THE EFFECT OF MALNUTRITION ON SALIVA COMPOSITION AND CARIES DEVELOPMENT

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av

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ABSTRACT


Starvation and protein deficiency increase susceptibility to infections in general and affect the function of various types of cells individually. Saliva contains substances which offer protection to the oral tissues. Its composition and secretion rate may be important in preventing the development of disease, e.g. dental caries. The aim of the present study was to evaluate the effects of starvation and protein deficiency on the secretion and composition of saliva and the effects possibly induced on dental caries.

The following results were found.

Short-term starvation on a liquid diet reduced the secretion rate and changed the composition of saliva in healthy humans. Chewing during starvation while it did not restore secretion rate, did partially restore the composition.

Total protein concentration was not greatly affected by gross malnutrition or protein deficiency. A more pronounced effect was observed on individual biologically active proteins. The salivary glands seem not to be prime targets for malnutrition but the rate of biosynthesis of a bacteria aggregating glycoprotein and the activity of salivary peroxidase were significantly reduced by a long-term protein deficiency and gross malnutrition respectively in the rat. In humans, short-term starvation on a liquid diet caused an impaired glycosylation of sialic acid to the protein and decreased lysozyme activity. Sialylation was partially restored by chewing.

In the rat the establishment of a cariogenic microorganism, S. cricetus (S. mutans) strain E 49 serotype a, was facilitated by protein deficiency.

Starvation in both man and the rat produced evident clinical effects. The rate of plaque formation was increased in man and in the rat there was significant increase in caries development induced by a standardized cariogenic challenge.

This study shows that nutrition is important for the secretion of saliva and that starvation and protein deficiency increase the cariogenicity of sucrose.

Key words: starvation, protein-deficiency, antibacterial proteins, saliva, caries, infection.

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PREFACE

This thesis is based on the following papers:


IV Johansson, I. and Ericson, T.: Saliva composition and caries development during protein deficiency and ß-receptor stimulation or inhibition. In manuscript.


In the thesis the papers will be referred to by the Roman numerals.
ABBREVIATIONS

BAGP - Bacteria agglutinating glycoprotein
CFU - Colony forming units
ER - Endoplasmic reticulum
PEM - Protein-energy malnutrition
RDA - Recommended dietary allowances
sIgA - Secretory immunoglobulin A
SP - Salivary peroxidase
UL - Uniform labelling
INTRODUCTION

Each cell and organ requires certain levels of energy and nutrients to function adequately. Within wide limits the organism can balance the requirements of energy and nutrients between cells and organs by mechanisms of storage and redistribution. The reserves - formed during periods of excess intake - are activated in situations of malnutrition.

Prolonged periods of malnutrition will affect tissues and organs in relation to their energy and nutrient requirements. The sensitivity of various organs to malnutrition varies from the extreme sensitivity of the muscles to the rather stable conditions of the neural system. Functional brain changes may occur but only as a temporary effect of acute carbohydrate deficiency. However, it must be assumed that a ranking of the effects including all organs is related to the type of deficiency and to the period of life at which it occurs. Permanent loss of function may appear when the deficiency occurs during the period of development (1, 2), whereas reversible changes may occur during growth periods (1, 3) and in the adult period (1). The response to nutritional deficiencies in different types of cells and organs is complicated and only partially understood.

PREVALENCE OF MALNUTRITION

The terms malnutrition and undernutrition, used synonymously in this work, describe a situation where an inadequate intake or poor absorption of energy and nutrients cause gross changes in an organism leading to loss of weight and ultimately to loss of function or to disease. The type of malnutrition which is characterized by a deficiency of specific nutrients is often less obvious and at an early stage difficult to detect without special analyses. Clinically, severe symptoms may appear only after a longer period of time. Protein-energy malnutrition (PEM), is usually combined with deficiencies in vitamins and minerals (4). It is the most common nutritional disorder and from a global perspective of overwhelming importance. In the developing countries up to 70% of the children are affected by serious or moderate PEM (5, 6).
In the industrialized countries several subpopulations risk malnutrition (6-13), not because of shortage of food but commonly in connection with alcohol and drug addiction or with the occurrence of disease, hospitalization, depression or loneliness. Patients with cancer (8), Anorexia Nervosa or oesophageal obstruction (2) are often malnourished. An English study published in 1972 showed that 3% of the population over 65 years of age was malnourished (9). When the same individuals were studied seven years later the prevalence had increased to 7% (10). In a Swedish study on the elderly (11) the protein intake (RDA 1974, stipulated by the Food and Nutrition Board, USA) was clearly below the recommended daily intake for 10% of the population. In a Swedish study it was recently reported that 22% of the elderly were already malnourished when admitted to hospital because of medical symptoms (12).

The Swedish recommendation for the daily intake of protein in healthy adults is 0.7 gm/kg body weight. When disease is present the recommended value ranges between 1.1-1.8 gm/kg body weight depending upon energy requirements. Hospitalized patients frequently exhibit a negative nitrogen balance (13) indicating too low an intake of protein to balance the increased need during illness and recovery.

SOME GENERAL EFFECTS OF MALNUTRITION

Metabolic changes during starvation

Dietary protein supplies nitrogen and essential amino acids. After enzymatic digestion of the protein to amino acids and small peptides and after active absorption the amino acids are transported to various cells in the body. The amino acids are needed for growth and repair of tissues and for the synthesis of soluble proteins, such as the biologically active enzymes, hormones, transport proteins and proteins with antibacterial properties.

There is a close connection between protein turnover and energy metabolism, which is more pronounced in man than in rodents (14, 15). Normally dietary carbohydrates and fat are used for energy production but dietary protein is used when these sources of energy are deficient. An excess of dietary energy is stored in depots of glycogen and fat tissue. The glycogen pool is rapidly depleted in the first phase
of starvation in man. The insulin level falls and the glucagon level rises (17-19). Metabolic processes are then activated to support cells, mainly in the brain, with glucose (16, for a review see 17). This occurs through catabolic processes involving muscles and fat tissue. Metabolites released from muscle tissue are used for gluconeogenesis and protein synthesis and blood glucose level can be maintained at a lower level (19). The oxidation of fatty acids released from fat tissue leads to formation of keton bodies which the brain and muscle cells can use for energy production (16, 19). On the third to seventh days of starvation the gluconeogenesis in the liver decreases but continues in the kidneys (19). These metabolic sequences protect the protein pool. The release of amino acids from the muscle mass is reduced and the renal excretion of nitrogen diminishes. A less negative nitrogen balance is obtained. After an initial 20 days of starvation this emergency situation can be maintained for a long time but it leads to a continuously decreased basal metabolism and loss of cell mass. Fasting also suppresses the sympathetic nervous system (20). Body proteins are saved and nitrogen excretion kept at a minimum. However, the daily loss of nitrogen in man is still equivalent to 20-25 gm of protein or 90-100 gm of muscle tissue (17).

The negative protein balance activates not only, although predominantly, catabolic processes in the skeletal and smooth muscles. Reductions in the biosynthesis of soluble proteins like prealbumin, retinol binding protein, albumin and transferrin have also been reported (for a review see 17). The formation of subcellular structures, involved in the synthesis of proteins, is defective during starvation (21) and periods of protein deficiency (22, 23). The content of the polyribosomes in the cells is decreased (21) as well as the incorporation of radioactively labelled amino acids into proteins (22, 24, 25). The transcription activity is reduced as a result of reduction of RNA polymerase II, involved in synthesis of messenger-RNA (26). This gives rise to a slower rate of protein synthesis.

The rate of protein synthesis normally varies among different organs and tissues. In malnutrition the reduction in protein synthesis also varies between organs. Protein synthesis in skeletal muscles is greatly reduced in young rats fed a protein-free diet for 6 days or more (23, 24). The reduction in protein synthesis occurs to a lesser
extent in other organs, e.g. liver, heart, kidney, brain (24), thymus, spleen and bone marrow (22, 25). The synthesis of proteins is also decreased in exocrine glands (27, 28 , for a review see 29).

The rapid initial loss of body weight during starvation is mainly caused by loss of water. In fasting the sodium intake is reduced. Sodium excretion is increased (30) as a result of lower aldosterone activity. The net effect is a reduction of extracellular body water content (30). Each gram of glycogen can release 2 gm of water, and in the catabolic process intracellular water and electrolytes are lost.

The immune system during malnutrition

The most frequent oral diseases are caused by bacteria. A special review of the antibacterial systems in malnutrition is therefore warranted. Malnutrition and susceptibility against infections are synergistic (31, 32, 33). The defence mechanisms against infections include the immune defence systems and non-immune systems such as proteins with antibacterial properties in exocrine secretions, eg. lactoferrin, lactoperoxidase, lysozyme and bacteria agglutinating glycoproteins.

Undernutrition seems to affect cell-mediated immunity particularly, but the humoral response, phagocytosis, and the complement system are also affected (for a review see 29 and 34).

PEM in man causes a reduction in the total number of T-lymphocytes and rosette forming T-lymphocytes in serum (35, 36), and a higher share of "null"-lymphocytes (36) whereas the level of B-lymphocytes is unchanged (36). It is suggested that PEM does not affect the level of immunoglobulins in serum unless T-lymphocytes are required (37). However, sIgA is decreased in exocrine secretions in man. Thus, the total amount secreted per minute of sIgA in milk was reduced in malnourished Pakistani women compared to well-nourished Swedish women (38). Reduced concentrations of sIgA were found in tears (6, 39), saliva (6) and in nasopharyngeal secretion (40, 41) from malnourished children, in vaginal secretion from protein-deficient guinea pigs (42) but not in intestinal secretion from undernourished Indonesian children compared to well nourished children (43). Immunoglobulin G (44, 45) or sIgA (45) have not been found to be affected by PEM in rat.
saliva. The levels of the different complements in serum are affected differently in times of PEM. Thus, the levels of C2 (46) and C3 (33, 46, 47) are reduced whereas C4 and C8 concentrations are not affected (46).

PHYSIOLOGY OF SALIVA SECRETION

The salivary glands are exocrine glands. There are basic morphological similarities between the secretory cells in salivary glands and other exocrine glands such as lacrimal glands, glands of the respiratory tract, lactation glands, goblet cells in the intestinal tract and secretory glands in the mucosa of the uro-genital tract. The salivary glands in man consist of three pairs of major glands, the parotid, the submandibular and the sublingual glands, as well as a number of minor glands in the mucosa on the inside of the lip, cheek and in the palate. Primary saliva is secreted from the acinar cells with some additional secretion from the intercalated duct cells. The acinar cells have a well developed endoplasmic reticulum (ER), Golgi apparatus and secretory granulae indicating the secretory function (48).

The formation of polypeptide chains and modifications to the different final proteins, like glycosylation of glycoproteins, are complicated, energy-requiring processes. The protein synthesis is the most energy-demanding biosynthetic process in the organism. Each peptide bond uses the energy from four high-energy phosphate bonds. The secretory proteins are synthetized on ribosomes bound to the surface of the ER. The genetic information is transcribed by messenger-RNA, translated by ribosomal-RNA and transfer-RNA. The nucleotide triplets of the m-RNA regulate the onset and termination of the protein synthesis. The traffic of proteins is guided into different directions by marker sequences of amino acids which determine where proteins shall end up, e.g. in secretory granulae or lysosomes (49). Many proteins are subjected to posttranslational modifications during these steps. Carbohydrates are attached by glycosyltransferases either one by one or by isoprene structures en bloc. This glycosylation takes place both in the ER and the Golgi apparatus (50). The secretory proteins are packaged in secretory granulae and expelled into the salivary duct. The production of secretory IgA does not follow this pattern completely (51).
The final composition of the electrolytes of saliva is regulated by a secretion-reabsorption mechanism which includes acinar cells and striated duct cells respectively. The flow of saliva through the salivary ducts is enhanced by means of myoepithelial cells outside the acinar cells.

The synthesis and release of saliva are very complicated processes and involve a number of different signal systems operating on different levels of synthesis and cellular transport (for a review see 52). According to the classical model parasympathetic and α-adrenergic stimulation regulates water and electrolytes in primary saliva (53, 54). The β-adrenergic component of the sympathetic system is thought to regulate the release of macromolecules (53, 55). Other factors which affect salivary secretion include physiological parameters like chewing (56), and several biologically active peptides (cf. 52). The peptides act directly on the secretory cells or indirectly via the capillary flow and on the distribution of water. The effects of gross or specific malnutrition on the function of the secretory cells are only partially known.

EFFECTS OF MALNUTRITION ON SALIVARY SECRETION

The reported effects of starvation or PEM on the morphology of the secretory cells, salivary glands and on saliva secretion are based on situations which vary widely with respect to species, developmental stage of the glands, saliva collection techniques, as well as degree and type of stimulation and starvation. Such variations affect the results significantly.

Salivary glands

The effects of starvation on the morphology of salivary glands depend on the degree and duration of starvation. Some authors report an enlargement of the glands (57-60) and increased accumulation of secretory granulae, and others a reduction in the size of the gland (61-65) and secretory granulae.

Some human studies, e.g. one epidemiological (57) and one presentation of case reports of patients with Anorexia nervosa (58), indicate that malnutrition leads to enlargement of the salivary glands. A standardi-
zation of human studies is however difficult to achieve and no conclusions can be drawn based on only these two reports.

A higher degree of standardization can be achieved in the rat. When the daily amount of food given to adult rats for 8 days was reduced from an initial amount of 24 gm/day to 16 gm/day there was an increase in the dry weight of the parotid glands (59). However, a further reduction of food led to reduction in gland weights. The maximum size reached at an intake of 16 gm/day was accompanied by an unchanged DNA content, reduced capacity for protein synthesis (RNA per gland weight) and reduced protein synthesis activity (ratio of RNA to DNA). Furthermore, an increase in the size and number of secretory granulae and an increase in the total amount of protein and amylase was found in the parotid gland and pilocarpine-isoproterenol stimulated parotid saliva. The combination of decreased synthesis and increased accumulation of protein was interpreted as a decrease in protein release. In another rat study (60), with a more moderate food reduction from 24 gm/day to 20 gm/day, the initial increase of the gland size reached a maximum after 12 days and returned to normal after another 12-day period.

In cases of more severe malnutrition - total starvation or PEM - reduced weights for the parotid (61, 62, 63) and the submandibular (64, 65) glands in rats have been reported. Rats subjected to total starvation already show morphological disturbances in the parotid gland after 16 hours (66). Thus, secretory granulae exhibit degenerative changes as well as signs of lysosomal digestion. Total inanition for 4 days (63) lead to a continuously decreasing gland weight and an initially increasing amylase content immediately followed by an increase in lysosomal enzymes, such as cathepsin D and acid phosphatase. Thereafter amylase activity decreased rapidly accompanied by an increase in lysosomal enzymes.

Already after two weeks, rats receiving 0,5 % protein diet (61) had a reduced parotid gland weight, DNA, RNA, total protein, total protein/mg DNA, amylase/mg DNA and dry weight/DNA. Severe morphological changes were also found in the submandibular glands of the rats when they were fed a 0.5 % protein diet (65). Atrophic, oedematous glands with severe changes in the morphology of the endoplasmic reticulum, the polyribosomes and secretory granulae occurred. This
parallels the findings in parotid glands after 72 hours of total starvation (66) and after a period of moderate protein deficiency (62). The disturbances in the morphological basis for cell function combined with the findings of reduced contents of DNA and RNA (61, 64) and reduced contents of total protein and amylase (61) in the gland indicate impaired conditions for protein synthesis in salivary glands during starvation or periods of PEM. It should be observed that even a mild reduction of food intake causes impaired conditions for protein synthesis (59, 60). A disturbed protein synthesis during malnutrition is also seen in other cells such as those from the liver (62) and the pancreas (61).

Saliva composition

In a study of severely malnourished Indian children, 1-10 years of age, a decreased secretion rate, concentration of protein and the activities of arginase and ferritin have been reported (67). A reduction of amylase and sIgA but not of protein or aminopeptidase was reported in malnourished Colombian children under two years (6, 68). To my knowledge no studies on the effect of starvation on saliva composition in adults have been published.

The effect of protein deficiency on saliva composition depends on the stage of development at which the deficiency is introduced (3, 27, 28, 44, 45). Gel diets containing 4 or 8 % protein were given to rat dams before conception through the lactating period. A control series of dams was given an adequate diet (25 % protein). After weaning the pups were given either the same protein deficient diet or the adequate diet. The results are contradictory and cannot be interpreted in detail. The impact on saliva secretion seems to be stronger in pups from protein deficient mothers (3, 28, 45). A similar study was made by Menaker and Navia (27). They observed significantly lower secretion rates in the pups of starved mothers as early as 16 days after birth. The effect was measured until day 35 and remained even though the pups received an adequate diet after weaning. The inference can be drawn that permanent damage to the pups was caused by the lower protein diet.

Reduced contents (mg/min) of total protein are found in rats fed low protein diets (27, 44) but the concentrations might remain unchanged
(45) or increase (27). After 47 days on an 8% protein diet the amylase activity decreased (28, 44) but when measured again after 91 days on the diet it had increased and surpassed the original level (28). A decrease in amylase activity was also found in saliva from rats fed a 4% protein diet for 35 days (3). Aminopeptidase was decreased when the protein deficiency was introduced before (3) but not after (28) weaning.

Nutrition has a profound effect on the development and morphology of the salivary glands and the secretory cells in the glands as well as saliva composition. Since saliva has implications for the development of dental caries further studies of the effects of malnutrition on caries development are warranted.

VARIABILITY IN CARIES DEVELOPMENT

Variations in caries development.

Dental caries is initiated by acids produced during the bacterial fermentation of carbohydrates. However, the rate of caries progress is dependent on a variety of host factors. The cariostatic effect of fluoride is an important example of a non-host factor which influences the direct sugar-bacteria-caries relationship.

It is important to recognize that wide individual variations in caries development occur in a healthy population given a reasonably standardized regimen of fermentable carbohydrates. Thus, the Vipeholm study (69) shows that caries development increased with an increasing number of intakes of fermentable carbohydrates, but also that patients subjected to the same standardized carbohydrate program developed caries at very different rates. Even in the group whose teeth were almost constantly exposed to sucrose, one third of the patients did not develop caries. The wide variation in caries development found in the Vipeholm study is often neglected. Some of the factors causing the variation are directly or indirectly related to saliva.

Role of saliva

The basic facts concerning dental caries are well established (70, 71). It is easy to anticipate interferences in these mechanisms by a number of salivary factors which affect bacterial adhesion and metabo-
lism, pH stability and de- and remineralization in the tooth. Surpris­
ingly weak correlations between individual specific factors and caries
incidence on the individual level have often been demonstrated (69,
72, 73). We must probably accept the fact that caries is not only a
multifactorial disease but that the factors can be divided into
categories. Only an evaluation of the concerted activity of different
categories provides a basis for caries prediction. This view has been
presented in a paper by Rundegren and Ericson (73).

In the present work the effect of malnutrition on one category - the
antibacterial factors - has been evaluated. Among the antibacterial
factors sIgA and bacteria agglutinating glycoprotein (BAGP) can affect
the conditions for bacterial adherence to surfaces in the mouth (74).
Secretory IgA is often ascribed a role in the local mucosal resistance
to infections (75, 76) and is referred to as an "antiseptic paint"
because of its presence on the mucosal surface perhaps as a complex
with other proteins (76). Secretory IgA and BAGP contribute to the
clearance of bacteria from the oral cavity by forming non-adherent
aggregates (77, 78). When salivary concentrations of sIgA and BAGP are
low, however, relatively high concentrations still are found in the
enamel pellicel due to the high affinity for the surface. Under these
conditions the uncoated bacteria are readily adsorbed to the agglutin­
inins in the pellicle and plaque formation rate is increased (79, 80).

Proteins with bacteriostatic or bacteriocidal properties (e.g. lyso­
zyme, salivary peroxidase and lactoferrin) suppress the metabolic
activity. The salivary peroxidase system consumes hydrogen peroxide
generated locally by bacteria (81, 82) and produces in the process
OSCN⁻. The OSCN-ion acts as a blocker of metabolism mainly in the
aerobic bacteria. Salivary peroxidase has a high affinity for bacteria
and enamel surfaces and binds in an enzymatically active form (83).
The oral mucosa, biologically active glycoproteins and anaerobic
microorganisms are protected by the consumption of oxygen radicals.

Lysozyme can function either by its agglutinating activity (84) or by
breaking down the glycosidic (β-1,4) bond between N-acetyl muramic
acid and N-acetyl glycosamine in the murein peptidoglycan of bacterial
cell walls (85). Its role in aggregating bacteria is negligible (86).
The lytic activity is most effective against gram positive bacteria
and less effective against gram negatives (89).

Lactoferrin limits the access to iron for the microbes. Bacteriocidal effects of lactoferrin on Streptococcus mutans have been reported in in vitro studies (e.g. 90).

Variability in saliva composition

The composition of saliva varies intra- and interindividually, but generally the protein content is about 0.1% and the electrolyte concentration is approximately 1/3 of the one in serum. The variations are related to the complicated conditions regulating the release and synthesis of proteins and the release of electrolytes and water (52).

Saliva composition shows circadian variations both in humans (91-93) and rats (94, 95). The circadian variations are secondarily affected by the gustatory and motoric stimulation of secretion. The composition of saliva is also dependent on the type of stimulation (96, 97). It is especially important to bear this in mind in experiments with rats where the secretion is usually stimulated. A standardization of the sampling time and procedure are essential if the effects of the biological variations are to be reduced.

EFFECT OF MALNUTRITION ON CARIES DEVELOPMENT

The effect of starvation or PEM on caries development in human populations has not yet been investigated. A diet containing 8% protein fed to rats during the fetal stage and kept during the lactating period resulted in increased caries development compared to the caries development in rats given the same diet containing 20% protein (98, 99, 100). No studies are published on caries development in the rat were malnutrition was introduced after weaning.
AIMS

The aims of the present investigations were to examine,

* the effect of an extreme low-nutrient liquid diet on secretion rate and composition of human saliva.

* the effect of a combination of chewing and a low-nutrient liquid diet on saliva composition and secretion.

* the effect of gross malnutrition in the rat started after weaning on saliva composition and the caries promoting effect of a given sucrose challenge.

* the effect of a moderate protein-deficient diet given after weaning on the saliva secretion rate and caries development in the rat.

* the synthesis and activity of a salivary bacteria agglutinating glycoprotein during a period of moderate protein deficiency.
METHODS

HUMAN STUDIES

Fasting for a period of eight days was chosen as a model for short-term starvation. Healthy female volunteers of normal weight participated in Study I. Study II was made on subjects staying at a health center where they were subjected to a low-calory diet regimen similar to the one in Study I.

The fast diet was a liquid diet consisting of fruit and vegetable juices and tea and broth. The diet provided 5.4 g of protein, 2.9 g of fat, 59.7 g of carbohydrates (mainly monosaccharides) and 3 liters of water per day. The consumption followed a strictly standardized schedule. In Study I the intake was checked at daily meetings with the participants. The juices were distributed day by day. In Study II the subjects were resident at the health center and were supervised by the personnel.

Medical recordings and serum analyses were made. Blood pressure and weight reductions in the participants were checked daily (I, II). In Study I, blood samples were taken before, during and after fasting. Serum concentrations of cholesterol, triglycerides, Na, K, Cl, CO₂, creatinine, uric acid, aspartate aminotransferase (ASAT, EC 2.6.1.1), alanine aminotransferase, (ALAT, EC 2.6.1.2), lactate dehydrogenase, (LD, EC 1.1.1.27) and IgG were measured according to standard procedures at the Department of Clinical Chemistry, University Hospital, University of Umeå, Umeå.

Saliva was collected before (I, II) during (I,II) and after (I) fasting. All human saliva samples were collected one hour after the morning meal. The participants were carefully instructed not to smoke or eat one hour prior to the saliva collection. Resting whole saliva and saliva stimulated by chewing on a one gram piece of paraffin were collected for standardized times. The donors were instructed to expire air above the saliva samples to preserve pCO₂. The test tubes containing the saliva were immediately covered with a plastic film and kept chilled with ice until the analyses were started within the space of 30 minutes.

The chewing program (II) contained five periods of ten minutes each of intense chewing on one piece of paraffin performed in connection with
ordinary meals. CFU of total streptococci, S. mutans and lactobacilli were determined in saliva (I) by cultivation on MS agar, MS agar with the addition of elkosin and Rogosa agar respectively. The plaque formation rate was studied (I) by checking the presence of plaque stained with Diaplac® (LIC Dental, Sweden) 16 hours after a careful cleaning of the teeth (79).

RAT STUDIES

The animals used in Study III were weaned Osborne-Mendel rats (NIH breeding colony, Bethesda) and in Studies IV and V weaned Sprague-Dawley rats and 46-day-old Sprague-Dawley rats (ALAB, Sweden) respectively. The rats were kept at 21° C, with a 12 hour daylight period starting at 6.00 a.m. The rats (III, IV) were kept in individual steel cages that were part of an automated feeding machine (101) with a scheduled feeding programme.

In Study V the rats were kept in plastic cages on plastic bottoms and food was served ad libitum. The basic rat diet was administered either by intubation (III), in a feeding machine (IV) or ad libitum (VI). In Study III the diet was given as three daily intubations of a supplemented baby-formula (102). It did not provide the optimal nutritional requirement (103) for growing rats (table 1). The experimental rats were given the same basic diet diluted with an equal volume of water. Experimental and control rats were given sucrose or starch as supplementations to be eaten by mouth. The carbohydrates were given as 17 portions of approximately 0.14 grams every 30 minutes starting at 10.00 p.m.

The rats in Studies IV and V were given slightly modified MIT 200 diets (104). Lactalbumin was replaced by casein and cotton seed oil by corn oil. The MIT 200 diet contains 20 % protein. In the low protein diet the protein content was reduced to 5 % by either substituting casein with α-cellulose (IV) or starch (V). Rats fed a protein-deficient diet eat less food (105). Since the vitamin and mineral content of Diet MIT 200 exceeds the needs of young adult rats (cf table 1) the vitamin and mineral supply was considered to be sufficient despite reduced consumption.

The high and low protein MIT 200 diets were served either in the
feeding machine 17 times a day in portions of 0.75 grams every 30 minutes starting at 10.00 p.m. (IV) or ad libitum (V). Distilled water was served ad libitum in all experiments.

Table 1. Nutritional requirements of growing rats compared to the nutrient content in diet MIT 200.

<table>
<thead>
<tr>
<th>Nutrients required per kg diet</th>
<th>Supply per kg diet MIT 200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross energy (kcal) 4,444</td>
<td>3,990</td>
</tr>
<tr>
<td>Fat (gm) 55</td>
<td>30</td>
</tr>
<tr>
<td>Protein (gm) 133*</td>
<td>200</td>
</tr>
<tr>
<td>Calcium (gm) 5.6</td>
<td>4.3</td>
</tr>
<tr>
<td>Iron (mg) 38.9</td>
<td>97</td>
</tr>
<tr>
<td>Zink (mg) 13.3</td>
<td>42</td>
</tr>
<tr>
<td>Vitamin A (mg) 0.67</td>
<td>30</td>
</tr>
<tr>
<td>Vitamin D (mg) 28.0</td>
<td>100</td>
</tr>
<tr>
<td>Vitamin E (mg) 39.0</td>
<td>100</td>
</tr>
<tr>
<td>Vitamin B1 (mg) 1.39</td>
<td>8</td>
</tr>
<tr>
<td>Vitamin B6 (mg) 2.8</td>
<td>6</td>
</tr>
<tr>
<td>Niacin (mg) 16.7</td>
<td>20</td>
</tr>
<tr>
<td>Vitamin B12 (mg) 7.8</td>
<td>3</td>
</tr>
<tr>
<td>Ca pantothenate (mg) 8.9</td>
<td>30</td>
</tr>
<tr>
<td>Choline (gm) 0.8</td>
<td>3</td>
</tr>
</tbody>
</table>

* Net protein

Caries experiments were conducted in an automated feeding machine where the amounts and frequency of food served and eaten could be controlled (101). The rats were placed in the individual cages within the machine immediately after weaning and inoculated with a heavy suspension of a streptomycin resistant *S. sobrinus* (*S. mutans*) 6715-15 serotype d strain in Study III (106) or an *S. cricetus* (*S. mutans*) E49 serotype a strain, in Study IV (107). Both these strains are cariogenic in rats. Care was taken to adjust the amounts of food served in order to ensure that the rats finished the meals. The number of meals was recorded. A meal was considered to be eaten if an estimated half was consumed. After decapitation at the end of the experiments (III and IV) dental plaque was scored (I) with a fluorescence microscopy method (108). The heads were gently autoclaved for 10 minutes and cleansed of soft tissues. Caries was scored (109) under a microscope (Nikon SMZGl). Smooth surface caries was registered. The jaws were stained with Murexin® and then separated and sulcal caries registered.
Saliva samples from the rats were collected between 9.00 a.m. and 11.00 a.m. Secretion was stimulated by subcutaneous injections of pilocarpine singly (IV) or in combination with isoproterenol(III, V). Fresh solutions of the agonists were made every hour. Contamination from nasal secretion and tears was carefully avoided by keeping the rats in a restrained position on a table that declined forward 10°. During saliva collection, the rats were anaesthetized with chloral hydrate 400 mg/kg b.w.(III) or pentobarbiturate 30-35 mg/kg b.w. (IV, V). Saliva was collected for 15 minutes starting when the first sign of secretion was noted. The collection was carefully supervised. All saliva samples were kept chilled with ice and covered with plastic film until used.

SALIVARY ANALYSES

Human saliva stimulated by chewing was diluted 1:1 with distilled water and centrifuged in a Beckman L5-65 ultracentrifuge (Beckman Instruments, Spinco Div., CA) at 20 000g for 30 (I) or 20 minutes (II) at 4°C (110). The volumes needed for each different analysis were pipetted into different test tubes before storing at -80°C.

Buffer capacity and pH were determined with a Beckman digital pH meter (Beckman Instrumentes, Fullerton, CA). Buffer capacity was determined according to Ericsson (111) by measuring the final pH after addition of 0.005M HCl.

Total protein was either determined according to Lowry (112) with tyrosine as a standard (I) or with a Commassie brilliant blue method (113) with bovine serum albumin as a standard (II, III, IV, and V). The ratio of Lowry protein value to Commassie brilliant blue value is approximately 2:1.

The electrolytes sodium, potassium and calcium were measured by atomic absorption spectroscopy (Varian Techtron AA6, Varian Associates, CA) with addition of caesium chloride to sodium and potassium samples and lanthanum chloride to calcium samples.

Phosphate was estimated spectrophotometrically (114). KHPO₄ was used as a standard.

Amylase was determined by the Phadebase kit method (Pharmacia, Sweden). The reaction was stopped by addition of 0.5M NaOH and the absorbance read at 620 nm.
Hexoses were measured according to the anthrone method as described by Jermyn (115). Glucose was used as a standard.

Fucose has been determined according to Gibbons (116) using L-fucose as a standard.

Sialic acid was analyzed using Warren's method (117). N-acetyleneuraminic acid was used as a standard.

Hexosamines were determined using a modified (118) Elson-Morgan method. Saliva was hydrolysed in 6M HCl for 3 hours at 100°C. The hydrolysate was lyophylized before addition of acetylacetone and Ehrlich reagent. Glucosamine-HCl was used as a standard.

Lysozyme in human saliva was measured by lysis of Micrococcus Lyso-deikticus using a Lysozyme Test Kit, (Kallestad, Chaska, USA). Lysozyme in rat saliva was determined in gel plates prepared in the laboratory as described by Osserman and Lawlor (119). The pH was adjusted to 5.5 which was found to be optimal pH for rat salivary lysozyme. Human lysozyme (Kallestad, Chaska, MN) was used as a standard.

Salivary peroxidase (SP) activity was measured by following the oxidation of 0.5 M pyrogallol by 1M H₂O₂ and saliva, in a spectrophotometer (120). The enzyme activity was expressed as A₄₀₀/min/ml.

Thiocyanate (SCN⁻) was measured spectrophotometrically by estimation of the iron salt formed with FeCl₃ (121), using a potassium cyanide solution as a standard.

Immunoglobulins in human saliva were measured using a radial diffusion method (122) with anti-human α-chain sera (Berinwerke, West Germany) and human serum IgA as a standard (Beringwerk, West Germany).

In rats (111) a solid-phase radioimmunoassay method with rabbit anti-rat α- and γ-chain sera was used to measure sIgA and IgG, respectively. Rat sIgA and rat IgG were used as a standard. The antisera and standard for rat IgA and IgG were prepared by Dr. M. Cole, NIH, Bethesda.

BAGP activity. Agglutination of a serotype c, S. mutans strain was measured as changes in turbidity of a suspension and followed in a spectrophotometer (123).

BAGP synthesis was estimated as the content of radiolabelled glucosamine incorporated (124) during two hours into glycoproteins adsorbable (125) to a serotype c strain of S. mutans.
RESULTS


Fasting on a liquid low calory diet was chosen as a model for starvation. Eight healthy females of normal weight fasted for eight days. The saliva secretion rate of whole stimulated saliva decreased in all participants. The means of the concentrations of phosphate, sialic acid and thiocyanate and the activity of lysozyme decreased significantly. The effect of fasting varied greatly among the participants but often there was an individual pattern. The total output in whole stimulated saliva reached significantly decreased levels for potassium, calcium, phosphate, hexoses, fucose, hexosamines and sialic acid. Since the secretion rate of resting saliva was not measured the total output per minute could not be calculated for substances analyzed in resting saliva.

The ratio of sialic acid to protein was decreased in stimulated saliva, indicating that the conjugation of the terminal carbohydrate was disturbed.

The participants developed an increased plaque formation rate. This might reflect the changes in saliva secretion rate and composition. There were no statistically significant changes in the CFU of total streptococci, of S. mutans or lactobacilli found in the saliva.

CONCLUSION. Short-term starvation or lack of chewing in adult humans affect the secretion rate and composition of saliva.

II. Effect of chewing on the secretion of salivary components during fasting.

Chewing was not checked in Study I and the lack of chewing might have contributed to the decrease in the secretion rate found. The effect of chewing in combination with a similar liquid low calory diet (I) on the secretion rate and composition of saliva was studied. A controlled, intense chewing program of five 10-minute chewing periods daily did not restore the secretion rate or the concentrations of calcium and thiocyanate or the activities of lysozyme and salivary peroxidase found during fasting. Chewing restored the changes found in concentrations of sodium, phosphate, fucose, hexosamine and partly the ratio of sialic acid to protein.

CONCLUSION. The reduced function of the salivary glands induced by short-term starvation on a liquid diet is not restored by chewing.

III. The effect of malnutrition on caries development and saliva composition in the rat.

Caries development and saliva composition was studied in grossly malnourished, weaned rats subjected to a standardized cariogenic challenge. The control rats were fed a diet containing double the amount of nutrients. The control rats did not show an optimal weight gain. Total salivary protein was reduced only in the grossly malnourished group supplemented with starch. The activity of salivary peroxidase was reduced in the grossly malnourished rats compared to
the better nourished rats. The choice of carbohydrate supplementation had an additive effect in combination with the effect of malnutrition on salivary peroxidase activity. Higher s-IgA values were found in saliva from the grossly malnourished rats given sucrose. No difference was found in the activity of lysozyme between the groups. The extent and depth of the caries lesions were five times higher for the grossly malnourished rats.

CONCLUSION. The cariogenic challenge of sucrose was potentiated by gross malnutrition introduced at weaning. Saliva composition was affected.

IV. Saliva composition and caries development during protein deficiency and β-receptor stimulation or inhibition.

In an experimental rat model the hypothesis was tested that a low protein diet (5%) would reduce the synthesis and release of saliva and salivary proteins and that such changes would increase caries development. The effect of an additional use of a β-receptor agonist or antagonist was also tested.

By the end of the experiment the rats receiving the protein-deficient diet had developed more dental caries and had higher numbers of inoculated S. cricetus (S. mutans). The secretion rate of pilocarpine-stimulated saliva was lower and the ratio of protein to amylase was reduced. The output of total protein was reduced.

A β-receptor agonist given to well-fed rats caused a reduction of protein concentration and amylase activity as well as a significantly increased caries development. No synergistic or antagonistic effect was seen between the agonist and protein deficiency. The β-receptor antagonist did not affect saliva composition or caries development.

CONCLUSION. A protein-deficient diet reduces the secretion rate of saliva and increases the number of cariogenic microorganisms as well as caries development. Treatment with a β-agonist causes the same effect in the well nourished rats.

V. Biosynthesis of a salivary bacteria agglutinating glycoprotein in the rat during protein deficiency.

In two independent series using different techniques we observed that rats fed a low protein diet (5%) showed a decreased activity of a salivary glycoprotein agglutinating a serotype c strain of S. mutans. In one series the agglutination of a saliva-bacteria mixture was measured as the decrease in optical density of a bacterial suspension. Agglutination induced by saliva from the rats fed the protein-deficient diet was reduced. In another series, the granulaes of the acinus cells were emptied by stimulation with pilocarpine one hour prior to administration of 10 μCi glucosamine (UL). Two hours later saliva was collected and adsorbed to S. mutans serotype c in a way that favours BAGP adsorption. PEM did not affect the total amount of glycoproteins secreted, measured as cpm, in saliva but the amount of radiolabelled glucosamine built into glycoproteins (BAGP) that could be adsorbed to the microorganisms was lower in saliva samples from the protein deficient rats.

CONCLUSION. Using an isotope technique we observed that the biosynthesis of a bacteria-agglutinating glycoprotein was reduced after induction of PEM.
GENERAL DISCUSSION

The present studies show that gross starvation and protein deficiency affect the secretion rate and composition of saliva especially when the effect is evaluated on the level of individual proteins. Such effects might explain the increase in the susceptibility to cariogenic microorganisms and dental caries.

Populations suffering from starvation or protein deficiency are found in developing countries (5) or among specific groups that risk malnutrition, such as the elderly (11) or the sick (12, 13). The discrete effects of malnutrition on oral health are difficult to estimate since starvation in humans is often combined with sickness and medication. This is an obvious problem in epidemiological studies. It would be difficult to interpret nutritional effects in a multivariate material when a multifactorial disease such as caries is studied. Therefore experimental conditions must be created minimizing the number of background variables. The effects of short-term starvation can be studied in human populations. I have chosen a group of healthy individuals of normal weight for these studies.

Some experimental conditions can only be created in animals. An animal system also offers an alternative model for studies of the effects of specific diets on health. Basic knowledge about dietary effects on salivary secretion and oral health is limited. It therefore seems advisable to study first the effects of gross dietary changes. The clear effects of gross undernutrition on the systems studied indicate the necessity for further studies of more marginal conditions of malnutrition, such as a moderate restriction or specific deficiencies.

The subjects who fasted followed a liquid low-calory diet based on fruit and vegetable juices providing 300 kcal per day. Under these conditions keton bodies are produced (16-18) which reduce the feeling of hunger and keep the number of drop-outs to a minimum.

The general reaction to starvation varied amongst the participants. One woman preferred not to continue fasting because of a temporary mild depression induced by the starvation. The participants as a whole, however, were very loyal to the experiment in spite of some
initial discomfort. During the second or third day of fasting some participants felt sick and occasionally vomited. This discomfort passed quickly and the water losses due to vomiting was negligible. Their experiences were discussed during daily meetings at which also the juices for the next day were given out. An objective measure of the compliance (cf. 19) was an average loss in body weight of 4.1 kg per person and an increase in serum values of uric acid from 297 μM to 464 μM (1).

A sudden and short period of starvation in healthy individuals is relatively unpleasant and might be expected to cause stress. Some stress situations cause an increase in serum catecholamine titers (17). One such example is when an increased requirement for energy and protein is not met after a trauma or during an infection (17). However, the titers of serum catecholamines and the turn-over of norepinephrine at nerve terminals are reported to decrease during experimental starvation (17, 20). The reduction in adrenergic transmitter substances during experimental fasting could contribute to the reduction in saliva secretion. Sympathetic stimulation has several direct or indirect effects on the metabolic processes, on the capillary flow and on the neurological control of α- and β-receptors in secretory (52) and myoepithelial cells (126). The interaction between the various types of receptors and transmitter substances is very complicated and far from completely understood. This is exemplified by the fact that circulating catecholamines also regulate the response to parasympathetic nerve stimulation in the rat (127).

It is well established that the lowered basal metabolism seen in hypothyroidism is accompanied by a decrease in the secretion rate of saliva and an increase in the incidence of dental caries (128). A lowered basal metabolism is also present during starvation not combined with trauma or infection.

A reduction of body water occurs during fasting (30, 129) through release of extracellular water and water from catabolized tissue and glucogen. Loss of body water leads to a decrease in the saliva secretion rate. An intake of 3 liters of water per day as in the present study cannot compensate for the loss of body water. The loss might have contributed to the lowered secretion rate found in starving humans (1, 11).
The females who took part in the present study (I) complained about having a dry mouth and some of them felt that their eyes were dry as well. In a similar study by Birkhed et al. (130) some patients complained about a dry mouth but no reduction in the resting saliva secretion rate was found during fasting. A majority of the test persons in the latter study had diseases known to affect saliva secretion although the median value for the secretion rates of resting saliva were remarkably high.

The reduction in the saliva secretion rate was found in all participants during starvation but saliva composition varied amongst individuals. The secretion rate and composition of saliva is normally enhanced by chewing (56). In rats fed a liquid diet the secretory cells are already affected within 24 hours (131) and the fast diet (I) which did not require chewing led to a decreased secretion rate and changes in the composition of the saliva. In a separate study a control group was given a fast diet and an experimental group used the liquid fast diet, combined with a chewing program (II) designed to provide the daily stimulation normally obtained in connection with meals. Study II cannot be directly compared to Study I since the age distribution, health condition and dental status differed between the studies. The effects of chewing in Study II partly restored the effects induced by the liquid diet alone. The changes in saliva secretion rate, the concentrations of calcium, thiocyanate and sialic acid and the activities of lysozyme and salivary peroxidase were still present but chewing normalized the values for sodium, phosphate, fucose, hexosamine and protein. This implies that chewing activates signal systems which influence the secretion and biosynthesis of salivary components but the changes observed during starvation are not exclusively induced by lack of chewing.

No statistically significant effect was found on the mean of total protein concentrations in humans but in Study I fasting reduced the ratio of sialic acid to protein. The lowered degree of sialylation is probably not a general phenomenon. In an earlier study it was observed (132) that removal of sialic acid from BAGP eliminates its activity. In the present study (I) it was observed that short-term starvation did not affect BAGP activity. It can be assumed that sialylation of BAGP was not reduced during the experimental period in Study I.
Starvation for eight days in humans did not affect the activity of BAGP whereas a longer period (18 days) on a protein-deficient diet reduced both synthesis and activity of BAGP in the rat (V). A pilot study (133) in the rat showed that the reduction was not present after 12 days on the 5% protein-deficient diet. This parallels the finding that SP was not affected during short-term starvation in Study I but a reduction was seen in the rat after a prolonged period of gross malnutrition. The activity of lysozyme was decreased during fasting in adult humans (I, II). This resembles the reduction found in tears from severely protein malnourished Colombian children (39), but not our findings in the rat. The increased activity of salivary peroxidase in Study II could not be explained by the decreased concentration of thiocyanate since thiocyanate present in saliva does not act as a competitor to the reagent we used in the SP assay system (Mansson-Rahemtulla, personal communication, cf. 134).

An increased plaque-formation rate was found in the participants at the end of the fasting period in Study I. The technique used to evaluate plaque formation was designed to measure the early stages of plaque which reflect bacterial adhesion more than conditions for bacterial growth. The amount of plaque was not considered, only the earliest macroscopic sign of bacterial colonization. The higher rate of plaque formation could be an effect of altered biological conditions following a decrease in the saliva secretion rate and a reduction in the secretion of proteins with antibacterial activity. Since the rate of formation was successively increased during the experimental period it is likely that the conditions for plaque formation were related to changes that occurred during the period. The presence of lectins in the diet (135) was identical during the two 16-hour periods when the plaque-formation rate was evaluated. The same vegetable broth and herbal tea were consumed but no juices. It is likely, however that the lectins in the diet (136) in combination with the high content of glucose and fructose (137, 138) gives the type of established plaque that people who fast describe as rough and which is thin and covers the teeth entirely. In the human study, therefore, the type of plaque, but not the rate of formation, can largely be explained by the special diet. In the animal studies (III, IV) where the diet, which comes in contact with the teeth, contains no lectins, we
recorded a changed microbial flora and saliva secretion and an increased caries activity. The changed flora and the increased susceptibility to dental caries induced by malnutrition seem to reflect salivary changes.

Ethical limitations make long-term experimental studies of malnutrition and the effect of malnutrition on caries development in humans impossible. Therefore rats were used for such studies (III, IV, V). In Study IV protein deficiency was combined with long-term treatment with a β-adrenergic receptor antagonist or a β-adrenergic receptor agonist. Among the elderly where malnutrition is often found the intake of β-blocking drugs is not uncommon. The β-ffectors did not have a synergistic effect with starvation but the β-agonist reduced the protein and amylase concentrations.

Several important factors must be taken into considerations when nutritional effects on animals are studied. Basically the intake of nutrients can be controlled and reproducibly varied only in animals. Ad libitum feeding is not generally suitable. Rats increase their consumption of a deficient diet to compensate for low energy content (139) but not for low protein content (105). The use of automated feeding machines or intubation of the diet allow intakes to be well controlled. In the present studies (III, IV) both techniques are used. There are disadvantages with both techniques. In the feeding machine the rats are kept in individual cages and the area is restrained. Furthermore, the controls also must be kept on a minimal diet to make them eat all meals offered. The control animals gain about 90% of the weight gain that ad libitum rats show on the same diet. The advantage of the feeding machine is that the amounts, frequency and sequence of intakes can be strictly controlled. The intubation method was chosen in Study III for two reasons both related to the fact that observations of caries activities were to be made. Firstly, contact between the teeth and the basic diet and, secondly, variations in stimulation caused by chewing, were avoided. Intubation seems to be accepted by the rats and the amounts deposited in the ventricles can be exactly estimated. The technique has some limitations. The diet must be given as a liquid and the volume of each meal often appears to be large due to difficulties in administering the required nutrients in denser solutions. It is not in tune with the rats physiology to
receive three large liquid meals a day. During the first few days they had diarrhea but the feces rapidly became solid again. The absorption of nutrients therefore appeared to be normal but all animals gained very little in weight. The intubation method garanties that no caries inhibiting components in the diet come in contact with the teeth. When the intention was to study the effect of the basic diet on the cariogenicity of carbohydrates the basic diet was supplemented with cariogenic carbohydrates administered at regular intervals in the feeding machine.

It is important to note that in the present studies in the rat no deficiency was induced in utero. The deficient diet was introduced at weaning, at the earliest on day 19 after birth (IV). In Study III it was started at day 21 and in Study V on day 46. The salivary glands are in an active period of development up to day 30-40 (140) and were thus not fully developed when the deficient diets were introduced in Studies III and IV. Malnutrition induced at weaning delays the development of the salivary glands in the experimental groups compared to the control groups, resulting in smaller parotid and submandibular glands (141). The delayed development might have contributed to the reduced saliva secretion rate in the PEM rats (IV). However, the reduction of the saliva secretion rate was of the same order of magnitude when the protein-deficient diet was given from an age of 46 days (Table 2). The object of these studies was to evaluate the effect of malnutrition on developed salivary gland tissue. The introduction of the deficient diet was made somewhat early in our studies but a compromise had to be made since studies on caries development must be started immediately after weaning.

Saliva in humans can easily be collected without pharmacological stimulation. Rats must be anaesthetized before saliva collection and the secretion must be stimulated by drugs or electrostimulation. In Studies III and V we chose to use both adrenergic stimulation by isoproterenol and cholinergic stimulation by pilocarpine. The use of an acute dose of isoproterenol results in higher amounts of protein secretion but the proportions of the proteins are reported to be the same (142). In Study IV, the reason for stimulating with pilocarpine alone was to avoid stimulation with a ß-receptor affecting drug when
the rats had been chronically treated with a β-receptor antagonist or agonist.

The secretion rate induced by pilocarpine stimulation is reduced by 50% when a long-term protein deficiency is induced at weaning (Table 2). A similar effect on secretion is observed when protein deficiency is introduced in 46-day-old rats. It is interesting to note that a combined stimulation with pilocarpine and isoproterenol results in significantly lower secretion rates regardless of protein deficiency. The effect of protein deficiency on salivary composition cannot be directly measured because of the techniques we have to apply to stimulate secretion. For this reason secretion was stimulated by both pilocarpine and pilocarpine-isoproterenol in combination. During a period of protein deficiency the mutual relationship between the cholinergic and adrenergic receptors seems to be affected.

Table 2  Saliva secretion rate in μl/min after stimulation with pilocarpine (P) alone or in combination with isoproterenol (I).

<table>
<thead>
<tr>
<th>Study</th>
<th>PEM introduced at</th>
<th>Deficiency period</th>
<th>Stimulation type</th>
<th>5 % diet</th>
<th>20 % diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>19 days</td>
<td>9 weeks</td>
<td>P</td>
<td>31 13</td>
<td>61 11</td>
</tr>
<tr>
<td>pilot</td>
<td>46 days</td>
<td>18 days</td>
<td>P</td>
<td>94 16</td>
<td>155 43</td>
</tr>
<tr>
<td>V</td>
<td>46 days</td>
<td>18 days</td>
<td>P+I</td>
<td>41 12</td>
<td>39 15</td>
</tr>
</tbody>
</table>

Amylase is often used as a marker of protein secretion in in vitro and in vivo studies of secretory mechanisms in exocrine cells even though the question of whether amylase is representative of other specific proteins in secretions is not settled. Many studies in which secretory mechanisms are investigated have been carried out in isolated cell systems. The involvement of the complex signal system present in in vivo studies complicates the picture. The effect reported in the PEM literature on salivary amylase activities seems somewhat conflicting.
Very few studies in humans are reported (39, 68). In PEM Colombian children reduced activities are reported in whole saliva (39, 68). This was not seen in adult humans after short-term starvation (I, II). Long-term protein deficiency in the monkey resulted in lower amylase activities in parotid saliva (143). In rats born of dams fed a protein-deficient and a high-starch diet and after weaning offered the same diet as the dams the reduction of amylase found at the age of 66 days had turned into an increase on the next sampling occasion at 110 days of age (28). In the present study (IV) an increase was found at the age of 111 days, when the rats had been fed a low protein high sucrose and cellulose diet since weaning. The results do not support the hypothesis that the high starch diet would induce an increased amylase production (28) but rather implies that more complex mechanisms are involved. Amylase is the quantitatively dominating protein in saliva. An increase in amylase content but an unchanged concentration of total protein support the finding that other individual proteins are reduced. Such proteins are e.g. the proteins with antibacterial properties.

The cariogenic potential of sucrose was increased during a period of gross starvation and protein deficiency. The frequency of the intake of fermentable carbohydrates is important in the development of caries (101, 102). This factor was largely controlled by putting the rats in a programmed, automated feeding machine. Still, the rats had the choice of eating a meal or not and in Studies III and IV there were slight variations in the number of meals eaten. In Study III some animals in the control group ate fewer meals. However, no animal in the control group reached the level of caries activity of the test animals regardless of the number of meals eaten. The rats in the experimental and control sucrose groups fell into two completely separate populations. In Study III gross malnutrition increases the cariogenicity from sucrose. In Study IV the rats fed the protein-deficient diet ate fewer meals but they developed more caries.

The studies presented here were performed in order to obtain information about the general effect of malnutrition on some basic salivary factors and caries development. It appears that it is not only the sucrose content of a diet that is of importance. It seems to be
clearly shown that short-term starvation in man induces impaired saliva secretion and that gross starvation and protein deficiency in the rat lead to changes which increase the cariogenic potential of sucrose. The anamnestic registration of sucrose intake is therefore not sufficient. A dietary registration and evaluation must be included in the collection of anamnestic information about the patient.
SUMMARY AND CONCLUSION

The nutritional status of man and the rat is important for the secretion rate and composition of saliva. Short-term starvation, on a liquid diet in healthy people of normal weight, reduced the saliva secretion rate and the concentrations of phosphate, sialic acid and thiocyanate and the activity of lysozyme as well as the amount of sialic acid conjugated to protein. Total protein concentration was not affected. The formation rate of dental plaque was increased.

Chewing during short-term starvation did not reverse the changes in the saliva secretion rate, or restore the concentrations of calcium and thiocyanate, or the activities of lysozyme and salivary peroxidase. The increased secretion of sodium and fucose, the reduction of phosphate concentration and partly the lowered glycosylation of sialic acids to protein were remedied by chewing.

The findings in humans were supported by results in rats. Thus, a moderately protein-deficient diet (5% protein) caused a reduced secretion rate of pilocarpine-stimulated saliva and an impaired synthesis and activity of a bacteria agglutinating glycoprotein. Total protein concentration was only slightly reduced in adult protein-deficient rats. The susceptibility to caries was significantly increased. Long-term gross malnutrition caused reduced activity of salivary peroxidase and a strong increase in caries activity.

Since both gross malnutrition and a moderate protein deficiency potentiated the cariogenic effect of sucrose, further studies of the effect of malnutrition on saliva secretion and composition are needed. The role of diet in caries development seems not to be limited to the effect of sucrose and fermentable carbohydrates, but to include the nutritional value as well.
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REFERENCES


