because the possibility of litter effect was excluded at the onset the experimental period by balancing the pups from the same dam among the experimental groups (II).

By the end of the experiment, the animals from the calcium-deficient group weighed less than the animals fed standard or sucrose diet, even though the differences in mean weights at the onset of the experiment or the differences in food intake during the experiment period were not notable (III).

No difference was observed in mean weights between the intragastric sucrose fed group (IGS) and the other experimental groups (CNT and DIS), even though the mean intake of IGS group was reduced when compared to CNT or DIS groups (IV).

5.2 Dentin apposition and the predentin layer

The reductive effect of sucrose on dentin apposition was independent of the length of exposure (II) or the route of sucrose ingestion (IV). The experimental high sucrose diet reduced dentin apposition in both mandibular (I-IV) and maxillary (I) molars when compared to the standard diet (controls). The maternal high sucrose diet during the lactation period reduced dentin apposition only in the first molars of their pups when compared to the pups of dams fed the standard diet (II). Pups from dams fed sucrose and then maintained on sucrose (Suc-Suc pups), had a significant reduction in dentin apposition in both molars when compared to the pups from dams fed the experimental

standard diet and then maintained on the same diet (Cnt-Cnt pups) or to the pups from dams fed the experimental standard diet and then maintained on the experimental sucrose diet (Cnt-Suc pups) (II). Dietary sucrose exposure (DIS) reduced dentin apposition more than the intragastric sucrose exposure (IGS). Intragastric sucrose feeding also caused a reduction in dentin apposition when compared to the standard diet (CNT) animals (IV). No difference in the width of the predentin layer was noticed between sucrose-exposed animals and the controls (III-IV).

Calcium-deficient diet reduced dentin apposition and increased the width of the predentin layer when compared to the control group. Compared to the sucrose-fed animals, the increase in the width of the predentin layer was significantly greater (III).

The affect of the caries process on the rate of secondary dentin formation under the measured fissures appeared to be minimal. Because the number of teeth available for analysis was small the value of statistical analysis was uncertain (I).

The reduction rates of dentin formation and induction rates of predentin width are presented as percentages in Tables 3 and 4.

Table 3. Experimental sucrose exposure and calcium-deficiency induced reduction rates on dentin formation and on predentin width (as percentage compared to controls) of offsprings in experiments I, II and IV.

Article	Dietary sucrose	Intragastric sucrose	Calcium-deficiency
I			
Dentin formation			
1st mandibular molars	-64% a	—	—
2nd mandibular molars	-39% a	—	—
1st maxillary molars	-33% a	—	—
2nd maxillary molars	-17% a	—	—
III			
Dentin formation			
1st mandibular molars	-24% a	—	-27% a
2nd mandibular molars	-40% a	—	-31% a
Predentin width			
1st mandibular molars	NS	—	+45% a
2nd mandibular molars	NS	—	+62% a
IV			
Dentin formation			
1st and 2nd mandibular molars	-23% a	-16% a	—
Predentin width			
1st and 2nd mandibular molars	NS	NS	—

Abbreviations: a = statistically significant difference to the controls ($P < 0.05$), NS = no statistically significant difference.

Table 4. Lactational and experimental sucrose exposure induced reduction rates on dentin formation (as percentage compared to Cnt-Cnt pups) of offsprings in experiment II.

Dentin formation	Cnt-Suc pups	Suc-Cnt pups	Suc-Suc pups
1st mandibular molars	-24% a	NS	-39% a
2nd mandibular molars	-40% a	NS	-47% a

Abbreviations: a = statistically significant difference to the Cnt-Cnt pups ($P < 0.05$), NS = no statistically significant difference.

5.3 Dental caries

The areas of dentinal caries lesions, seen as a change in fluorescence, were increased in dietary sucrose feeding groups (I, IV). The areas of dentinal lesions were significantly larger in the mandibular than in the maxillary molars, both in the sucrose and standard diet animals (I).

Dietary sucrose feeding also increased the occurence of dental caries. Fewer intact teeth and more dentinal caries lesions were noticed in the dietary sucrose groups than with the standard diet (II) or intragastric sucrose feeding groups (IV). Also the maternal sucrose feeding appeared to predispose their pups to the later occurrence of caries. However, the experimental exposure to sucrose had the major role for the increased occurrence and progression of caries (II).

Intragastric sucrose feeding had no increasing affect on either the areas of dentinal caries lesions or the occurrence of caries lesions when compared to the controls (IV).

5.4 Serum mineral and insulin levels

Dietary sucrose had no affect on the serum mineral levels of the pups (II, III) or dams (II). No significant differences in serum insulin levels were found and dietary sucrose elevated insulin levels only slightly when compared to the standard or intragastric sucrose groups (IV).

The calcium-deficient diet reduced serum calcium and increased serum sodium levels significantly compared to the sucrose or standard diet (III).

5.5 Urinary mineral levels and excretions

Dietary sucrose feeding did not affect the volume of urine excretion (II, III), but intragastric sucrose feeding increased the volume of urine excreted (IV). The high

sucrose diet was associated with increased calcium and decreased phosphorus excretion both in dams (II) and pups (II-IV). Reduced urinary phosphorus, potassium and sodium levels were also found in dietary sucrose-fed animals (III). Intragastric sucrose exposure increased calcium, and decreased phosphorus, potassium and sodium excretion similar to what was observed with dietary sucrose, althought the differences compared to the controls were not significant (IV).

Calcium-deficiency reduced both urinary mineral levels and mineral excretions of calcium, phosphorus, potassium and sodium when compared to the controls. The differences in urinary calcium and potassium levels, and excretions, were decreased in the calcium-deficient group when compared to the sucrose group (III).

6 Discussion

6.1 Methodological considerations

6.1.1 Animal handling and feeding procedures

In experiment I all animals were inoculated with a fresh suspension of *Streptococcus sobrinus* (ATCC 27531 K 1 Fitzgerald), which has been reported to be cariogenic (De Soet *et al.* 1991). Because the main interest of the other experiments (II-IV) was the systemic effects of sucrose or low calcium diet on dentinogenesis, instead of caries, no contamination with specific cariogenic bacteria was performed. Despite the lack of specific cariogenic bacteria, a large number of dental caries lesions occurred, especially in high sucrose groups (II, IV), which supports the previous finding that the relationship between dietary sucrose and dental caries is stronger than the relationship between bacterial counts and caries (Mundorff-Shrestha *et al.* 1994).

Dietary protein restriction of rat dams has been reported to decrease their milk production and reduce the weight gain of their litters (Grigor *et al.* 1987). Balancing the amount of protein with casein in the high sucrose diet was considered to be succesful, because no diffences in the general health of the dams (II) and pups were noticed (I-IV). Moreover, the mean weights and growth rates of sucrose and standard diet groups were similar (I-IV). Despite compositional differences, the amounts of micro and macro nutrients (Table 2.) met the demands of the National Research Council (National Research Council 1972) which minimized the effect of food intake on the general health, growth and dentinogenesis during the experiment. Therefore, the reduced mean weights of calcium-deficient animals (III) (Tjäderhane *et al.* 1995a) was concluded to be the outcome from inaquate calcium reserves.

Dental caries was one of the main variables studied in the present works (I, II, IV). *Ad libitum* feeding maintains better conditions for caries development than does controlled feeding conditions (König *et al.* 1968). From a cariological point of view, *ad libitum*

feeding, corresponding to an eating frequency of 24 or 30 times daily resulted in the same number of fissural caries in the rat (König *et al.* 1968).

It is noteable that the experimental diets used in these studies (I-IV) were slightly below the recommendent energy level (Table 2.), but because food was freely available no deprivation of energy occurred as the rats adjusted their food intake to meet their energy demand (Rogers 1979). The capability of rats to balance their energy intake could also be seen in experiment IV, where the food intake of intragastic sucrose-fed (IGS) animals was reduced compared to standard (CNT) or sucrose (DIS) animals. Also the relatively normal growth rate of the animals among the tests groups (I-IV) suggests that all vital dietary elements were present in the experimental diets, the exeption was the low calcium diet (III). The ideal situation in dietary studies would be that only one dietary variable (sucrose or calcium amount) was under observation (I-IV). The other compositional differences in the diets, other than the factor under observation, points out one of the main difficulties of dietary experiments, the balancing of the basic elements of the diets.

However, the amount of a few vitamins was notably different among the diets (Table 2.). All of them were analogues of vitamin B (B_1, B_3, B_5 and B_6; respectively), which are water soluble vitamins and so rarely accumulate in toxic concentrations. Deficiency of a single vitamin of the B complex is rare, since poor diets are often associated with multiple deficiency states. Because our experimental diets (I-IV) fulfilled the nutritional requirements (RDA) of National Research Council (1972) fairly well, the differences in nutritional values were generally marginal. The diets were fed *ad libitum* without any evidence of vitamin B deficiency or overdose. The differences in the amounts of vitamin B analogues among the test diets had no significant impact on the general health or dentinogenesis of test animals, even though dietary sucrose theoretically increases vitamin B requirements compared to the other test diets (Table 2.). Only completely synthetic diets are such that the effect of one ingredient can be studied, but they have their own limitations as well (including economicones).

Increasing intragastric sucrose load, and gradually increasing the liquid amount introduced directly into the stomach did not cause any health problems to the test animals (IV). The experimental procedure resulted in a reduction in dentin apposition; therefore, the borderline concentration of sucrose required for the reduction of dentinogenesis (Huumonen *et al.* 1997) was exceeded. Intragastric sucrose feeding also diminished the occurence and the progression of dental caries, which is accordance with the earlier findings of Toyry and Mechin (1976).

6.1.2 Measuring the area of dentin formed

The sagittal bisectioning of the mandibular (I-IV) and maxillary rat molars (Keyes 1958) is fast, easy to perform, widely used and accepted in caries experiments (Larson 1981). The problem with this method is the possibility that the tooth is not divided into equal halves and the areas of formed dentin or dentinal caries may appear smaller than they actually are. Also some dental material is lost during cutting or small carious lesions are

not registered in either section. Some of these problems might be avoided by serial sectioning of the jaws, which was used in predentin analyses (III, IV). However, the same problems with the curvature of the dental archs and the cutting of molars sagittally in the mid-line would be present anyway. Because of these methodological limitations, the areas of dentin formations and dentinal caries lesions in the third molars were not measured in these studies.

Measuring the dentin formed during the experimental period from mandibular or maxillary halves, by using tetracycline labeling, is a relatively painless method for experimental animals, easy and reproducible to examine with minor intra-examiner variability (less than 3.5%) and systematic percentage error (Larmas & Kortelainen 1989, Hietala *et al.* 1993). In addition, the reduced rate of caries progression in the maxillary molars might offer the possibility to observe the response of the orginal odontoblasts to the systemic challenges without the disturbance caused by large caries lesions. However, most measurements in earlier experiments (Larmas & Kortelainen 1989, Larmas *et al.* 1992, Hietala *et al.* 1993, Tjäderhane *et al.* 1994, Huumonen *et al.* 2001) were made on mandibular molars and therefore comparison to earlier studies might be difficult if only maxillary molars are analysed.

6.1.3 Caries scoring

Dentinal caries were analyzed using two methods. The occurence and progression of caries was quantified after Schiff's staining (II, IV) (König *et al.* 1958, Hietala *et al.* 1993) and the areas of dentinal lesions by measuring the autofluorescing areas in the carious dentin (I, IV) (Larmas & Kortelainen 1989, Hietala *et al.* 1993, Banerjee & Boyde 1998). The scoring of lesions is dependent on the staining reagent used (Larson 1981) and on the cut-off point established to differentiate the type of lesion (II, IV). The autofluorescence method is a more sensitive indicator of the carious process in dentin for revealing the areas of mineral loss (I, IV), than the Schiff's reaction (Hietala *et al.* 1993). However, the number of enamel caries lesions can not be counted accurately.

Supplementing the protein content of the high sucrose diet (R 642) with casein may have had a reductive effect on the rate of dentinal caries progression because casein may prevent the colonization of *Streptococcus sobrinus* and thereby inhibit the formation of advanced dentinal caries (Guggenheim *et al.* 1999). However, when the occurring caries or the areas of dentinal caries lesion was measured, the enhancing affect of sucrose on cariogenesis was substantial in all cases (II, IV).

6.1.4 Statistical analyses

The variation in urinary phosphorus excretion in control dams was substantial (II). This variability was probably caused by the small number of dams in the groups. However, all excretion data points for each dam were checked, but no single erroneous high value,

which could cause skewing, was found. Measuring the area under the urinary mineral excretion curve for each animal takes into account the fact that values at different time points are from the same animal. The arithmetic mean for urinary phosphorus excretion includes four separate values from three different dams from each experimental group (II). The same urinary mineral excretion method used for the dams, was used for the pups (Matthews *et al.* 1990), with an exception that the measurement time in pups was longer than in dams to ensure adequate collection of the urine specimen (II-IV).

No significant difference in predentin thickness among the sucrose and control groups were noted (III,IV). The mean values, with SD, of the groups in question would give some reason to presume that differences might exist. Tukey's HSD t-test was used to test the differences between groups. This test compares the biggest difference between groups and if this difference is not statistically significant, none of the smaller differences will be. Significant differences were noted between the calcium-deficient and other experimental groups (III).

Because the areas of dentinal caries lesions did not meet the assumption of homogeneity of variances, the nonparametric Kruskal-Wallis ANOVA was used to detect the differences between groups (IV). When differences were detected, the analysis was done using the nonparametric Mann-Whitney U-test (II, IV).

6.2 Carbohydrates on the host side

A sucrose rich diet has been reported to increase serum insulin levels and alter calcium balance in humans by increasing urine calcium levels (Holl & Allen 1987). The calciuric effect of sucrose is thought to be the result of reduced tubular reabsorbtion (Lemann *et al.* 1970, Nguyen *et al.* 1993), possibly induced by elevated serum insulin levels (DeFrozo *et al.* 1975). Similarly in rats, high sucrose intake promotes insulinemia (Wright *et al.* 1983), deterioration of glucose tolerance (Wright *et al.* 1983, Hallfrisch *et al.* 1979) and hypercalciuria (Wood & Allen 1987). The calciuric effect, with reduced phosphorus, potassium and sodium excretions, was observed in sucrose-exposed animals (III, IV). However, the urinary mineral level was not associated with an elevated serum insulin level (IV). A high carbohydrate intake during the suckling period of rat pups cause hypertrophy of pancreatic β-cells, and induces insulinemia during the postweaning life (Vadlamudi *et al.* 1993). Alterations in insulin balance develop faster the more rapidly dietary glucose is absorbed (Higgins *et al.* 1996). Rats fed a high starch diet did not develop insulin resistance (Lockwood & Eckhert 1992) or severe reduction of dentin apposition (Hietala & Larmas 1995b).

In the present work, no clear evidence was noticed for a direct insulin effect on odontoblasts or an adverse effect causing a reduction in dentinogenesis. However, it should be noted the insulin level were analyzed in only one study (IV). The direct insulin effect on the odontoblasts has not been proven, but Välikangas *et al.* (2001) found that insulin per se does not affect odontoblast collagen type I synthesis *in vitro*. Insulin injections in rats neither reduced normal dentinogenesis or normalized reduced dentinogenesis following dietary sucrose exposure (Pekkala *et al.* 2002). The reduced

dentinogenesis in calcium-deficient animals (III) was clearly the outcome of different mechanism(s) other than those in dentinogenesis in sucrose exposed animals (I-IV). Calciuria occured only in sucrose-loaded animals (II-IV). Sucrose feeding reduced dentin calcium, phosphorus, and also the total mineral content during the postweaning period when compared with the preweaning period (Huumonen & Larmas 1999). Because a disturbed calcium balance does not necessarily explain the reduction of dentin apposition, caused by the high dietary sucrose load, the exact mechanisms for reduced dentinogenesis still remains unknown. Final conclusions can not be drawn.

6.3 Dentinal response to diet and caries

In the rat, dentin formation slows down without apparent transition between primary and secondary dentinogenesis (Johannessen 1961, Hietala & Larmas 1992, Kortelainen & Larmas 1994). Considering the duration of experiments (3 to 6 weeks) and the young age of the animals at the end of the experimental periods (42 to 56 days), the dentin formed rapidly during this period was thought to be primary because the reduction in dentin formation, caused by the experimental diets, was found to be significant (I-IV). There is some evidence that the odontoblasts of rat molars may atrophy after the formation of primary dentin (Romagnoli *et al.* 1990). During secondary dentinogenesis, the experimental procedures might be ineffective because of the slower rate of dentin formation.

Increasing the sucrose load depresses odontoblast activity, which reduces dentin formation during primary dentinogenesis in the rat (Larmas *et al.* 1992, Tjäderhane *et al.* 1994, Huumonen *et al.* 2001). The sucrose concentration needed for the dietary sucrose effect is between 30 to 40% (Huumonen *et al.* 1997), although similar reductions in dentinogenesis can be shown with other variations in sucrose diets (Autio *et al.* 1997) or via intragastric sucrose feeding (IV). Reduction of dentinogenesis was observed both in maxillary and mandibular molars (I). However, mandibular molars are commonly used for dentin measurements (Larmas *et al.* 1992, Tjäderhane *et al.* 1994, Huumonen *et al.* 2001) because mandibular molars are more susceptible to cariogenesis in the rat (König *et al.* 1958). One of the main interests has been the correlation between reduced dentinogenesis and the induced progression of dentinal caries (Tjäderhane *et al.* 1995b). Differences in caries rates among the jaws can be explained via morphological differences between the mandibular and maxillary molars of the rat (König *et al.* 1958, Hunt *et al.* 1970a). Maxillary molars have lower levels of dental plaque and food debris in the fissures of (Hunt *et al.*1970b) and decreased caries progression compared to mandibular molars. Also, early weaning (Hietala *et al.* 1997) and maternal sucrose diet (II), not only predisposes for the reduction in dentin formation, but also elevates the rate of dental caries progression in rat pups (Navia & Lopez 1977) (II).

In previous studies, the improper mineralization of dentin or predentin was thought to be the only cause for the reduction in dentinogenesis, because sucrose feeding stimulated the widening of predentin layer compared to control fed rats (Hietala & Larmas 1995ab, Autio *et al.* 1997). Lower levels of dentin mineral elements, especially calcium, in

sucrose-fed animals (Tjäderhane 1996, Huumonen & Larmas 1999) support the theory of impaired mineralization of dentin. However, a low calcium diet will cause widening of the predentin layer in the rat (Engström *et al.* 1977, Rasmussen 1977) and reduced dentin formation (Lozupone & Favia 1994, Tjäderhane *et al.* 1995a). In the present study no evidence of similar widening of unmineralized predentin layer or disturbance in calcium balance, as in a calcium-deficient animal, was found in sucrose-exposed animals (III, IV). Therefore, it was concluded that the reduced dentinogenesis observed in sucrose-fed animals was an outcome from impaired synthesis of the dentin organic matrix. Moreover, the roles of calcium balance (III, IV) and insulin (IV) on sucrose-induced effects of dentinogenesis and the function of the pulp-dentin organ need further studies in the future.

High dietary sucrose intake increases the progression of dental caries in rats (Huxley 1977, Hefti & Schmid 1979). The caries rate is dependent on the developmental stage of tooth, so that the earlier caries occur, the larger are the carious lesions in dentin (Larmas *et al.* 1992, Hietala *et al.* 1997). Resistance to the carious process also increases with age (Larson & Fitzgerald 1964). Also, the eruption phase of teeth is the most critical time for caries initiation and progression (Carvalho *et al.* 1989, Ekstrand & Björndal 1997, Björndal *et al.* 1998), especially in human molars (Larmas *et al.* 1995, Härkänen *et al.* 2002). Rapid progression of caries in posteruptive teeth could be explained by incomplete maturation of enamel, but advanced progression of caries in dentin, associated with high dietary sucrose load and reduced primary dentinogenesis, supports the importance of a systemic defence response of the pulpo-dentinal compex on caries irritation (Huumonen 1999). The capability of the pulpo-dentinal complex to respond to caries irritation, even its initial stage, (Massler 1967, Brännström & Garberoglio 1972, Björndal *et al.* 1998) might offer a chance to evaluate the effects of systemic factors on caries response in the future. However, intragastric sucrose feeding reduces primary dentinogenesis without increasing the occurrence of dental caries (IV). Also the occurrence of the same rate of caries among the control and intragastric sucrose-fed animals (IV) points out the importance of local sucrose challenge in the initiation of dental caries.

From the traditional point of view, enhanced caries progression should induce secondary dentin formation to prevent pulpal exposure (Massler 1967). Unfortunately, the number of teeth used for dentin and dentinal caries measurements was too small to establish a causal relationship between reduced dentinogenesis and induced dentinal caries progression. However, Huumonen *et al.* (2001) by collecting numerous dentin and caries measurements has shown that a high sucrose diet abolishes the ability of the pulpo-dentinal complex to induce defensive reactions against dentinal caries. The reduced response of odontoblasts during secondary dentinogenesis becomes evident as a reduction in dentin formation under the carious fissures of sucrose fed rats, whereas in animals fed the control diet, the effect of caries on dentin formation was inductive (Huumonen *et al.* 2001). These result are in accordance with the finding that the relationship between dietary sucrose and dental caries is stronger than the relationship between the caries and bacterial counts (Mundorff-Shrestha *et al.* 1994, van Palenstein Helderman *et al.* 1996).

It is appears that dentin formation and dentinal response to caries irritation are modulated by mechanisms within the pulpo-dentinal complex. Physiologically, the odontoblasts, as part of the pulpo-dentinal complex, have an essential role in affecting the dentinal response. According to Björndal & Mjör (2001), optimal assessment to

prevailing clinical conditions can only be made through knowledge of the biology of the pulp-dentin organ.

7 Conclusions

When the high sucrose exposure was maintained, the reductive effect of sucrose on primary dentinogenesis was found in both mandibular and maxillary molars. However, the rate of dentinal caries progression was slower in maxillary teeth.

Sucrose feeding affected the function of primary odontoblasts in the second generation of the animals, as evidenced by decreased dentinogenesis. In addition, a close connection between reduced dentin formation and enhanced dental caries occurrence was noticed. Direct experimental exposure of the pups to the sucrose diet reduced dentinogenesis and enhanced the progression of dental caries more than indirect exposure through sucrose-fed dams. Exposing the dams to sucrose predisposed the pups to reduced dentinogenesis and enhanced the occurrence of dental caries. The maternal and experimental sucrose exposures together reduced the rate dentinogenesis and induced the occurence of dental caries cumulatively during the postweaning period. Dietary sucrose reduced urinary calcium excretion but induced phosphorus excretion, especially in pups exposed to the maternal and experimental sucrose diet.

The high sucrose diet reduced the rate of primary dentinogenesis as much as a dietary calcium deficiency, but the mineralization of predentin was not affected by sucrose even though the calciuric effect of sucrose was evident. Therefore the sucrose-induced effect on dentinogenesis could not be explained as the consequence of disturbed calcium homeostasis.

The sucrose exposure, dietary or intragastric, affects the function of the pulp-dentin organ in growing rats. Both intragastic and dietary sucrose feeding had a reductive effect on the rate of primary dentinogenesis. With the exception of a calciuric effect, sucrose did not affect predentin mineralization or insulin blood levels. The intragastric sucrose feeding excluded the local cariogenic challenge in the oral cavity, which demonstrates the importance of external sucrose in caries initiation.

These results demonstrate that reduced dentinogenesis, caused by a sucrose load, is the outcome of reduced synthesis of the dentin organic matrix rather than a reduced rate of predentin mineralization.

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