AN INVESTIGATION OF THE LEVELS OF SALIVARY ALPHA AMYLASE ACTIVITY IN INFANTS FROM BIRTH TO FIVE MONTHS OF AGE AND ITS RELATIONSHIP TO INFANT FEEDING

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CHRISTINE L.A. HOLODINSKY

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ABSTRACT

The purpose of this study was to investigate the levels of salivary α-amylase in infants from birth to 5 months of age and their relationships to the introduction of starch containing foods, age and weight.

Thirty infants born at the Health Sciences Centre, Winnipeg Manitoba, between July 15 and August 15, 1980 were subjects. Saliva was collected at birth and at monthly intervals for 5 months. At each collection period diet questionnaires were administered and height and weight measurements were taken. Saliva was extracted and analyzed for α-amylase and protein. A wide range of salivary α-amylase levels was observed at birth (9.9±9.2 units/ml). By 4-6 months of age the rate of increase was not as great as from birth to three months of age. There was a rapid increase in activity but from 4-6 months the activity increased more slowly and averaged 50 units/ml. Amylase activity was not related to the introduction of starch-containing foods into the diet. The absolute increase in weight was not related to the absolute increase in salivary α-amylase levels from birth to 5 months of age. Salivary α-amylase levels and the independent variables of age and weight were significantly related when linear regression analysis was performed on the total collections from birth to 5 months. Part of the variation of salivary α-amylase levels could be explained by the factors of age and weight over time.

Among the infants fed starch, those infants experiencing low levels of salivary α-amylase and low growth rates were not significantly different from the infants who had normal amylase levels and normal

growth rates. No infants were apparently affected by being fed starch too early which would be indicated by low growth rates.

Further research is needed to determine the levels of salivary α-amylase that will allow adequate hydrolization of starch and the conditions that lead to iatrogenic diarrhea.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDICIES

DEDICATION:

TO MY MOTHER AND FATHER

1. INTRODUCTION

During the last 5 decades, technological and scientific advances in the Western World, coupled with social changes have profoundly affected infant feeding practices (Health and Welfare, 1980). Currently, the type of solid introduced into the infant diet and the time of the initial introduction are varied (Clark, 1979). Over the past 50 years the advice given to parents and the reasons for the advice concerned with the introduction of solids has followed a cyclical pattern (Table 1).

The time of introduction of solid foods into the infant diet can influence the nutritional status of the infant. The premature introduction of solid foods has been related to food allergies, obesity and a potential for dietary nutrient imbalance (American Academy of Pediatrics, 1980). The Canadian Paediatric Society (1979) recommends that the age of introduction of solid foods should be determined by the physiologic maturity of the nervous system, intestinal tract and kidneys. This same society states that no nutritional advantages are achieved by supplementing the infant diet with solid food before 4 to 6 months of age. The American Pediatric Society (1980) has also issued a similar statement.

Despite the advice given by professionals, a considerable number of Canadians feed their infants solid foods before three months of age (Table 2). Generally, the first solid food introduced to the infant diet is cereal (Nutrition Canada, 1970). Since starch is the major constituent of cereal, development of the infant's physiological ability to digest starch must be a critical factor in determining the optimum time

TABLE 1. Advice given to parents regarding the introduction of solid food into the infant diet.

Year	Author	Advice	
		Age for Introduction of Solids	Reasons Given for Delaying Introduction of Solids
1936	Guy	18 mos	breast milk should be sufficient
1938	Platt & Gin	6 - 8 mos	infant has no salivary enzymes
1948	Spock	3 - 6 mos	at less than 3 months solids would cause digestive problems
1963	Spock and Lowenberg	$1\frac{1}{2}$ - 2 mos	no reason given
1977	Ontario Public Health Nutritionists	3 - 4 mos	physiologically unready before 3 months
1979	Health and Welfare Canada	3 - 4 mos	no ability to transfer food to the back of the mouth before 3-4 months
1979	Manitoba Home Economics Directorate	4 -6 mos	no nutritional benefits result from earlier solids intake

TABLE 2. Percent Canadian infants less than 3 months of age receiving solid foods.

Source	Sample Size	% receiving solids < 3 mos of age
Nutrition Canada, 1970	35	88%
Clark, L. 1979	423	73%
Fabro, J.[1] 1979	49	67%
Hogue, L. and McDaniel, M. 1980	48	63%

1. Infants were between 2 - 4 months of age.

for the introduction of cereals.

The objective of this study was to investigate the levels of salivary α-amylase, the digestive enzyme which hydrolyzes starch in the infant, and relate these levels to the age, weight and diet of the infant. Insight into the development of salivary α-amylase in infants will aid health professionals to an understanding of the degree of physiological maturity required for the digestion of starch-containing foods. The extent of development of salivary α-amylase may have direct implications for the advice on weaning practices given to the public by health professionals.

2. REVIEW OF LITERATURE

STARCH DIGESTION IN THE INFANT

Starch is a polysaccharide composed of a linear fraction, amylose, and a branched fraction, amylopectin. Amylose consists of a straight chain of glucose molecules with the fourth carbon of one molecule attached to the first carbon of the next molecule by an oxygen bridge. This is referred to as ar. α-1,4 glucosidic bond. Amylopectin is similar in structure to amylose, containing chains of glucose units with an α-1,4 glucosidic bond linked at the branching points by an α-1,6 glucosidic bond.

Alpha amylase (1,4-α-D-glucan glucanohydrolase, E C 3.2.1.1.) hydrolyzes the α-1,4 glucosidic bond of the starch molecule. The enzyme is activated by chloride ions. The enzyme action of α-amylase is confined to the α-1,4 glucosidic bonds which are either non-terminal or not in the vicinity of an α-1,6 glucosidic bond. The end products of amylose hydrolysis are maltose, glucose and maltotriose. The end products from amylopectin hydrolysis are the same as those from amylose hydrolysis together with branched oligosaccharides, alpha-limit dextrins.

Several factors can affect the enzymatic hydrolysis of starch. Some of the factors are cooking and the type of starch. Cooking disrupts the granular structure increasing the susceptibility of the starch to α-amylolysis. The type of starch also influences the

enzymatic hydrolysis. Cereal starches are more easily attacked than root starches.

Alpha amylase is secreted in the body by both the salivary and pancreatic glands. Salivary α-amylase has been reported to be low or negligible during the first few months of life (Rossiter et al., 1974). Pancreatic α-amylase has been reported to be low but not completely absent in children below six months of age (Hadorn et al., 1968).

The role of salivary α-amylase may be more significant in the infant than it is in the adult in starch digestion. In the infant, salivary α-amylase is thought to have more prolonged activity than is normally found in adults due to a higher gastric pH of the infant stomach (Younoszai, 1974). Salivary α-amylase is essentially non-active as an enzyme once the pH falls below 4 (Guyton, 1976). In a study by Mason (1962), the actual pH of the stomach contents of the infants were reported to be dependent on the food digested. The acidity was found to fluctuate from pH 3.5 at zero time increasing to pH 6.8 by 60 minutes after breast feeding and return to pH 3.5 after 210 minutes.

Borgstrom et al. (1960) studied intestinal α-amylase in premature and full term infants by collecting duodenal contents. Borgstrom concluded the amylase activity found in the intestinal content was derived from salivary amylase that had reached the intestine in the active form due to the abscence of free hydrochloric acid in the infant during digestion of a meal. Electrophoretic studies by Norman et al. (1972) of the duodenal juice of 3 to 15-day-old newborns after feeding indicated that the amylase was of salivary origin.

Hadorn et al. (1968) observed pancreatic amylase activity to be age dependent. In this research pancreatic amylase was found to be

lowered but present in all children under six months of age. Auricchio et al. (1967) attributed the incomplete digestion of amylopectin by most infants under six months of age to the low levels of α-amylase found in the duodenal juice after a meal.

SECRETION OF SALIVARY ALPHA AMYLASE

Salivary α-amylase is found in the secretions of the major salivary glands: the parotid, submandibular, sublingual and the minor mucous glands. These combined secretions form whole saliva. Alpha amylase accounts for approximately 30% of the total protein in whole saliva (Benedek-Spat, 1973).

Schneyer (1956) reported several relationships between the secretion of amylase from the three major salivary glands. Firstly, under conditions of reduced stimulation (resting), the concentration of amylase (mg/ml) was the greatest from the parotid gland with a mean of 1.03 mg/ml (Table 3). The mean resting concentration of amylase from the submandibular and sublingual glands was 0.25 and 0.26 mg/ml respectively (Table 3).

Secondly, the flow rate (mg/hr) from these three glands also varies under resting conditions. The parotid secretes 54% of the total amylase with a flow rate 5.4 mg/hr. The sublingual only contributes 2.4% with a minimal flow rate of 0.24 mg/hr (Table 4). The above relationships are altered when there was mild or substantial stimulation of saliva production. When the flow rate of saliva was increased

TABLE 3. Amylase concentrations of salivary secretions under resting conditions.

Secretion	Submandibular	Sublingual	Parotid	Total
	(mg amylase/ml)			
Mean	0.25	0.26	1.03	0.42
S.E.	0.06	0.08	0.11	0.06

Schneyer, 1956

TABLE 4. Rate of amylase secretion by resting salivary glands.

Secretion	Submandibular	Sublingual	Parotid	Total
	(mg amylase/hr)			
Mean	4.5	0.24	5.4	10.1
S.E.	1.5	0.06	1.5	2.4
	% Total amylase/hr			
Mean	44.6	2.4	53.5	100

Schneyer, 1956

slightly above the unstimulated rate, the protein content decreased, while at higher flow rates, the protein content increased (Dawes, 1978).

The concentration of amylase in the parotid gland decreased from 1.03 mg/ml to 0.95 mg/ml during stimulated conditions. The rate of transport of amylase from the parotid increased during stimulation from 5.4 mg/hr to 17.9 mg/hr (Table 5).

In the adult, the average flow rate of saliva was reported to be 0.3 ml/min (Becks and Wainwright, 1943). Flow rate increased with age throughout childhood until adolescence, then appeared to decrease (Buchnar, 1972; Waterhouse et al., 1973). Dawes (1978) reported the flow rate of unstimulated saliva to follow a circadian rhythm. During sleep virtually no saliva was produced in adults. The peak flow rate of 0.6 ml/min occurred at 6 p.m. (Dawes, 1978). This circadian rhythm does not develop in infants until a regular sleep-wake pattern develops (Dawes, personal communications).

THE INFLUENCE OF DIETARY CARBOHYDRATE ON ALPHA AMYLASE PRODUCTION

Few studies have been conducted regarding the influence of dietary carbohydrate on the production of salivary α-amylase. The effect of dietary starch on the rate of synthesis of salivary α-amylase is unclear. High carbohydrate diets were associated with increased amylolytic activity in some studies (Squires, 1953; Wesley-Hadzija and Pignon, 1972) but not in others (Behall et al., 1973). Squires (1953)

TABLE 5. Concentration and rate of transport of amylase in stimulated parotid gland secretions.

	Mg Amylase/ml (concentration)	Mg Amylase/hr (rate)
Mean	0.95 (1.03)	17.9 (5.4)
S.E.	0.15	3.5

Schneyer, 1956

reported on five Kalahari bushmen who were fed a diet much higher in carbohydrate than they consumed normally. He reported their salivary α-amylase activity to be 4 times that of bushmen when normally consumed the high meat diet in the habitat. However Squires (1953) does not state what type of saliva was examined or what time of day it was collected. Dawes (1970) questions the validity of these results since the analyses were done under field conditions which may have caused the temperature of the incubation mixtures to fluctuate. Also the method of determining the amylase concentration, the achromic end point method, is now considered unsatisfactory (Searcy et al., 1907). Wesley-Hadzija and Pignon (1972) studied 3 groups of young adults in Kumasi Ghana. The first group were Ghanaians consuming a high carbohydrate diet. The second group were Ghanaian students consuming an adequate protein diet. Group three was composed of Europeans consuming a well balanced diet. Amylase activity was expressed in units defined as mg of maltose released per unit of saliva. Group I with the predominately carbohydrate diet had a mean amylase activity level of 1869 while group II and group III had mean levels of 541 units and 431 units respectively. These results support an association between the concentration of amylase in man and the diet consumed. Behall et al. (1973) observed the effect of different carbohydrates fed in load doses on the amylase activity. Seven different carbohydrates were fed to seven women in the amounts of 60 g in 250 ml of distilled water. Only parotid saliva was collected. The load doses were fed after a 12 hour fast and 1 and 2 hours after each carbohydrate load. The amylase activity increased 1 and 2 hours after the carbohydrate load dose but the increase could not be attributed to

the carbohydrate load doses.

Hall and co-workers (1967) found that after a 7 day diet of metrecal[1], the total volume of the parotid secretions was decreased along with total protein and the rate of amylase secretions. However, Hall attributed his results to the decrease in mastication, as chewing stimulated the salivary glands (Winsor and Bayne, 1929). Dawes (1970) suggested that in the study by Hall, subjects experienced a reduction in flow rate thus causing a decrease in salivary amylase concentration. Recently, Lieberman and co-workers (1977), studied Samoan migrants who traditionally have a carbohydrate-rich diet. When compared to the other migrants in Hawaii consuming less starch, the Samoans had the same salivary α-amylase levels. These results of Lieberman were in agreement with Dawes (1970) who stated that there was little evidence to suggest that salivary α-amylase levels could be altered by changing the diet.

PRODUCTION OF SALIVARY AND PANCREATIC AMYLASE IN INFANTS.

Published research findings on the production of salivary α-amylase in infants under one year of age are scarce. MacKeith and Wood (1971) claim that at birth, there is minimal salivary α-amylase activity and suggested that newborns have "comparative" difficulty digesting starch. Collares and Brasil (1978) report the amount of

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1. Liquid diet containing 900 calories per 32 fl. oz., 11.6% carbohydrate (26.1 g). Supplied by Mead Johnson, Cransville, Ind.

saliva at birth to be small, reducing the amount of α-amylase present. Platt and Gin (1938) claim salivary α-amylase levels increased with age, with more saliva in "thriving" infants compared to "sickly" infants.

The secretion of pancreatic α-amylase has been reported to be a function of age (Hadorn et al., 1968). Low levels of pancreatic α-amylase in the duodenum of infants under 6 months of age, have been reported by Anderson et al. (1972) and Aurricho et al. (1967).

Apart from directly measuring the amount of enzyme present, other researchers have indirectly observed pancreatic α-amylase activity by studying carbohydrate digestion. The most accurate method for determining completeness of digestion is to measure blood glucose levels (Anderson et al., 1974). Measuring the starch content of the feces offers erroneous results due to the bacterial digestion of starch in the colon (Bond et al., 1972).

Aurrichio et al. (1967) investigated in vivo hydrolysis of amylopectin. They reported larger amounts of dextrins than normal in the duodenal contents of infants under 6 months of age. This would seem to indicate incomplete starch digestion due to low levels of α-amylase in the duodenal juice.

Husband et al. (1970) studied the gastric emptying of isocaloric starch and glucose test meals in newborn infants. The starch test meals emptied more rapidly than did the glucose meals. It has been shown in adults that starch and glucose test meals with the same caloric value leave the stomach at the same rate even though the osmotic pressure of the starch was zero and no starch was digested in the stomach (Hunt, 1960). The post-pyloric receptor responds to starch after it has been converted to maltose and then glucose by pancreatic

amylase, slowing gastric emptying. Rapid gastric emptying of starch relative to glucose as shown by this research suggests that there was slow hydrolysis of starch due to low secretion levels of pancreatic α-amylase.

With low levels of both pancreatic and salivary α-amylase being reported at birth, the premature introduction of starch-containing foods may result in iatrogenic diarrhea and failure to grow. Lillibridge and Townes (1975) reported a case study where an infant was fed cereal at the age of one week. Weight gain was unsatisfactory and the mother increased the amount of cereal. Copious and loose stools continued. The child failed to gain weight and was eventually admitted to the hospital. Laboratory tests at the age of four months revealed the absence of pancreatic α-amylase. Starch was eliminated from the diet and as a result the caloric content reduced. Growth improved markedly following this intervention even though the total caloric content of the diet was decreased (Table 6).

This example demonstrates the importance of the infants physiological ability to digest starch. This is a critical factor in determining the optimum time for the introduction of starch containing foods.

TABLE 6. Relationship of dietary intake to growth rate in a reported case study.

	Age (months) 0-1	1-2	2-4	4-11
Total Calories / Day	515	760	1735	1030
Calories / Kg / Actual weight	140	190	300	120
Calories Contributed By Starch (%)	7	9	16	0
Observed Growth Rate (grams/week)	140	140	145	310
Expected Growth Rate (grams/week)	250	170	125	60
			↑ time of intervention	

Lillibridge and Townes, 1975

3. PURPOSE

This study investigates the development of salivary α-amylase in infants and its relationship to the individual growth rates of infants and to the presence of starch-containing foods in the diets of infants.

4. HYPOTHESES

Ho_1: There will be no difference between the levels of salivary α-amylase in the infants fed starch-containing foods compared to the infants not fed starch-containing foods at each monthly collection of saliva.

Ho_2: There will be no association between the absolute increase in weight and the absolute increase in salivary α-amylase activity from birth to 5 months of age.

Ho_3: There will be no association between salivary α-amylase activity and the individual growth rates (g/day) of infants less than 5 months of age.

Ho_4: There will be no difference between the predicted and actual growth rates (g/day) of those infants fed starch-containing foods who have low levels of salivary α-amylase.

5. RESEARCH DESIGN

The sample investigated comprised two groups, which will be referred to as Group I and Group II.

Group I numbered nineteen subjects. Saliva was collected and analyzed from this group at ages three, four and five months plus or minus 7 days. Group II numbered ten subjects. Saliva was collected and analyzed from this group at ages birth, one, two, three, four and five months, plus or minus 7 days.

A standardized environment for the collection of saliva was developed to eliminate variations in saliva composition resulting from the time of collection or from stimulation.

Dawes (1978) reported the flow rate of unstimulated saliva to follow a circadian rhythm in adults. The peak flow rate occurred between 1 p.m. and 6 p.m. with the highest rate at 6 p.m. The infant does not develop a circadian rhythm until a regular sleep-wake pattern develops (Dawes, personal communication). To control for the various sleep-wake patterns of the infants, all collections of saliva were taken at the adult peak flow time of between 1 p.m. and 6 p.m.

Whole saliva is a mixture of the secretions from the three paired major salivary glands, the parotid, sublingual and submandibular plus a small contribution from numerous minor salivary glands. The flow rate of amylase (mg/hr) under "resting conditions" (unstimulated) is highest in the parotid saliva. However it would be misleading to measure the amylase content of the parotid gland only since the saliva in the mouth is a mixture of the secretions from the various glands. Therefore the

collection of saliva from each infant's oral cavity was a sample of whole saliva.

Under conditions of stimulation, chemical or mechanical, the flow rate of saliva is increased above normal. The protein content (mg/100 ml) of the gland secretions decreases initially when stimulated and then increases at higher flow rates. The concentration of amylase in the parotid saliva (the major contributor of amylase) decreases during stimulation. This would then cause a subsequent decrease in the α-amylase concentration.

Since many variables must be considered under stimulated conditions, all attempts were made to collect unstimulated saliva or saliva under "resting conditions". No chemical stimulants were used to induce the flow rate, nor was the oral cavity intentionally mechanically stimulated. Saliva was obtained by the use of a suction catheter. This device caused no or minimal mechanical stimulation to the oral cavity. Any mild forms of stimulation caused by this device were random throughout all infants at all collections.

Dawes (1978) reported that in adults the "most acceptable procedure" was to collect saliva 2 hours after feeding. For this research all saliva samples were taken $1\frac{1}{2}$ to 2 hours post feeding.

METHODS OF CHEMICAL ANALYSES

Saliva was analyzed for amylase activity and protein. By determining the protein content of saliva the amylase levels of saliva

could also be expressed in terms of specific activity that is as amylase activity per milligram of protein. This provided the opportunity to test for significant relationships using both the activity per unit volume or the activity per milligram of protein.

DIET RECORDS AND ANTHROPOMETRIC RECORDS

Starch is the main component of cereals, and cereals have been reported to be the major first solid food introduced to infants. Starch is also present in fruits and vegetables. Therefore all types of solid foods introduced to the infant diet were recorded to take into consideration the starch found in these latter two foods.

An overview of the data collection plan is given in Table 7.

TABLE 7. Data collection periods for Group I and Group II.

Period	Age of Infant	Group I (n=19)	Group II (n=11)
C_1	Birth-3 days		•
C_2	30 days±7		•
C_3	60 days±7		•
C_4	90 days±7	•	•
C_5	120 days±7	•	•
C_6	150 days±7	•	•

(• when collections occurred)

6. MATERIALS AND METHODS

SUBJECT SELECTION

All of the infants in the study were born at the Health Sciences Centre, Winnipeg, Manitoba. Initially 28 mothers agreed to have their infants as subjects for Group I and 12 others agreed to have their infants as subjects for Group II. At the end of the study 9 infants from Group I had dropped out and 1 infant from Group II dropped out, leaving a total sample size of 30 (19 + 11). An additional infant in Group II left the study for the last two collection periods. Reasons given for leaving the study were illness, moving out of the city, the mother being too busy and the mother returning to work.

All mothers of the infants were fully informed about the study and the procedure for saliva collection. Consent forms were signed in the hospital (Appendix 1) prior to the first sample collection.

All infants were "pre-screened" by the head nurse of the pediatric ward for eligibility. Only those infants without complications and considered healthy by the head nurse were asked to participate in the study. Infants living outside Winnipeg were not considered.

DATA COLLECTION PROCEDURE

Whole saliva samples were collected from the oral cavity of the infants at monthly intervals. The collections commenced at 3 months of age for Group I and at birth for Group II, and continued until both Groups reached the age of 5 months.

When saliva collections were made, anthropometric (height and weight) and diet information were also recorded (Appendix 2 and 3). Collection of saliva took place between the hours of one and six p.m. for all infants.

The sample collections were made at monthly intervals, plus or minus seven days. The analysis of data was done using the absolute age of the infant in days to allow for this seven day leeway. All samples of whole saliva were collected one and a half to two hours post feeding.

ANTHROPOMETRIC MEASUREMENTS

The height and weight of each infant at each monthly collection period was recorded (Appendix 2). Height was measured to the nearest 0.5 centimeter using an Infantometer.[2] The infant was placed in a supine position on a level surface and the Infantometer was then placed

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2. Grafco Infantometer, #2867-1334, Graham-Field Surgical Co. Inc. New Hyde Park, New York 11040.

parallel to the infant with the two end pieces perpendicular to the infant. The top end of the Infantometer contacted the top of the head and the bottom end contacted the heel of the foot. The measurement was then taken twice, and the mean value recorded.

Weight was measured to the nearest gram using a beam balance scale. The scale was zeroed with a cloth on the weighing surface for the comfort of the infant. Infants were measured nude or with a diaper on. The weight of the diaper was then taken and accounted for. All diapers were dry.

DIETARY RECORDS

At each monthly collection it was ascertained whether the infant was breast and/or bottle fed. The introduction of solid foods was documented along with the kind, amount and frequency of intake (Appendix 3).

METHOD OF SALIVA COLLECTION

Whole saliva was collected from the oral cavity by a suction catheter.[3] The trap attached to the catheter was not used to collect the

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3. Argle De Lee Suction Catheter. Size 10FR, Trap Size 10cc. Mfg. by Sherwood Medical Industries, St. Louis Missouri, 63103.

saliva. The saliva was drawn up the tube by suction. The saliva was forced out of the catheter into a plastic microcentrifuge tube and transported to the laboratory for analysis where it was refrigerated at 4°C. Samples were prepared for chemical analysis by centrifugation at 2000xg for an hour to separate the sediment from the supernatant. The supernatant was used for the chemical analysis. No measurement was made of saliva flow rate.

CHEMICAL ANALYSIS OF SALIVA: ALPHA AMYLASE

Alpha amylase activity of the saliva samples was assayed by the method of Bernfeld (1955) with adaptations by Strumeyer (1967). This method relies on the colour reaction between the reagent dinitrosalicylic acid and maltose liberated through the action of the enzyme. Saliva samples were diluted (1:250 to 1:500) with a phosphate buffer, pH 6.9 containing sodium chloride (0.06M NaCl). Five ml of the saliva solution was then added to an equal volume of the substrate solution. The substrate was a 1% starch solution prepared with the same phosphate buffer, pH 6.9 containing sodium chloride (0.06M NaCl).

Duplicate aliquots of the saliva sample were made of each saliva sample. One sample was incubated for 3 minutes and the other for 6 minutes in a water bath at 30°C. This was done to determine if the saliva dilution was appropriate. If the 6 minute sample released twice as much maltose as the three minute value then the enzyme activity curve would be linear. This confirmed that the saliva dilution was

appropriate. The reaction between the enzyme and the substrate was terminated by the addition of dinitrosalicylic acid. The mixture was then boiled for 5 minutes. Cooling water was added (10 mls) and the absorbance was read spectrophotometrically at 540 nm.[4]

A standard curve was made with maltose (0.1, 0.2, 0.5, 1.0 mg in 1 ml of water). The maltose values for each sample were read off the calibration curve and converted into Units of Activity.[5]

CHEMICAL ANALYSIS OF SALIVA: PROTEIN

Protein analysis was undertaken to calculate the specific activity, defined as α-amylase activity per milligram of protein. Protein content of the saliva was analyzed by the method of Lowry et al. (1951). This method is based on a colour reaction formed by (1) biuret reaction of protein with copper ion in alkali and (2) reduction of the phosphomolybdic-phosphotungstic reagent (Folin-Ciocalteau Reagent) by tyrosine and tryptophan present in the treated protein.

A standard curve was prepared with casein (32.0, 64.0, 96.0 mg in 1000 ml of water and 64.0, 96.0 128.0 mg in 500 ml of water). Sufficient amounts of 1N sodium hydroxide was added to solubilize the casein. For protein analysis of saliva samples, five µl of centrifuged saliva was added to tubes (10x75mm) with 200 µl of water and mixed. Then 1.0 ml of the alkaline copper solution was added by pipette and

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4. SP6-300 Pye Unicam Spectrophotometer.
5. One Unit of Activity = 1 mg maltose / minute at 30°C, pH 6.9.

mixed thoroughly. This solution was left for ten minutes and then 100 µl of the phenol reagent was added directly into the protein and alkaline copper solution, mixing immediately. After 30 minutes the optical density was read at 600 nm. Protein values were recorded as mg/100 ml (mg%).

7. RESULTS

A total of 29 infants participated in the study. Table 8 refers to the numbers of subjects in each group participating at each collection period.

In Group I, one subject did not have saliva collected at age 4 mos and in Group II there was a "drop out" at age 4 mos and 5 mos. The total number of saliva samples collected by the end of the study was 56 for Group I and 64 for Group II.

Tables 9 and 10 give the number of infants receiving each type of diet at each monthly saliva collection. Breast feeding was the main source of nourishment in both groups from the beginning of the study until the end, 5 months of age.

Starch-containing foods were fed as early as one month of age in Group II. Three infants (27%) consumed cereal at this age. By three months of age 4 infants (36%) consumed cereal and at 5 months of age 8 infants (72%) consumed cereal in Group II. In Group I there is no data for before 3 months of age. At three months of age 7 infants (36%) consumed cereal and at 5 months 14 infants (73%) consumed cereal in Group I. Comparing Group I to Group II these percentages are quite similar for 3 months and 5 months of age.

Growth rates were calculated by subtracting the weight of the previous month from the current month then dividing by the number of days between the two measurements, rendering a value reported in grams per day. The average growth rate and weight of the infants at each saliva collection period for both groups are listed in Table 11.

TABLE 8. Sample size by age for each group.

Age	Number of Samples Collected	
	Group I	Group II
Birth	-	11
1 mos	-	11
2 mos	-	11
3 mos	19	11
4 mos	18	10
5 mos	19	10

TABLE 9. Dietary pattern of infants at each monthly saliva collection - Group I.

Dietary Pattern	Age		
	3 Mos	4 Mos	5 Mos
Breast	12	11	10
Bottle	10	11	3
Cereal	7	8	14
Fruit	2	7	10
Vegetables	1	3	4
Meat	1	2	2
	n=19	n=18	n=19

TABLE 10. Dietary pattern of infants at each monthly saliva collections - Group II.

Dietary Pattern	Age					
	Birth	1 Mos	2 Mos	3Mos	4 Mos	5 Mos
Breast	10	10	8	8	8	7
Bottle	1	1	3	3	4	4
Cereal	0	3	3	4	5	8
Fruit	0	0	1	1	1	5
Vegetables	0	0	0	1	1	1
Meat	0	0	0	0	0	0

n=11

TABLE 11. Average[1] weight and growth rate at each collection period.

Collection Num / Age	Weight (g)		Growth (g/day)[2]	
	Group I	Group II	Group I	Group II
1. Birth		3627±567	-	-
2. 1 Mos	-	5221±832	-	33±13
3. 2 Mos	-	5754±818	-	30±15
4. 3 Mos	6108±1034	6299±882	21±11	25±8
5. 4 Mos	6974±1047	7102±1021	22±20	23±8
6. 5 Mos	7401±1257	7644±977	9±20	20±7

1. Averages are recorded with ± the standard deviation.
2. Growth rates are taken from the weight of the previous month subtracted from the weight of the current month divided by the number of days between the two measurements.

The mean values for the biochemical variables, amylase, protein and specific activity, are recorded in Table 12 and 13 for Groups I and II respectively.

At age 5 months the values for amylase and the specific activity are almost identical for both Groups. There is more of a difference between Group I and II for the amylase values of 3 and 4 months of age.

STATISTICAL ANALYSIS

The four hypotheses were tested using the computer based statistical package "SAS"[6]. All testing was done at the 5% level, $p \leq 0.05$.

Hypothesis #1

To test for difference between the mean amylase values of those infants fed starch-containing foods and those infants not fed starch-containing foods, a Students' t-test was performed on the mean amylase levels (Table 14 and 15). The same procedure was also done between the mean specific activity values of those infants fed starch-containing foods and those infants not fed starch-containing foods (Table 16 and 17).

In Group II the t-tests were not performed on the amylase or specific activity levels for the saliva samples at birth, since no infants were fed starch at this time. In some cases the number of infants (n)

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6. Statistical Analysis System, Raleigh, N.C., USA.

TABLE 12. Mean values[1] for biochemical variables at each collection period by age - Group I.

Age	Amylase Activity (Units/ml saliva)		Protein (mg/100 ml)		Specific Activity (amylase activity per mg protein)	
	n		n		n	
Three Mos	19	31.3±22.0	11	75.4±28.2	11	35.01±22.17
Four Mos	18	41.0±28.8	11	90.8±25.9	18	44.43±26.75
Five Mos	19	59.2±39.7	19	106.2±62.0	19	59.87±35.91

1. Means reported with standard deviations.

TABLE 13. Mean values[1] for biochemical variables at each collection period by age - Group II.

Age	Amylase Activity (Units/ml saliva)		Protein (mg/100 ml)		Specific Activity (amylase activity per mg protein)	
	n		n		n	
Birth	11	9.9±9.2	7	294.0±70.9	7	4.49±5.03
One Mos	11	28.3±27.3	9	240.4±98.9	9	14.3±11.1
Two Mos	11	48.7±46.4	10	143.3±76.6	10	34.46±26.0
Three Mos	11	57.2±40.7	10	140.2±45.5	10	43.0±26.6
Four Mos	10	52.6±37.4	10	112.5±34.9	10	50.1±40.8
Five Mos	10	58.1±28.3	10	138.5±58.0	10	51.1±29.8

1. Means reported with standard deviations.

TABLE 14. t-test results on mean amylase activity levels between those infants fed starch and not fed starch-containing foods for Group I.

	n	Mean Amylase Activity (Units/ml saliva)	t-value
3 Months			
Starch	7	28.0±22.7	0.47
No starch	12	33.1±22.4	
4 Months			
Starch	8	38.1±24.8	0.37
No starch	10	43.3±32.8	
5 Months			
Starch	15	53.4±28.4	0.75
No starch	4	80.7±70.2	

TABLE 15. t-test results on mean amylase activity levels between those infants fed starch and not fed starch-containing foods for Group II.

	n	Mean Amylase Activity (Units/ml saliva)	t-value
1 Month			
Starch	3	13.0±6.2	1.16
No starch	8	34.1±30.2	
2 Months			
Starch	3	23.0±6.4	1.80
No starch	8	58.3±51.7	
3 Months			
Starch	4	48.9±36.4	0.49
No starch	7	61.9±45.1	
4 Months			
Starch	5	43.5±19.4	0.19
No starch	5	35.2±15.7	
5 Months			
Starch	8	52.6±23.3	1.29
No starch	2	80.5±46.6	

TABLE 16. t-test results on mean amylase specific activity levels between those infants fed starch and not fed starch-containing foods for Group I.

	n	Mean Specific Activity (amylase activity per mg protein)	t-value
3 Months			
Starch	5	33.12±23.40	0.29
No starch	6	37.27±23.06	
4 Months			
Starch	10	36.63±14.79	1.20
No starch	8	50.65±32.92	
5 Months			
Starch	4	51.65±24.98	0.27
No starch	15	90.68±56.74	

TABLE 17. t-test results on mean amylase specific activity levels between those infants fed starch and not fed stach-containing foods for Group II.

	n	Mean Specific Activity (amylase activity per mg protein)	t-value
1 Month			
Starch	2	14.00±1.13	0.05
No starch	7	14.44±12.86	
2 Months			
Starch	2	23.50±4.94	0.64
No starch	8	37.20±28.77	
3 Months			
Starch	3	46.01±29.79	0.52
No starch	7	35.96±20.82	
4 Months			
Starch	5	58.04±49.65	0.59
No starch	5	42.26±33.68	
5 Months			
Starch	8	53.21±30.47	0.41
No starch	2	43.00±36.76	

on which the t-tests were performed may differ at the same collection period for the amylase activity and specific activity of the infants fed starch and not fed starch. This is due to the fact that in some cases there was not enough saliva after analyzing for amylase to perform protein analysis.

No significant differences in amylase measured as amylase activity or specific activity were found between those infants fed starch-containing foods and those infants not fed starch-containing foods. This result was true for each collection period for both Group I and Group II. Hence, the null hypothesis was not rejected.

Hypothesis #2

To test hypothesis #2, that there will be no association between the absolute increase in weight and the absolute increase in salivary α amylase activity from birth to 5 months of age linear regression analysis was performed. Amylase levels were reported as amylase activity and specific activity. Linear regression analysis could only be used on Group II. The data in Group I was collected starting at 3 months of age, not birth. Hence the absolute increase in weight and amylase levels could not be computed from birth. Assuming linearity, linear regression analysis measures the degree to which the dependent variable amylase activity, increases or decreases linearly as the independent variable, growth increases.

The results (Table 18) revealed no significant association between the absolute increase in weight and the absolute increase in amylase activity or the absolute increase in specific activity. There was not enough evidence to reject the null hypothesis that the absolute increase in weight is not related to the absolute increase in amylase activity.

TABLE 18. Linear regression analysis between the absolute increase in amylase activity and the absolute increase in weight from birth to 5 months of age in Group II.

	Dependent Variable			
	Amylase Activity (units/ml saliva)		Specific Activity (amylase activity per mg protein)	
	r^2	p	r^2	p
Weight(g)	0.08	0.41	0.00	0.99

Hypothesis #3

To test that there was no association between amylase levels and individual growth rates (g/day) coefficients of determination (r^2) and their respective significance values (p) were computed for both Group I and Group II. These values were computed for each collection period. In addition to growth rate (g/day) results are also given for weight (g). Tables 19 and 20 are a summary of the coefficients of determination for Group I and Group II respectively.

The coefficient of determination, r^2, is a measure of the amount of total variation of amylase activity, explained by the auxillary variables growth and weight. This measurement interprets the strength of the relationship between amylase activity with growth and weight. No significance ($p \leq .05$) was reported for any of the r^2 values. Since there were no significant values at each collection, linear regression analysis was done on the total collections for both Group I and Group II to see if as a group there were any relationships over time. The results of linear regression analysis are recorded in Table 21 and 22 for Group I and Group II respectively.

By looking at the whole group, at all collection periods, linear regression analysis revealed some positive significant relationships between amylase activity and the independent variables of age and weight over time. In Group I (Table 21), age was significantly related to amylase activity ($r^2=0.42$, $p=0.001$). Forty-two per cent of the variation of amylase activity could be attributed to age in Group I.

Weight was significantly related to amylase activity ($r^2=0.27$, $p=0.001$). Twenty-seven percent of the variation of amylase activity could be attributed to weight in Group I. Over time growth (g/day)

TABLE 19. Coefficients of determination for growth and weight correlated with amylase activity for Group I.

Independent Variable	Dependent Variable: Amylase Activity (units/ml saliva)			Dependent Variable: Specific Activity (amylase activity per mg protein)		
	3 Mos	4 Mos	5 Mos	3 Mos	4 Mos	5 Mos
Growth (g/day)	0.02	0.13	0.02	0.02	0.03	0.07
Weight (g)	0.02	0.00	0.00	0.13	0.00	0.04

No p values ≤0.05

TABLE 20. Coefficients of determination for growth and weight correlated with amylase activity for Group II.

Independent Variable	Dependent Variable Amylase Activity (units/ml saliva)					
	Birth	1 Mos	2 Mos	3 Mos	4 Mos	5 Mos
Growth (g/day)	-	0.00	0.46	0.00	0.06	0.06
Weight (g)	0.08	0.01	0.10	0.06	0.14	0.29

Independent Variable	Dependent Variable Specific Activity (amylase activity/mg protein)					
	Birth	1 Mos	2 Mos	3 Mos	4 Mos	5 Mos
Growth (g/day)	-	0.02	0.00	0.00	0.12	0.24
Weight (g)	0.05	0.00	0.08	0.03	0.08	0.01

No p values ≤ 0.05

TABLE 21. Linear regression analysis for Group I.

Independent Variable	Dependent Variables: Amylase Activity (units/ml saliva) r^2	Amylase Activity (units/ml saliva) p value	Specific Activity (amylase activity per mg protein) r^2	Specific Activity (amylase activity per mg protein) p value
Age (days)	0.42	0.001	0.05	0.10
Weight (g)	0.27	0.001	0.002	0.71
Growth (g/day)	0.04	0.05	0.02	0.28

TABLE 22. Linear regression analysis for Group II.

Independent Variable	Dependent Variables: Amylase Activity (units/ml saliva) r^2	p value	Specific Activity (amylase activity per mg protein) r^2	p value
Age (days)	0.16	0.0008	0.24	0.0001
Weight (g)	0.26	0.0001	0.27	0.001
Growth (g/day)	0.00	0.815	0.003	0.70

was not significantly related to amylase activity or specific activity in Group I. In Group II (Table 22) age was related significantly to amylase activity and specific activity (r^2=0.16, p=0.0008 and r^2=0.24, p=0.0001 respectively). Weight was significantly related to amylase activity (r^2=0.26, p=0.0001) and significantly related to specific activity (r^2=0.27, p=0.001). Growth (g/day) was not found to be significantly related to amylase activity in Group II.

Hypothesis #4

Fisher's Exact Test was used to test the difference between the proportion of infants with low amylase values and low growth rates with the proportion of infants having normal amylase values and normal growth rates. Groups I and II were combined for this test and all infants had been introduced to starch-containing foods. The Fisher's Exact Test yields the exact probability value for a given or more extreme value than the one observed.

A 2x2 contingency table was constructed as follows; growth (g/day) was divided into low and normal. Amylase activity was also divided into low and normal. The cut off points for low growth were taken from Lillibridge and Townes (1975). In their paper expected growth rates for infants less than 1 year old were given (Table 23). Any infants with a growth rate below the rate given in Table 23 for the appropriate age were classified into the low growth category. Choosing a cut off point for low amylase was not based on any published values as there are none with which the data here could be compared. Thus the point at which an infant was said to have low amylase levels was arbitrarily chosen to be 50 units/ml saliva. This is a "working definition" of low amylase taken from the average levels of amylase

TABLE 23. Expected growth rates for infants less than one year of age[1].

Age (months)	Expected Rate (g/day)
0-1	35.7
1-2	24.2
2-4	17.8
4-11	8.5

1. Adapted from Lillibridge and Townes 1975.

activity for months 3,4 and 5 of this research.

The infants fed starch were then classified into one of four cells of the table; normal growth and normal amylase, low growth and normal amylase, low amylase and normal growth and finally low amylase and low growth. Appendix 6 is a summary of the growth rates and amylase levels of all infants fed starch-containing food. Fisher's Exact Test was performed on 3 groups based on age; 3 months, 4 months and 5 months of age. There was insufficient data for the age groups one and two months to perform the Fisher's Exact Test.

The results for the age groups 3, 4, and 5 months are given in Table 24, 25 and 26 respectively.

The probabilities (p-values) obtained from the Fisher's Exact Test, for the ages of 3, 4 and 5 months, support the null hypothesis of no difference between the predicted and actual growth rates of those infants fed starch-containing foods while having low levels of salivary α-amylase (<50 units/ml saliva).

There is no evidence to support any difference between the four cells analyzed; normal amylase, normal growth; normal amylase, low growth; low amylase, normal growth; low amylase, low growth.

TABLE 24. Fisher's Exact Test for infants fed starch-containing foods at 3 months of age.

		Growth (g/day)	
		Low	Normal
Amylase (units/ml saliva)	Low	3	5
	Normal	1	2

Exact p value=0.72, n=11.

TABLE 25. Fishers Exact Test for infants fed starch containing foods at 4 months of age.

		Growth (g/day)	
		Low	Normal
Amylase (units/ml saliva)	Low	4	5
	Normal	2	4

Exact p value=0.55, n=15.

TABLE 26. Fishers Exact Test for infants fed starch containing foods at 5 months of age.

		Growth (g/day)	
		Low	Normal
Amylase (units/ml saliva)	Low	3	10
	Normal	3	10

Exact p value=0.68, n=26.

8. DISCUSSION

Salivary α-amylase, the enzyme secreted by the three salivary glands and the minor mucous glands is responsible for 40% of starch hydrolysis in the adult (Guyton, 1976). Pancreatic α-amylase hydrolyzes 50% of the total starch-consumed by adults with the remaining 10% being hydrolyzed by intestinal amylase (Guyton, 1976). Even though food does not remain in the oral cavity very long, the action of salivary α-amylase continues for several hours after food has entered the stomach (Guyton, 1976).

Once the contents of the stomach are mixed with the acid of the gastric secretions, salivary α-amylase is rendered inactive as a result of the pH falling below 4.0. The remaining starches that have not been split are digested by pancreatic α-amylase which is secreted into the duodenum by the pancreas.

The above processes concerned with the digestion of starch are affected by the maturity of the gastro-intestinal tract. Therefore, the introduction of starch into the infant diet is partially dependent on the physiological maturity of the infant's digestive system. More specifically, it is known that pancreatic α-amylase activity is low at birth, increasing with age (Hadorn et al., 1968), placing more importance on the activity of salivary α-amylase to hydrolyze starch in the infant's digestive tract. Also, due to the low gastric acidity of the infant, the activity of salivary α-amylase may be prolonged in the stomach (Mason, 1962). Amylase activity found in the intestinal tract of full term infants has been reported to be salivary in nature

(Borgstrom et al., 1960). Salivary α-amylase activity, like pancreatic has been reported to be minimal at birth (Makeith and Wood, 1971). Hence it is probable that those infants fed starch-containing foods in the first few months of life will experience difficulty digesting the starch.

The levels of salivary α-amylase activity during the first months of life could serve as contributing information for the recommendations concerned with infant weaning practices. It has been documented that infants fed starch before α-amylase activity has reached "appropriate levels" can suffer from iatrogenic diarrhea (Lillibridge et al., 1975).

In an attempt to study the levels of salivary α-amylase activity in the infant, four hypotheses were tested to examine if there were factors which affected these levels. Table 13 reports the mean α-amylase activity, specific activity levels and protein levels of both groups.

Comparing Group I to Group II from month three to month five, mean values for α-amylase activity and specific activity at each monthly collection were similar. In both groups the range of α-amylase activity and specific activity at each monthly collection is large as denoted by the standard deviation. In some cases the standard deviations of the mean amylase and specific activities were higher that the absolute values. A wide range of salivary α-amylase levels between individual subjects, is true of adults as well as infants (Dawes, personal communication).

The question remains, why do some infants have higher salivary α-amylase levels than others? If this variation could be understood then possibly this information could be used to advise mothers regarding the introduction of starch-containing foods into the infant

diet.

The major factor influencing the composition of saliva from an individual is flow rate (Dawes, 1978). Movements of the oral musculature result in an increase in salivary flow rate (Dawes, 1978). As the flow rate increases so does the time rate of amylase transfer from the parotid gland (5.4 to 17.9 mg/hr). The net result is more amylase being secreted in the same amount of time as compared to resting conditions. The collection procedure utilized for saliva collection in this study may have caused some periods of mechanical stimulation resulting in increased α-amylase being secreted by the parotid gland. Crying and sucking on the catheter could have resulted in movements of the oral musculature. The method was thought to be sufficiently consistent and it was not felt necessary by the researcher to control for the chance of stimulation of the oral cavity. The degree of variation in salivary α-amylase levels at each monthly collection was high as shown by the large standard deviations (Table 11). This could possibly have been due to the effect of the mechanical stimulation of the oral cavity during collection periods. Since this could not be controlled, therefore it can be mentioned only as a possibility.

The first hypothesis tested the variability of α-amylase activity as it related to the introduction of starch-containing foods. The data did not support any difference in the levels of salivary α-amylase between those infants fed starch-containing foods and those infants not fed starch-containing foods at each monthly collection. There was no evidence to reject the null hypothesis. Higher levels of salivary α-amylase acitivity were not due to the introduction of starch-containing foods. This result does not support the genetotrophic model of

enzymes as described by Lieberman et al.(1977). The genetotrophic model views diet and specific dietary elements as exerting selective pressure on digestive enzymes. This model should suggest that a diet high in starch would be positively correlated with a high level of salivary α-amylase. This relationship was not shown in this study which supports the research findings of Dawes and Shaw (1965) and Lillibridge et al. (1975).

Pancreatic α-amylase has been reported to be influenced by diet. Zoppi et al. (1972) reported that the introduction of starch to the diet from birth enhanced pancreatic α-amylase production. Contrary to the findings of Zoppi et al. (1972), Lillibridge et al. (1975) found amylase activity to be absent in the duodenum of an infant fed starch from age 1 week to 4 months. They suggest that the infant's ability to digest starch correlates with the appearance of pancreatic α-amylase.

Possibly the deciding factor in pancreatic α-amylase induction is the amount of starch fed. Zoppi et al. (1972) fed their infants a commercial skim milk formula with the addition of 2g soluable starch per 100 ml. The individual infant reported on by Lillibridge et al (1975) was fed 1 tablespoon of rice cereal powder twice a day at one week of age. This amount increased progressively until the infant was 4 months of age. Ten grams of starch is the average amount given to one month old infants fed milk formulas while 23 grams of starch corresponds to that given a 3 month old infant receiving commercially prepared cereals (Devizia et al., 1975). Forty grams of starch per day fed to a one month old infant has been reported to cause iatrogenic diarrhea (Devizia et al., 1975). The tolerance of a growing infant to very large quantities of starch is limited due to low levels of salivary and

pancreatic α-amylase secretions. Too much starch could be responsible for over saturation of the minimal amount of α-amylase present during the first few months. Lillibridge and Townes (1975) reported that at 4 months of age the duodenal aspirate of their infant revealed no sign of amylase activity with the infant being fed 69g a day from starch. Lillibridge and Townes (1975) referred to this as a "physiologic" amylase deficiency for when starch was elliminated from the diet, amylase activity was low but detectable.

The infant early in life exists on a liquid diet, breast or formula. Possibly it is not the starch-containing food but the physical structure of the feed which is associated with the increased salivary α-amylase activity. Hall et al. (1967) reported that total liquid diets fed to adult males changed the functional status of the parotid gland. The liquid diet resulted in a decrease in mastication, decreasing the volume of parotid secretions. Total protein and total amylase levels decreased 30% and 29% respectively (Hall et al., 1967). Work by Winsor and Bayne (1929) also supports the view that mastication stimulates the salivary glands in man. Therefore the development of salivary α-amylase may be related to the development of teeth. This would be the time when an infant is teething or chewing causing the stimulation of the parotid gland by the action of mastication.

The second hypothesis that there will be no association between the absolute increase in weight and the absolute increase in salivary α-amylase activity from birth to 5 months of age in Group II was supported by the data. The absolute change in α-amylase levels over the 5 month period compared to the absolute change in weight over the same period took into account the initial difference imposed by the

values obtained for these two measures at birth. The development of salivary α-amylase is therefore not associated with the increase in weight of the infant.

Contrary to what many parents believe, the larger infant does not necessarily require solid food (starch-containing) earlier than the smaller infant. Amylase activity does increase with the weight of the infant. However, the amount of weight gained by 5 months of age did not have a significant relationship with the level of salivary α-amylase activity (absolute increase) by 5 months of age. The infant who gained more weight over the 5 month period did not have a higher absolute change in salivary α-amylase as compared to the infant who gained less weight over the same time period.

The third hypothesis was designed to test the association between salivary α-amylase levels and individual growth rates and weight at each collection period. No associations, between salivary α-amylase and growth or weight as determined by r^2 were significant ($p \leq 0.05$), in any of the collection periods for Group I or Group II. This can be interpreted as the total amount of variation of amylase activity explained by growth or weight is not of any significant value at the monthly collection periods. The various levels of salivary α-amylase at each month were independent of the infant's weight or growth rate. These results are in agreement with the previous discussion of the work done by Collares (1978) who reported on infants (20 to 36 months) suffering from Kwashiorkor. Between day ten and day thirty of nutritional intervention a significant positive correlation ($p \leq 0.001$, $r^2=0.85$) was observed between growth and salivary α-amylase. The decrease in amylase activity of these infants suffering from Kwashiorkor was

attributed to amino acid deficiencies, manifesting itself in a decrease in digestive enzyme synthesis.

This positive relationship between growth and amylase activity was an isolated event occuring under therapeutic conditions. Once the infants recuperated from Kwashiorkor their amylase levels were the same as those in the control group. Only under conditions of recuperation from malnutrition has growth been found to be positively correlated with salivary α-amylase levels.

Several significant relationships were found when linear regression analysis was performed on the Groups as a whole rather than on individual collection periods. In Group I over all collection periods salivary α-amylase activity was positively related to the independent values of age and weight. In Group II positive associations were also found between salivary α-amylase activity and age and weight. Also in Group II specific activity was significantly positively associated with age.

In an infant, age and weight are highly biologically correlated[7]. It is difficult to separate out the effect of one on the other. Therefore it would seem quite logical that if age was significantly related to amylase levels that weight would also be significantly related over time and vice versa. This result was observed in this data. Both age and weight were found to have a significant positive relationship with salivary α-amylase levels in both Group I and Group II (Table 21 and 22). Age and weight can partially explain the increase in salivary α-amylase levels in infants from birth to 5 months of age. Salivary

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7. C. Young, Statistical Consultant, Dept. of Statistics and Computer Science, University of Waterloo.

α-amylase cannot be induced by starch-containing foods nor is it affected by the rate of growth of the infant. The bigger infant proved not to have significantly higher levels of salivary α-amylase activity.

Lillibridge and Townes (1975) suggest that the ability of an infant to digest starch correlates with the appearance of pancreatic amylase. Pancreatic amylase has been found to be age dependent (Hadorn et al., 1968). Pancreatic amylase is low in children below 6 months of age (Hadorn et al., 1968). It is interesting to note that the current recommendation for introduction of solid foods is between the ages of 4 to 6 months (The Canadian Paediatric Society, 1979).

The data generated from this research gives the average salivary α-amylase values for ages 3 to 5 months to be 44.5, 46.8 and 58.6 units/ml saliva, respectively. The average of these three figures is 50 units/ml saliva. Based on this research possibly one could hypothesize that by between the ages of 4 to 6 months infants might have enough salivary α-amylase activity to tolerate starch-containing foods. The amount of activity corresponding to this age would be 50 units/ml saliva. The rate of increase of amylase activity is quite rapid in the first 3 months of life whereas in the second three months of life the rate of increase is much slower. Devizia et al. (1975) suggest introducing starch-containing food between 4 to 6 months of age with more emphasis given to the latter age. A safe level of starch suggested by Devizia et al. (1975) would be 10-23 g/day.

What would happen to the infant who was fed cereal at an early age who had insufficient salivary α-amylase activity? Lillibridge and Townes (1975) reported that an infant fed starch too early in life resulted in failure to thrive due to iatrogenic diarrhea. The fourth

hypothesis dealt with this theory by testing if there were any differences between the actual and predicted growth rates of all infants fed starch-containing foods while having low levels of salivary α-amylase. Fisher's Exact Test revealed that there was no evidence to support the hypothesis that infants with low growth rates and low amylase levels being significantly different from the infants classified as low growth and normal amylase, normal growth and low amylase, and normal growth and normal amylase. Although Fisher's Exact Test is specific to small samples, the samples from this research were very small and perhaps a larger size could have revealed more information. Also the cut off points for low growth were adapted from Lillibridge and Townes (1975) and the value for low amylase (<50 units/ml saliva) was a "working value" taken from the average levels of the collection for months 3, 4 and 5 of this research. These results might have been different if the cut off point was 20 units/ml of saliva. However the sample size using this value was too small for statistical analysis.

Appendix 6 is a chart of all the collection periods when an infant was fed starch. The amylase levels and growth rates (actual and expected) are given. This was the basis for dividing the infants into the four groups of low and normal levels of amylase and growth. The dots represent periods of low growth low amylase. Of the 64 times starch was fed to 30 infants, 13 infants (48%) experienced periods of low growth accompanied by low salivary amylase.

The youngest age that these infants were introduced to starch-containing foods was 1 month. Five infants were fed starch-containing foods at this age. The source of starch was cereal for all five and the amount was between 1 and $1\frac{1}{2}$ tablespoons per day. It is hard to say

how much of this was consumed since most mothers experienced the child having difficulty taking in solid foods.

The amount of starch in 1 tablespoon of rice cereal is approximately 1.6 grams.[8] Devizia et al. (1975) reported that 10 to 23 grams of starch was absorbed by 1 and 3 month old infants when served as cooked flours. Starch in the amounts of 40 grams per day or 25 tablespoons has been reported to cause fermentitive diarrhea (Devizia et al., 1975). There seems to be no difference between starch varieties if the starch is cooked since the granular structure is destroyed by cooking making the starch more readily hydrolyzed (Devizia et al., 1975).

According to the research of Devizia et al. (1975), the concern of feeding starch in young infants lies in the amount of starch. There were no mothers who took part in this research who fed their infant 25 tablespoons of cereal per day. The highest recorded level of starch fed to an infant in this study was about 20 tablespoons per day when the infant was 5 months of age. It appears as though there is a tolerance or a threshold level of young infants to large quantities of starch. This is probably a consequence of low intestinal and salivary α-amylase levels. If α-amylase is somewhat dependent on age and weight then more security lies in feeding the older infant starch as opposed to the younger infant.

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8. Calculated from Gerber Rice Cereal, Gerber (Canada) Inc., Niagara Falls, Canada.

9. SUMMARY AND CONCLUSIONS

The age at which starch-containing foods should be introduced into the infant diet has been subject to various recommendations over the past 50 years. As the recommendations have changed so has the advice supporting them.

The advice given to mothers on infant feeding is partially based on the nutrient requirements of the infants. Additional guidance for advice on infant feeding patterns and weaning practices has come from studies on the development of digestive enzymes during early childhood.

The major first food fed to Canadian infants is cereal (Nutrition Canada, 1970). With starch being the major constituent of cereal it would seem justified to examine the level of activity of the enzyme responsible for starch hydrolysis, salivary α-amylase.

This research was undertaken to investigate the levels of salivary α-amylase activity in infants from birth until 5 months of age. It was hypothesized that the variables associated with the levels of amylase activity were age, weight, growth rates and the induction of the enzyme by it's substrate, starch. The results indicated that the enzyme salivary α-amylase was not apparently induced by starch. The variation of α-amylase activity could not be explained by the introduction of starch-containing foods. The absolute weight (g) of the infant from birth to 5 months of age was hypothesized to correlate with the absolute increase in the levels of salivary α-amylase activity. This could be interpreted as the larger the infant the more salivary α-amylase would be produced. This theory was not supported by the

data. It is a common practice of mothers to feed more solid food to their infants rationalizing with the theory "he/she is a big baby and needs more". According to this research the large infant does not produce any more salivary α-amylase than the smaller infant. The mother who tries to feed a larger infant more starch-containing foods in the first few months of life may cause the infant to experience iatrogenic diarrhea. This could also affect the infant who is smaller than normal and the mother wishes to see it heavier.

The next theory tested to explain the levels of salivary α-amylase activity was it's possible relationship to individual monthly growth rates (g/day) and weight (g). Salivary α-amylase levels were found not to be related to either weight or growth rates at any of the monthly collection periods. This result is in agreement with research by Brasil (1978) who found that growth rates only correlated with salivary α-amylase activity under conditions of recuperation from malnutrition.

When the salivary α-amylase levels at all collection periods were correlated with age, weight, and growth, age and weight were significantly related to amylase activity in both Group I and Group II. The amount of salivary α-amylase development which could be explained by weight was similar for both groups. Group I had a correlation coefficient of 0.27 and Group II had a correlation coefficient of 0.26. Both were significant at the 5 percent level. Approximately one quarter of the variations in salivary α-amylase levels could be explained by weight. In Group I age explained 42% of the variation in amylase levels while in Group II it could only explain 16%.

From these conclusions it can be shown that the age and the weight of the infant are reasonable indicators for initiating starch into

the diet with respect to time. Growth which measures the weight gain over time has no significant relationship to salivary α-amylase. Therefore the infant at 3 months of age who weighs the same as the infant at 6 months of age (with the same birth weight) does not have higher levels of salivary α-amylase activity. The chronological age of the infant seems to play a part in development of salivary α-amylase levels.

An attempt was made to see if those infants who had low levels of salivary α-amylase activity (<50 units/ml saliva) and fed starch-containing foods experienced low growth; below the predicted rate for their age. No significant results were obtained to support this theory. However of the 64 starch feeding periods of the 30 infants, 13 of the infants (48%) did experience low growth associated with low levels of salivary α-amylase (Appendix 6).

Some other factors which may be related to salivary α-amylase activity is the development of teeth. Mastication causes movement of the oral musculature. This in turn stimulates an increase n salivary flow rate. As the flow rate increases so does the time rate of amylase transfer from the parotid gland. Therefore the development of teeth may be associated with increased levels of salivary α-amylase. Teeth usually erupt around the age of 6 months which would be a safe time to introduce starch-containing foods as both salivary and pancreatic α-amylase levels are well developed (Hadorn, 1968).

Not only should the timing of the introduction of starch be considered but also the amount of starch fed to the infant. All infants had detectable levels of salivary α-amylase in this research, even at birth. Whether or not the levels of salivary α-amylase in the infants

were high enough to hydrolyze the starch is unknown. One teaspoon of rice cereal contains 1.6g of starch. Divizia (1975) reported that 40 grams of starch (25 teaspoons) per day could not be tolerated in the infant between one to three months of age.

The purpose of this research was to investigate the levels of salivary α-amylase in infants and its possible relationship to the addition of starch-containing foods to the diet, growth rates, weight, and the age of infant. Salivary α-amylase was found to be significantly related to the age and the weight of the infant but not the growth rate which is a combination of age and weight.

It was anticipated that the results of this research would aid the Health Professional when giving advice regarding the introduction of solids - especially starch-containing foods. From this reseach and the present literature two factors should be considered before introducing starch-containing foods:

1) The age and corresponding weight of the infant

2) The amount of starch-consumed by the infant per day.

Between the ages of 4 to 6 months the infant has salivary α-amylase levels of approximately 50 units/ml of saliva. Also at this time teeth are erupting which may cause an increase in α-amylase activity due to stimulation of the salivary glands by the movement of the oral musculature.

The second point dealing with the amount of starch reflects the work of Divizia (1975). This research indicates that infants less than three months of age can tolerate 10 to 23g of starch per day and at amounts of 40g per day they experience diarrhea and consequent loss of weight. This result was also shown by Lillibridge and Townes (1975)

who reported on an infant being fed 69g of starch per dat at the age of 4 months. This value is well over the 40g threshold expressed by Divizia (1975).

Every infant is an individual and from this research one can see the inherent variability of salivary α-amylase levels between infants and at each monthly collection. Divizia (1975), based on his research suggests the introduction of starch-containing foods be between the ages of 4 to 6 months, to guard mothers from feeding stach too early or from feeding too much starch.

This research was an attempt to investigate the levels of salivary α-amylase in infants with respect to the introduction of starch-containing foods. Knowledge on the development of salivary α-amylase and activity levels in humans is minimal if non-existant in the literature. Yet several authors acknowledge low amylase levels as being the reason for delaying starch-containing food into the diet of infant (Ontario Public Health Nutritionists, 1977; Health and Welfare Canada, 1979, Manitoba Home Economics Directorate, 1979). The data generated from the study can act as a base or ground work for further researchers investigating the physiologic maturity of the infant's digestive tract; specifically salivary α-amylase development. Further questions to be answered are how much amylase activity is needed to hydrolize a unit of starch and at what point do infants develop iatrogenic diarrhea?

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APPENDIX 1

Consent Form

It has been explained to me by _____ of the Dept. of Foods and Nutrition, University of Manitoba that she is conducting research on the saliva of infants.

I understand that saliva will be collected from my infant in my home at monthly intervals for _____ months starting _____. Also body measurements will be taken and questions will be asked on the baby's diet.

I am fully aware of the procedure that will be used and I agree to have my infant participate in this study. Although there will be no direct benefit to me or my infant, I am aware that I can receive the results of this study if I so desire. I can terminate my participation and my infant's participation in this study at any time without penalty or changes in the health service to me or my child.

Date: _____ Name: _____

Signature: _____ Address: _____

APPENDIX 2

Anthropometric Data Saliva Study

MOTHER'S NAME # _____

_____

INFANT'S NAME INFANT'S AGE

_____ _____

DATE _____

HT _____ cm

WT _____ grams

APPENDIX 3

Diet Questionnaire for Saliva Study of Infants

YOUR NAME _____ # _____

INFANT'S NAME _____

INFANT'S AGE _____

DATE _____

The following information is needed to further analyze your infant's saliva. Just check the appropriate answer.

1) How are you feeding your infant?

_____ Breast

_____ Bottle

_____ Both

Additional information? _____

_____

2) Have you introduced your infant to solid foods?

_____ yes

_____ no

if yes then

a) list the kinds _____

b) Amounts _____

c) How frequently _____

Thank you very much for your time.

Chris Holodinsky

APPENDIX 4

Description of the Sample by Type of Diet at Each Collection Time - Group I.

Infant Number	Collection Age	Breast	Bottle	Cereal	Fruit	Veg[1]	Meat
1	3 mos	•					
	4 mos	•					
	5 mos	•			•		
7	3 mos	•					
	4 mos	•					
	5 mos	•			•		
8	3 mos		•	•			
	4 mos		•	•			
	5 mos		•	•			
9	3 mos	•					
	4 mos	•			•		
	5 mos	•		•	•		
10	3 mos		•				
	4 mos		•				
	5 mos		•	•			
11	3 mos	•					
	4 mos	•					
	5 mos	•		•	•		
12	3 mos	•					
	4 mos	•					
	5 mos	•		•			
14	3 mos	•					
	4 mos	•					
	5 mos	•		•			
15	3 mos	•	•				
	4 mos	•	•	•		•	
	5 mos	•	•	•		•	
16	3 mos	•	•				
	4 mos		•	•			
	5 mos		•	•			
18	3 mos	•	•	•			
	4 mos	•	•	•	•	•	
	5 mos	•	•	•	•	•	

APPENDIX 4 (continued)

Infant Number	Collection Age	Breast	Bottle	Cereal	Fruit	Veg[1]	Meat
19	3 mos	•	•	•			
	4 mos	•	•	•			
	5 mos		•	•		•	
20	3 mos		•	•		•	
	4 mos		•	•	•	•	
	5 mos		•	•	•	•	
22	3 mos		•	•			
	4 mos		•	•	•		
	5 mos		•	•	•	•	
23	3 mos		•	•	•		•
	4 mos		•	•	•		•
	5 mos		•	•	•		•
24	3 mos		•	•	•		
	4 mos		•	•	•		•
	5 mos		•	•	•		•
25	3 mos	•					
	4 mos	•			•		
	5 mos	•	•		•		
28	3 mos	•					
	4 mos	•	•				
	5 mos	•	•	•	•		

1. Veg = vegetables.

APPENDIX 5

Description of the Sample by Type of Diet at Each Collection Time - Group II.

Infant Number	Collection Age	Breast	Bottle	Cereal	Fruit	Veg[1]	Meat
32	Birth	•					
	1 mo	•					
	2 mos	•					
	3 mos	•					
	4 mos	•					
	5 mos	•		•			
33	Birth		•				
	1 mo		•	•			
	2 mos		•	•			
	3 mos		•	•	•	•	
	4 mos		•	•	•	•	
	5 mos		•	•	•	•	
34	Birth	•					
	1 mo	•					
	2 mos	•					
	3 mos	•		•			
	4 mos	•		•			
	5 mos	•			•	•	
35	Birth	•					
	1 mo	•					
	2 mos		•				
	3 mos		•		•		
	4 mos		•		•		
	5 mos		•	•	•		
36	Birth	•					
	1 mo	•					
	2 mos	•					
	3 mos	•					
	4 mos	•		•			
	5 mos	•		•			
37	Birth	•					
	1 mo	•					
	2 mos	•					
	3 mos	•					
	4 mos	•					
	5 mos	•					

APPENDIX 5 (continued)

Infant Number	Collection Age	Breast	Bottle	Cereal	Fruit	Veg[1]	Meat
38	Birth	•					
	1 mo	•					
	2 mos	•					
	3 mos	•					
	4 mos	•	•				
	5 mos		•	•			
39	Birth		•				
	1 mo		•	•			
	2 mos		•	•	•		
	3 mos		•	•			
	4 mos						
	5 mos						
40	Birth	•					
	1 mo	•					
	2 mos	•					
	3 mos	•			•		
	4 mos	•	•	•			
	5 mos	•	•	•	•		
42	Birth	•					
	1 mo	•					
	2 mos	•					
	3 mos	•					
	4 mos	•					
	5 mos	•					
43	Birth	•					
	1 mo	•		•			
	2 mos	•		•			
	3 mos	•		•			
	4 mos	•		•			
	5 mos	•		•	•		

1. Veg = vegetables.

APPENDIX 6

All Infants Fed Starch Containing Food.

ID #	Type of Solid[1]	Age	Amylase Level (IU/ml)	Growth Rate (g/day)	
				Actual	Expected[2]
1	FR	5 mos	28.4	-11.4	8.5 •
7	FR	5 mos	30.8	8.5	8.5 •
8	CE	3 mos	68.5	11.6	17.8
	CE	4 mos	84.9	68.8	17.8
	CE	5 mos	107.8	-2.0	8.5
9	FR	4 mos	38.6	38.6	17.8
	FR,CE	5 mos	31.8	-21.6	8.5 •
10	CE	1 mo	3.0	38.7	35.7
	CE	2 mos	7.8	18.1	24.2 •
	CE	5 mos	30.5	21.2	8.5
11	FR,CE	5 mos	52.8	9.1	8.5
12	CE	5 mos	40.7	23.0	8.5
14	CE	5 mos	96.5	-1.2	8.5
15	CE,VG	4 mos	59.5	10.5	17.8
	CE,VG	5 mos	84.2	8.5	8.5
16	CE	4 mos	26.0	13.8	17.8 •
	CE,VG	5 mos	65.6	10.2	8.5
18	CE	3 mos	12.7	16.5	17.8 •
	CE,VG,FR	4 mos	-	10.0	17.8
	CE,VG,FR	5 mos	56.0	20.7	8.5
19	CE	3 mos	13.8	25.0	17.8
	CE	4 mos	10.5	-18.0	17.8 •
	CE,VG	5 mos	9.9	13.1	8.5
20	CE	1 mo	1.4	21.2	35.7 •
	CE	2 mos	6.7	44.7	24.2
	CE,VG	3 mos	11.2	7.9	17.8 •
	CE,VG,FR	4 mos	26.2	29.0	17.8
	CE,VG,FR	5 mos	31.6	34.1	8.5

APPENDIX 6 (continued)

ID #	Type of Solid[1]	Age	Amylase Level (IU/ml)	Growth Rate (g/day)	
				Actual	Expected[2]
22	CE	2 mos	12.8	61.0	24.2
	CE	3 mos	21.9	25.5	17.8
	CE,FR	4 mos	30.7	21.2	17.8
	CE,FR,VG	5 mos	44.0	15.4	8.5
23	CE,FR	2 mos	16.6	27.4	24.2
	CE,FR	3 mos	52.0	24.1	17.8
	CE,FR	4 mos	50.0	43.2	17.8
	CE,FR	5 mos	83.7	47.3	8.5
24	CE,VG,FR	3 mos	16.3	6.6	17.8 •
	CE,VG,FR	4 mos	17.4	35.1	17.8
	CE,VG,FR	5 mos	24.5	12.9	8.5
25	FR	4 mos	82.2	25.4	17.8
	FR	5 mos	178.5	6.5	8.5
28	CE,FR	5 mos	43.0	21.2	8.5
32	CE	5 mos	59.2	15.7	8.5
33	CE	1 mo	18.4	37.7	35.7
	CE	2 mos	27.2	27.7	24.2
	CE,VG,FR	3 mos	102.8	22.9	17.8
	CE,VG,FR	4 mos	74.8	25.6	17.8
	CE,VG,FR	5 mos	47.1	25.7	8.5
34	CE	3 mos	22.1	23.5	17.8
	CE	4 mos	17.1	14.0	17.8 •
	CE,FR	5 mos	42.1	23.5	8.5
35	CE,FR	5 mos	69.9	12.3	8.5
36	CE	4 mos	107.0	25.2	17.8
	CE	5 mos	75.5	22.2	8.5
37	CE	5 mos	52.7	35.0	8.5
38	CE	1 mo	6.2	31.7	35.7 •
39	CE	2 mos	15.6	32.0	24.2
	CE	3 mos	33.2	25.3	17.8
40	CE	4 mos	2.4	14.3	17.8 •
	CE,FR	5 mos	4.8	15.3	8.5

APPENDIX 6 (continued)

ID #	Type of Solid[1]	Age	Amylase Level (IU/ml)	Growth Rate (g/day)	
				Actual	Expected[2]
43	CE	1 mo	14.5	25.5	35.7 •
	CE	2 mos	26.4	55.2	24.2
	CE	3 mos	37.3	24.2	17.8
	CE	4 mos	73.1	15.1	17.8
	CE,FR	5 mos	42.5	22.4	8.5

1. CE = cereal, FR = fruit, VG = vegetables.
2. Taken from Lillibridge and Townes, 1975.
• - periods of low growth, associated with low α-amylase levels.