LACTASE RESTRICTION AND LACTASE PERSISTENCE

A study of Sri Lankans and a ~~British~~ paediatric population

SHANTHI THOMAS

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Department of Clinical Biochemistry
Institute of Child Health
University of London

ABSTRACT

Lactase is an enzyme, located on the small intestinal brush border, that hydrolyses the disaccharide lactose into its monosaccharides glucose and galactose, which are then absorbed. In mammals the enzyme is present at birth and disappears after weaning. In all human neonates the enzyme is also present at birth. In the majority of the world population the enzyme activity declines during childhood (lactase restriction phenotype) and only in a minority does the enzyme activity persist in adults (lactase persistence phenotype).

It is most likely that genetic and environmental factors are involved in determining these phenotypes. All the evidence suggests that lactase persistence is a result of genetic mutation conferring a selective advantage among some populations. The enzyme lactase has been characterised and cloned, and the gene is located on Chromosome 2. There is some evidence that the molecular mechanism of the genetic expression of the phenotypes include post-transcriptional processing of the enzyme precursor.

In this study, 291 oral lactose loads and lactase activities of 41 small intestinal biopsies have been used to investigate the progression and prevalence rate of the lactase phenotypes in Sri Lankans. A comparison was made by studying the lactase activities of 170 histologically normal small intestinal biopsies on British children of mixed ethnic origin.

Lactase was present at birth in Sri Lankan children and declined around the age of eight years, the majority (73-95%) of the adults belonging to the lactase restriction phenotype. In contrast the majority of the British children belonged to the lactase persistence phenotype throughout childhood. The lactase restriction phenotype found among British children was largely contributed by children of Asian and African origin.

Although most adult Sri Lankans demonstrated low intestinal lactase activities, this did not lead to any significant clinical problems. They did not suffer from clinical lactose intolerance or other related clinical complaints in the majority of the cases.

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INTRODUCTION

The absorption of lactose in children and adults has aroused world-wide interest. Lactose was established as a constituent of milk more than 350 years ago (Bartolettus 1633). Its chemical synthesis was completed 300 years later (Haworth et al 1927). The realisation that lactose could be associated with the pathogenesis of diarrhoea was noted at the beginning of this century (Jacobi 1901). However, some of the questions concerning this subject still remain unanswered. Based mainly on the observation that the prevalence rate of lactase restriction is high among the African and Asian populations, it was proposed that this is genetic. On the other hand it has been debated that there may be an adaptive aetiology based mainly on the observation that the prevalence of lactase restriction was high among the non-milk drinking populations. This question has been reasonably settled and the consensus, based on some extensive family studies and a study of the phenotypes among twins, is that lactase restriction is inherited by an autosomal recessive gene (Sahi 1974a, Flatz 1987, Metneki et al 1984).

The per capita milk intake in Sri Lanka is low (Simoons 1970a). Bolin et al (1970a and b), who think that lactase restriction is a consequence of low milk intake, predicted that countries such as Sri Lanka would have a high prevalence of lactase restriction. This prediction prompted the present study to determine the true prevalence rate of lactase restriction in Sri Lanka.

The work reported in this thesis first critically evaluates the various investigative procedures for the diagnosis of the lactase phenotypes, determines the prevalence rate* of lactase restriction in the Sri Lankan population and the age at which it is expressed and discusses the clinical importance of this condition in that country. Part of the results in this thesis has already been published (Senewiratne et al 1977, Thomas et al 1990).

This is compared with the prevalence rate and age of expression among the paediatric population in Britain.

The study in Sri Lanka was approved by the ethical committee of the University of Sri Lanka. Informed consent was obtained from the subjects or the patients. The tests carried out on the British children were part of their routine investigation.

* For definition of terms please see Appendix VIII.

CHAPTER 2

PHYSIOLOGICAL AND BIOCHEMICAL ASPECTS OF LACTOSE AND LACTASE

1. MILK

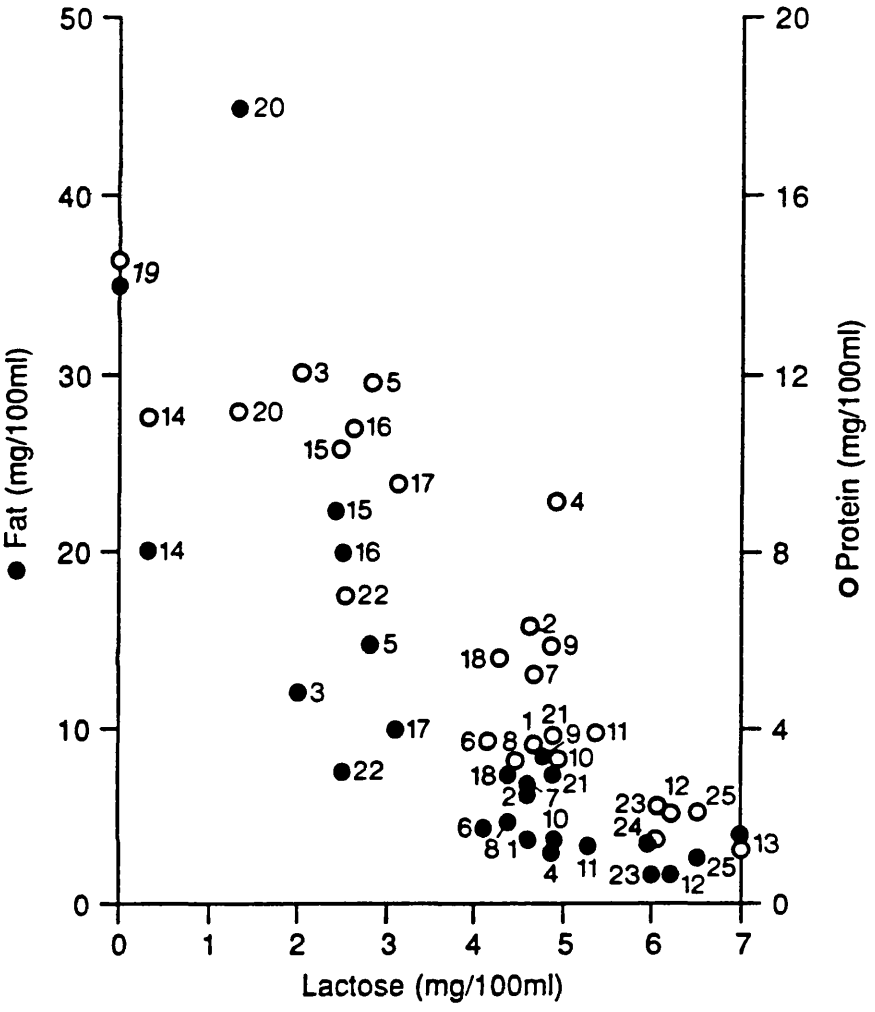
Milk is a specific product of the mammary glands, only present in mammals (Hazid and Ballou 1957). Lactose is a disaccharide that occurs in large amounts and is a freely diffusible molecule in all mammalian milk with the exception of that of the Californian sea-lion (Zalophus californianus). The suckling sea-lion cub lacks intestinal lactase, the enzyme necessary to hydrolyse lactose, and depends on milk fat for its energy metabolism (Sunshine and Kretchmer 1964).

Lactose is the principal carbohydrate constituent of milk. The lactose content of milk varies from species to species and is negatively correlated with the amount of fat and protein (Fig 2.1). In human milk the lactose content is 7% and accounts for 40% of the energy intake of the infant. In adult Caucasians lactose accounts for 10% of the carbohydrate intake and 5% of calories ingested (Gray 1971, Gray 1975).

1.1 Milk Product - Yoghurt

Yoghurt is a fermented milk product. It is described here as the properties of yoghurt are different from those of milk. The lactose in yogurt is better absorbed by individuals with low intestinal lactase activity - the enzyme necessary for the hydrolysis of lactose.

Yogurt is manufactured by incubating milk with lactobacillus bulgaricus and streptococcus thermophilus. These organisms contain beta-galactosidase which metabolise the lactose in the milk to organic acids, thus decreasing the lactose content and decreasing the pH to about 4.6 (Kolars et al 1984). Nearly eighty years ago it was suggested that ingestion of yoghurt was beneficial and could prolong life (Metchnikoff 1907). It is not surprising that yoghurt is favoured by some populations with low intestinal lactase (eg the Greeks). The desirability of yoghurt for individuals with low intestinal lactase is thought to be due to 1) the reduced content of lactose (Alm 1982), 2) the ingestion of the bacterial beta-galactosidase aiding the hydrolysis of lactose in the gut. The second suggestion has not been fully examined. Studies examining the



1 ox	10 cow	*19 sea lion*
2 fox	11 llama	20 dolphin
3 rabbit	12 ass	21 buffalo
4 cat	13 human	22 guinea pig
5 rat	14 anteater	23 horse
6 camel	15 reindeer	24 orangutan
7 yak	16 deer	25 monkey
8 goat	17 dog	

Adapted from Palmiter 1969

Figure 2.1

Inverse relationship of fat and protein content to lactose content in milk from various animals.

colonisation of the intestine by lactobacilli in yoghurt fed rats were not convincing (Garvie et al 1984). However it has been shown, using the breath hydrogen test, that the absorption of lactose in yoghurt ingested by humans with low intestinal lactase is better than when equal amounts of lactose is ingested in milk (Kolars et al 1984) and that the intraluminal lactase activity of the duodenal contents increased after ingestion of yoghurt. Although there is no lactase activity in stored yoghurt because of the low pH and temperature, if the pH and temperature are artifically raised the bacterial lactase rapidly hydrolyses lactose. The intracellular location of lactase in these bacteria protects the enzyme from the acid pH of the stomach. The ability to absorb lactose in yoghurt differs in the different preparations of yoghurt possibly because of the varying beta-galactosidic activity in them (Wytock and Dipalma 1988).

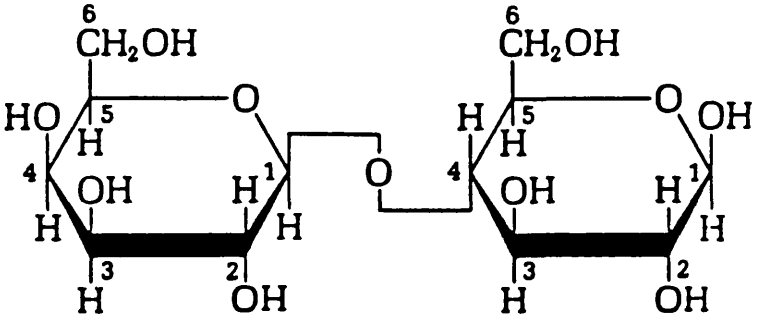
2. LACTOSE

Lactose is a disaccharide, (O-β-D-galacto-pyranosyl-(1-4)-B-D-glucopyranose). The monosaccharides are linked via a 1-4 glycosidic bond (Fig 2.2). it is a beta-glycoside, unlike other nutritionally important disaccharides, maltose and sucrose, which are alpha-glycosides. Lactose is synthesised in the mammary gland from glucose and UDP-galactose. This reaction is catalysed by a compound enzyme system consisting of UDG-galactosyl transferase and alpha-lactalbumin (Brew et al 1968).

2.1 The metabolism of lactose

2.1.1 Metabolism of lactose in individuals with high intestinal lactase: In Caucasian adults with high intestinal lactase, the ingested lactose is hydrolysed by the lactase into glucose and galactose, when it reaches the small intestine. The enzyme is located on the luminal side of the apical membrane of villous enterocytes of the small intestine. The active enzyme sites appear to be available to the disaccharide molecules which make contact with the surface of the microvillus. The monosaccharides resulting from hydrolysis require a specific active transport mechanism for absorption. The hydrolysis of lactose occurs only half as rapidly as other dietary disaccharides and monosaccharides are produced at rates insufficient to saturate the glucose-galactose

Lactose



(*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranose)

Figure 2.2

Structure of lactose β form

transport system (Gray and Santiago 1966). This makes hydrolysis the rate-limiting step for the overall digestion and absorption of lactose (Dawson et al 1986). After hydrolysis, glucose and galactose are absorbed into the portal circulation.

2.1.2 Metabolism of lactose in individuals with low intestinal lactase: Carbohydrate intolerance as a cause of diarrhoea was suggested by Althausen et al in 1935. It was only after 1966, however, that experiments were done to study this mechanism (Kern and Struthers 1966, Bedine and Bayless 1973) by perfusing lactose and sucrose through a loop of intestine in man (both lactase deficient and control subjects). Perfusates containing 9% lactose or sucrose and 0.1% polyethylene glycol (a non-absorbable marker) at an osmolarity of 290 mosm/kg were perfused through a tube into the proximal jejunum at a rate of 9-10 ml/minute. The intestinal contents were aspirated 100 cm distal to the point of perfusion and the content of sugar and electrolyte analysed. In the control subjects both sugars were absorbed, water being absorbed at a rate of 5 ml/minute. In the lactase deficient subject sucrose was absorbed normally. However, when the lactose solution was perfused there was an efflux of fluid into the lumen at a rate of 5 ml/minute, the total amount of water lost into the lumen being as large as 2 l and providing the basis for watery diarrhoea. The watery solution was iso-osmolar showing that there was an efflux of sodium and chloride ions but no potassium ions (Kern and Struthers 1966, Bedine and Bayless 1973). The lactose solution that moves into the colon is metabolised by the colonic bacteria to organic acids, producing osmotic diarrhoea, abdominal colic and borborygmi.

2.1.3 In Bacteria: It is interesting to compare the metabolism of lactose in bacteria, as bacterial metabolism of unabsorbed lactose in the gut is important in the symptomatology of lactose intolerance in man. In bacteria (eg pseudomonas, lactobacillus bulgarias, streptoccocus thermophilus) lactose is converted into organic acid which is then metabolised to CO_2 and H_2O (Nishizuka and Hayaishi 1962). This process could occur in the intestinal lumen of the mammals in whom the micro-organism is a normal inhabitant (Chapter 8, 2.3.1).

The bacterial beta-galactosidase is intracellular and aid in the fermentation of milk, and the production of yoghurt.

3. LACTASE

Lactase, responsible for the hydrolysis of lactose in man, is a beta-galactosidase. This enzyme is classified as EC3.2.1.23. The beta-galactosidase occurs as a complex with two active sites; one hydrolyses lactose (EC 3.2.1.23) and the other hydrolyses phlorizin (EC 3.2.1.62). The physicochemical characterisation of the lactase-phlorizin complex (LPH) is described below (3.3).

3.1 Classification of lactase

Density gradient ultracentrifugation and gel filtration chromatography have revealed that there are three types of beta-galactosidases in the small intestinal mucosal homogenates (Gray and Santiago 1969, Asp et al 1969, Asp 1971). Speficic assays for these lactases have been developed based on the differences between the three enzymes regarding pH optimum, substrate specificity and sensitivity to p-chloromercuribenzoate (PCMB) (Asp and Dahlqvist 1972, Table 2.1).

Beta-galactosidase I (neutral lactase): This enzyme is thought to be principally responsible for the hydrolysis of ingested lactose and termed "neutral lactase". It is intimately associated with the brush-border of the small intestinal epithelial cells, has a pH optimum of 5.5-6.0 and specificity for lactose is at least five times that for synthetic substrates or cellobiose. It is not inhibited by 0.2 mM PCMB. The highest activity is present in the jejunum, with lower activity in the duodenum and the terminal ileum. The enzyme can be detected by immunochemical and histochemical methods in the differentiated non-dividing villus cells of the epithelium (Doell et al 1965, Fortin-Magna et al 1970).

Beta-galactosidase II (acid lactase): A second lactase with an acid pH optimum (3.5 - 4.5) is found in the intestine, liver and kidney of all mammals, and is localised in the lysomes. It hydrolyses lactose as well as the synthetic substrates. 2-naphthyl beta-galactoside is a specific substrate hydrolysed only by this enzyme. It is inhibited by PCMB. This enzyme has been shown to be deficient in patients with gangliosidosis (Okada and O'Brien 1968), and it has been suggested that it is important in the metabolism of gangliosides (Gray et al 1969).

TABLE 2.1

TYPES OF HUMAN LACTASE

Enzyme	pH optimum	Subcellular localization	Function	Substrate	Reaction with PCMB
Lactase I (Neutral beta-galactosidase)	5.5-6.0	brush border	Hydrolysis of ingested lactose	Lactose, cellobiose, synthetic substrate* less specific	not inhibited
Lactase II (Acid beta-galactosidase)	3.5-4.5	lysosomal	does not participate in digestion of lactose	Synthetic galactosides, lactose less specific	inhibited
Lactase III (Hetero beta-galactosidase)	5.5-6.0	cytoplasmic	Function not understood	Synthetic galactosides only	inhibited

* Synthetic galactosides = o - nitrophenyl beta-galactoside
p - nitrophenyl beta-galactoside
phenyl beta-galactoside
2 - naphthyl beta-galactoside
6 - bromo-2-naphthyl beta-galactoside

PCMB = p-chloromecuribenzoate

Beta-galactosidase III: A third type is found in the cytosol of the enterocyte of man as well as monkeys and rabbits (Gray and Santiago 1969). This enzyme also has a pH activity curve identical with that of enzyme I. It shows specificity only for synthetic beta-galactosides, and is inhibited by PCMB. Its activity is greatest in the jejunum. Because of the many similarities between this type and the neutral lactase, Gray et al (1969) have suggested that Type III may be a precursor or a fragment of neutral lactase. This suggestion has not been confirmed. Similar patterns of activities of the different lactases in infants and adults with lactase restriction and lactase persistence do not support the suggestion (Lebenthal et al 1974) that these two types of lactases represent an infantile form and the adult form and that the latter is either persistent in lactase persistence or switched off in lactase restriction.

3.2 Localisation of lactase

For many years lactase was thought to be a constituent of intestinal secretions - "succus entericus" - and that the hydrolysis of disaccharide took place in the intestinal lumen.

As far back as 1890 Dastre (Dastre 1890), in experiments on rabbits, dogs and cattle, found no lactase activity in the "succus entericus", the digestive juice secreted by the small intestine. Forty years later Cajori (1935) showed, in dogs, that disaccharidases were not secreted. The current concept that hydrolysis of disaccharides occurs in the mucosal cell was established by Borgstrom and his co-workers (Borgstom and Dahlqvist 1958, Dahlqvist and Borgstrom 1961), who showed that there were only traces of disaccharidase activity in the luminal contents of the intestine. It is now well established that the disaccharidases are located in the brush-border of the small intestinal epithelial cells, a subject reviewed in detail by Dahlqvist and Gray (Dahlqvist 1967, Gray 1970 and 1975), and recently by Semenza and Alpers (Semenza 1981, Alpers 1987).

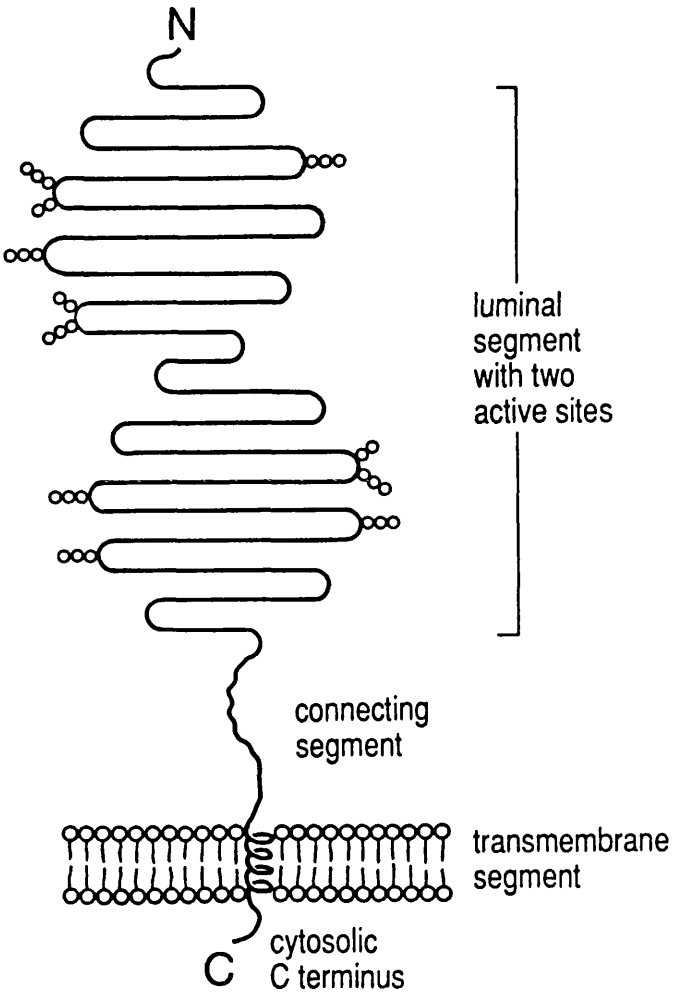
In the human, enzyme activity increases from the proximal to the distal duodenum (Welsh et al 1966) with peak activity in the jejunum or proximal ileum (Triadou et al 1983). Lactase levels in the distal ileum

are low (Auricchio et al 1963a, Newcomer and McGill 1966) with no appreciable activity in the normal stomach or colon (Auricchio et al 1963a, Sheehy and Anderson 1965).

The cellular location of lactase was earlier thought to be various intracellular sub-fractions, but was shown in 1961 by differential centrifugation of mucosal homogenates to be localised in the brush-border of the intestine (Miller and Crane 1961a). Doell et al (1965), using fluorescent antibody techniques, showed that the enzyme was located at the luminal surface of the brush-border and localised to the plasma membrane of the microvillus. A simple method for sub-cellular fractionation of the intestinal mucosa of peroral biopsy (Peters 1976) and a highly sensitive fluorigenic assay for enzyme activity (Peters et al 1976) has confirmed the intestinal brush-border location of the lactase. After homogenisation and centrifugation a cytosolic fraction and total membrane fraction are obtained. Calcium precipitation of the membrane fraction separates this into a microvillar membrane fraction (containing the brush border enzymes) and a non-microvillar fraction (containing the cytosolic membranes and basolateral membranes) (Danielsen 1982). The ability to prepare brush border vesicles and subcellular fractionation techniques have helped in determining the brush border location of the enzyme, a subject reviewed by Semenza (Semenza 1986). The enzyme is an intrinsic membrane protein in that it cannot be extracted from the membrane by solutions of low or high ionic strength or moderately acidic or alkaline pH values. Papain treatment of the lactase-phlorizin complex has demonstrated that there is a hydrophobic segment (40,000 daltons) which anchors the complex to the brush border membrane (Skovbjerg et al 1981). The single polypeptide carrying the two catalytic sites is anchored to the brush border membrane via the hydrophobic sequence of some nineteen amino acids long which is located near the C-terminal end. This anchoring segment crosses the membrane bilayer only once in the direction of N_{out} - C_{in}, the short hydrophylic C terminus being located in the cytosol (Mantei et al 1988, Fig 2.3).

3.3 Physico chemical characterization of lactase

Using monoclonal and polyclonal antibodies (Potter et al 1985, Skovbjerg et al 1981, Swallow et al 1985) and immunoaffinity



Adapted from Mantei et al 1988

Figure 2.3

Diagramatic representation of lactase-phlorizin hydrolase.

chromatography, purified lactase has been produced (Lau 1987). This has helped in the physicochemical characterisation of the enzyme protein. The beta-galactosidase complex consists of two active sites which can be differentiated by heat inactivation and by kinetic criteria (Dahlqvist and Semenza 1985, Skovbjerg et al 1981, 1982). One hydrolyses lactose into glucose and galactose (EC 3.2.1.23) and the other hydrolyses phlorizin into glucose and phloretin (EC 3.2.1.62). The "phlorizin hydrolase" activity has been shown by many workers to be present in many species including man (Malathi and Crane 1969, Schlegel-Haueter et al 1972, Colombo et al 1973, Leese and Semenza 1973). In purified preparations of lactase, the phlorizin hydrolase activity is also present and the ratio of lactase to phlorizine hydrolase activity is the same at all stages of purification, indicating that the two active sites are closely associated with each other (Schlegel-Haueter et al 1972). The age dependency of the phlorizin hydrolase activity is similar to that of lactase (Colombo et al 1973) and is low in adult humans with low lactase activities (Lorenz-Meyer et al 1972).

However the two activities have different heat stabilities and are inhibited differently by Tris (Schlegel-Haueter et al 1972, Skovbjerg et al 1982). Lactose does not inhibit phlorizin hydrolase activity, whereas phlorizin inhibits competitively lactase activity. These two enzyme activities are now called the lactase-phlorizin hydrolase complex (LPH, EC 3.2.1.23/62). It was demonstrated that the natural substrate for the phlorozin hydrolase activity to be glucosylceramides and galactosyl ceramides, both of which are components of the fat globules in milk. Thus the phlorizin hydrolase activity is also (Leese and Semenza 1973) referred to as the "glycosylceramidase activity". However the ceramides are not practical substrates for investigation as they are expensive and not water soluble, and thus phlorizin is used in the investigation of the enzyme complex.

Gel filtration and polyacrylamide gel electrophoresis of purified lactase suggest that the molecular weight of the native human enzyme complex determined without any denaturing is approximately 320,000 daltons (Skovbjerg et al 1981). However under denaturing conditions this complex yields a single band with an apparent molecular weight of 160,000 daltons. This indicates that the two subunits of the complex

have identical molecular size and are non-covalently bound to each other. This has been confirmed by electron microscopic studies of the purified lactase-phlorizin hydrolase complex which shows that the molecule is composed of two identical structures (Skovbjerg et al 1981). Recently the LPH gene has been cloned (Mantei et al 1988) and the molecular weight of the mature enzyme calculated from the cDNA deduced aminoacid sequence is approximately 120,000 daltons which is close to the figure of 160,000 obtained from gel filtration studies. It has also confirmed that both lactase and phlorizin hydrolase active sites are present on the single polypeptide chain. Thus it is possible that the enzyme polypeptide dimerises to yield the 320,000 dalton form seen by gel filtration studies and electron microscopy (Skovbjerg et al 1981).

3.4 Biosynthesis of lactase

Raising of polyclonal and monoclonal antibodies and effective methods of isolating microvillar membranes from the intestinal biopsy specimens (Skovbjerg et al 1981, Potter et al 1985, Swallow et al 1985, Lau 1987) together with the availability of translatable mRNA have aided the study of the biosynthetic stages of microvillar proteins (Sjostrom et al 1983, Danielsen et al 1984a and b).

The precursor forms of lactase has been studied using Ca^{++} precipitation method which is useful in the study of non-microvillar proteins (Skovbjerg et al 1984). The gene coding for the LPH complex is localised on chromosome 2 (Kruse et al 1988) and the cloning of the cDNA of the LPH complex (Mantei et al 1988) has also established the primary structure of the pre-pro form of the enzyme. The long pre-pro LPH with 1927 amino acid residues is made up of different segments: (1) a cleaved signal sequence of 19 amino acids; (2) a large 'pro' portion accounting for about 40% of the pre-pro form which does not appear in the mature enzyme complex; (3) the portion that finally forms the mature enzyme complex with both catalytic sites; (4) the membrane spanning hydrophobic segment near the C terminus which serves as the membrane anchor; and (5) a hydrophilic C terminus which becomes the cytosolic end of the polypeptide.

In the endoplasmic reticulum the pre-pro form is glycosylated to a high mannose form which then proceeds through the Golgi membranes where trimming and complex glycosylation takes place (Naim et al 1987). Unlike sucrase-isomaltase which is cleaved extracellularly, lactase is acted on by intracellular proteases, as it has been demonstrated that the pancreatic proteases are not responsible for the cleaving of the lactase precursor (Skovbjerg et al 1982). Of the 1927 amino acid residues of the pre-pro LPH only about 60% of the C terminal end appear in the brush border membrane. The role of the 847 amino acid segment ('pro' segment) is not understood.

In tissue cultures it has been shown that the movement from the endoplasmic reticulum to the Golgi membrane takes 60-90 minutes (Danielsen 1982, Danielsen et al 1984(a), Hauri et al 1985). From the Golgi complex the 160,000 mature enzyme complex passes directly to the luminal pole of the enterocyte where it is inserted to the microvillar membrane.

Thus it can be seen that the biosynthesis of the beta-glycosidase is in some ways similar to the alpha-glucosidases in that a single polypeptide precursor with two active sites is formed which undergoes complex glycosylation through its passage in endoplasmic reticulum and the Golgi complex and is targetted directly onto the brush border membrane. However, in several respects it differs from the alpha-glucosidases: (1) the LPH pre-pro form consists of a cleavable signal which is absent in the alpha-glucosidase precursor; (2) LPH precursor undergoes proteolytic cleavage intracellularly, whereas the sucrase-isomaltase is cleaved by pancreatic proteases; (3) anchoring of the LPH to the brush border membrane is different. That is, the C terminal end is anchored with a C_{in} - N_{out} direction whereas the N terminal end of the alpha-glucosidases is anchored to the membrane with N_{in} - C_{out} direction.

3.5 Lactase and ageing

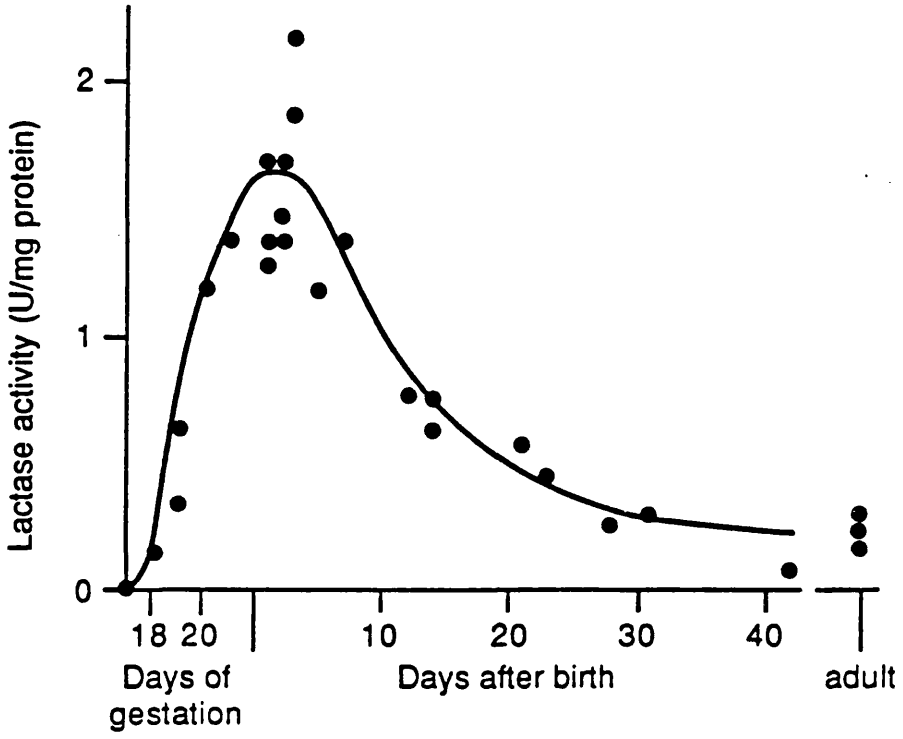
In all the mammals and in the majority of the humans, intestinal lactase activity decreases with age. In 1907 Mendel and Mitchell showed that lactase was present in the intestine of infant animals but was absent or

diminished in the adult animal. In the human foetus lactase can be detected from the third month of gestation (Auricchio et al 1965, Antonowicz and Leventhal 1977) and increases throughout gestation, reaching a maximum at delivery (Mobassaleh et al 1988). In all mammals except humans, lactase activity then declines (Henning 1981). Low levels are reached after the first three weeks of life in the rat (Alvarez and Sas 1961), after five weeks in the pig (Bailey et al 1956) and after nine months in the cow (Heilskov 1952). Lactase activity in a mammal at various ages is shown in Fig 2.4. In humans this course is variable. In some racial groups the adult intestine does not possess adequate levels of lactase, whereas in some others the intestinal lactase persists into adult life (Auricchio et al 1963a, Haemmerli et al 1965). Further information is provided in this thesis (Chapter 3, 2.2, Chapter 8, 4).

4. OTHER DISACCHARIDASES (OLIGOSACCARIDASES)

The disaccharidases other than lactase are capable of hydrolysing oligosaccharides of more than two glucose residues and hence the preferred terminology is oligosaccharidases (Table 2.2). They form integral constituents of the intestinal brush border membrane. They are all large glycoproteins having pH optima around 6 and apparent Km values in the low millimolar range (1-20 mM). They are all (except lactase) alpha-glucosidases. These can be separated and identified by gel electrophoresis (Maestracci et al 1975) or sucrose gradient fractionation (Peters 1976) of the brush border membrane. The two alpha-glucosidases have also been characterised, and reviewed recently (Semenza and Auricchio 1989).

Maltase-glucoamylase: This enzyme complex consists of two subunits which have similar but not identical substrate specifities. They hydrolyse 1-4 alpha-glucosidic bonds from the non-reducing ends of amylose, amylopectin and glycogen and 1-4 alpha-glucosidic bonds of oligosaccharides and maltose.



Adapted from Doell & Kretchmer 1962

Figure 2.4

Lactase activity in the developing rat.

TABLE 2.2

OTHER DISACCHARIDASES (OLIGOSACCHARIDASES)

Name	Other names	Substrate	Products of hydrolysis
Gluco-amylase EC 3.2.1.20	maltase	maltose a (1-4) links of oligosaccharides	glucose residual oligosaccharides with (1-6) links
Sucrase-Isomaltase EC 3.2.1.48/10	Sucrase subunit (Invertase) EC 3.2.1.48	Sucrose maltose, maltoriose, a-(1-4) links of a-dextrins	Glucose, fructose, residual oligosaccharides
	Isomaltase subunit (a-dextrinase) EC 3.2.1.10	a-(1-4) links of oligosaccharides, a-(1-6) links of a-dextrins	glucose
Trehalase EC 3.2.1.28	Trehalase	Trehalose	glucose

Sucrase-Isomaltase: This complex with two active sites is responsible for the hydrolysis of maltose, maltotriose and sucrose. The isomaltase subunit is important in the final digestion of carbohydrates in that it is responsible for the hydrolysis of 1-6 linkages of alpha-dextrins. Isomaltose is not a physiological substrate in man. Hence it is sometimes referred to as "Sucrase-alpha-Dextrinase".

Sucrase-isomaltase deficiency is a rare inherited disorder which presents with intolerance to starch and sugar in children (Weijers et al 1961, Cooper et al 1979) and rarely in adults (Cooper et al 1983, Naim et al 1988).

Trehalase: Hydrolyses trehalose, a disaccharide found in mushrooms.

The oligosaccharidase activity can be measured by methods described by Dahlqvust (1964). Recently a more sensitive fluorometric modification has been described (Peters et al 1976, 1975).

As a compound system the beta-galactosidase and the several alpha-glucosidases mentioned above promote the hydrolysis of all oligosaccharides presented to the brush border, to monosaccharides (glucose, galactose and fructose) which are then absorbed by specific transport systems.

5. MONOSACCHARIDE TRANSPORT SYSTEMS

Glucose accounts for 80% of the monosaccharides (glucose, fructose, galactose) released after sequential hydrolysis of starch oligosaccharides and disaccharides. The hydrolysis of lactose gives rise to glucose and galactose. Glucose and galactose share a specific active sodium dependent transport system. Fructose on the other hand is transported across the intestine by a non-active sodium independent system (Gray 1975).

5.1 Glucose-Galactose transport

Both glucose and galactose are transported by the same transporter. The hypothetic model for sodium coupled active transport of glucose and galactose was proposed by Crane in 1962 (Crane 1962). This model, now known as the sodium-gradient hypothesis, has been tested from many experimental standpoints (Semenza et al 1984) and has accommodated some twenty-five years of research without much modification (Hopfer 1987, Sacktor 1989).

The concentrative uphill transport of glucose is located at the brush border membrane and is as a result of co-transport wth Na^+. The influx of Na^+ is driven by its electrochemical gradient across the brush border membrane which is maintained by the activity of the Na^+-K^+-ATPase pumping mechanism located at the basolateral membrane that extrudes sodium into the serosal environment (Fig 2.5). The efflux of the accumulated glucose from the intestinal cell into the blood stream is by facilitated diffusion located at the basolateral membrane driven by the chemical gradient.

The energetics of Na-cotransport has been researched both on intact epithelia and brush border membrane vesicles and it is now established that the Na^+ electrochemical gradient can indeed energise the uphill accumulation of the monosaccharides (Freel and Goldner 1981, Semenza et al 1984). A mechanistic model has been detailed in which Na/glucose transporter being functionally asymmetric with respect to the membrane operates as a gated channel with a snip-snap mechanism (Semenza et al 1984). Kinetic studies (Peerce and Wright 1984a and b) have suggested that sodium acts as a competitive activator of glucose transport by inducing a conformational change and increases the affinity for glucose. Inside the cell the lower sodium concentration and high potassium concentration has the opposite effect thus releasing the glucose into the cytoplasm.

Sodium dodecyl-sulphate gel-electrophoresis and photoaffinity differential labelling using monoclonal antibody techniques of the brush border membrane vesicles has revealed that the functional unit of the Na^{++} glucose carrier is a 72,000 dalton protein (Hosang et al 1981, Schmidt et al 1983, Peerce and Wright 1984a and b).

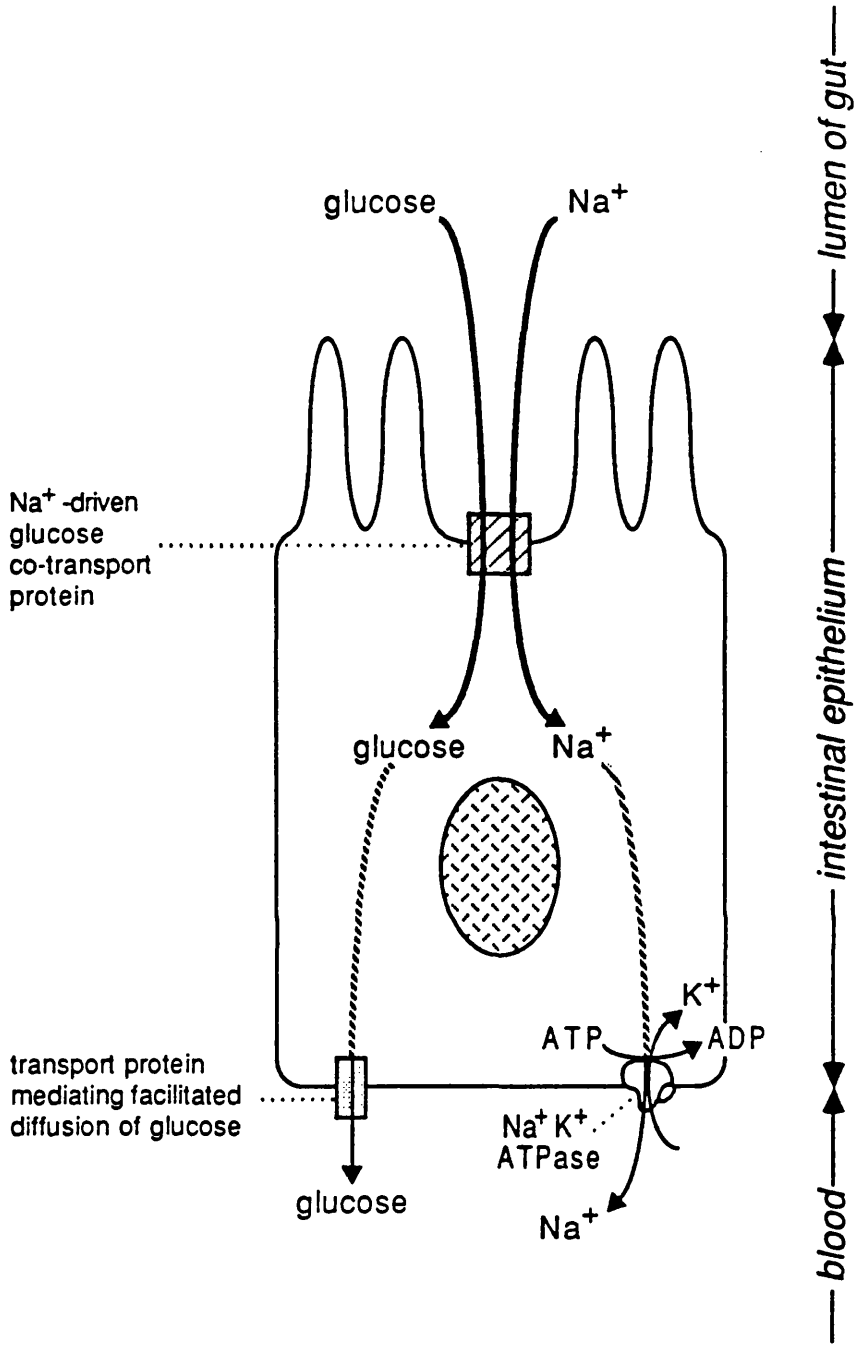


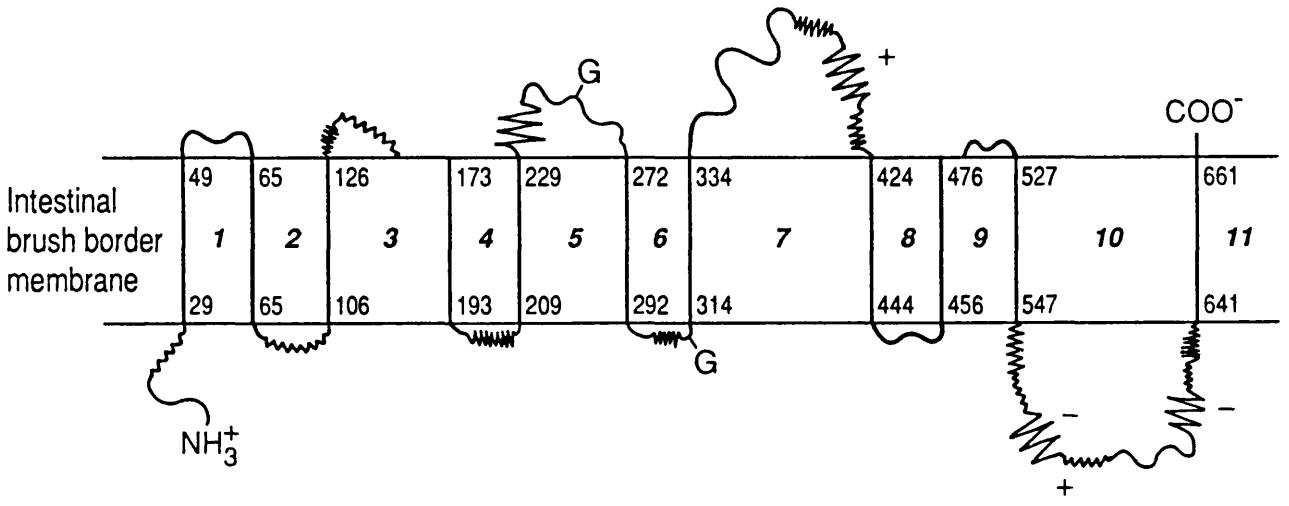
Figure 2.5

Schematic representation of a small intestinal epithelial cell showing transport of glucose from the gut to the blood stream.

Isolation of the mRNA from rabbit intestinal mucosa (Hediger et al 1987b) was a significant advancement. Subsequently the Na/glucose transporter has been cloned (Hediger et al 1987) and the amino acid sequence calculated from cDNA indicate that the Na^+/glucose co-transporter consists of a 662 amino acid peptide with a relative molecular mass of approximately 73,000 daltons (Hediger et al 1987) which is consistent with the estimation of 72,000 based on SDS gel electrophoresis (Peerce and Wright 1984a and b). From the predicted amino acid sequence the hydrophobicity plot has been generated. This indicates that the structure of the transporter consists of eleven membrane-associated beta-helices. Some of these sequences help to stabilise the structure by forming H-bonds and salt bridges (Fig 2.6).

BBV studies on sodium and glucose flux rates at different glucose concentrations and in vitro studies on intestinal mucosa have been studied using glucalogues which are monosaccharides of the glucose group. These studies (Honegger and Semenza 1973) indicate that there are two types of Na/glucose transporters on the intestinal brush border. Carrier 1 is the major high capacity low affinity saturable system with sodium adding first and is characterised by an apparent 1:1 (sodium/glucose) coupling stoichiometry (Kaunitz and Wright 1984). This also has a broad specificity (it interacts with D-glucose, D-galactose, deoxy-D-glucose, 3-methyl-D-glucose and arbutin) and is more prominent at birth (Honnegar and Semenza 1973). The Carrier 2 is a low capacity high affinity system which is multivalent for sodium (Kaunitz and Wright 1983). It does not interact with other glucalogues and is less prominent at birth. In addition a hydrolase associated glucose transport system has been suggested (Malathi et al 1973, Ramaswamy et al 1984). However infusion studies on patients with glucose-galactose malabsorption (who do not have a functional Na/glucose transporter) do not support the presence of this hydrolase associated glucose transport (Fairclough et al 1978, Beyreiss et al 1985).

The distribution of the Na-glucose carrier along the small intestine has been studied recently (Harig et al 1989) which suggests that the order of frequency is distal jejunum > proximal jejunum > distal ileum. It has also been suggested that the two Na/glucose transporters of different



Adapted from Hediger et al 1987

Figure 2.6

Proposed model for the orientation of the Na^+-glucose cotransporter.

kinetic properties, described above, are distributed differently in the small intestine. That is the major high capacity system is distributed in the proximal part where the luminal glucose concentration is high, and the low capacity high affinity transporter is present in the distal intestine which is well suited for the low luminal glucose concentrations. It has also been suggested that one may be present at birth and the other appears later (Honegger and Semenza 1973).

Deficiency of the Na^+-glucose transporter is a rare inherited disorder (Laplane et al 1962, Lindqvist and Meenuwisse 1962) which presents with profuse neonatal diarrhoea. Using brush border vesicles it has been shown that the specific defect in this disorder is the sodium dependent glucose-galactose transporter (Booth et al 1988). The improvement in glucose intolerance seen with age in human glucose-galactose malabsorption supported by the change in relative activities of Carrier 1 and Carrier 2 seen in hamster with age, suggest that there is one glucalogue carrier present at birth and a second develops with age and that in glucose-galactose malabsorption it is the Carrier 1 that is affected (Honegger and Semenza 1973, Evans et al 1985).

The purification and preparation of basolateral membrane vesicles (Wright et al 1980) has provided evidence that the glucose transporter on this membrane is Na^+ independent and operates by facilitating the diffusion of the monosaccharide down their chemical gradient (Hopfer et al 1976).

6. FACTORS AFFECTING THE BLOOD GLUCOSE CONCENTRATION AFTER AN ORAL LOAD OF LACTOSE

The blood glucose concentration is a function of the appearance rate and disappearance rate of glucose from the blood compartment. Factors that influence the appearance rate of glucose into the blood compartment are:

1) The absorption of glucose from the gut. A part of this glucose is produced by the hydrolysis of lactose.
2) Glycogenolysis.
3) Gluconeogenesis.

Factors that influence the disappearance rate of glucose from the blood compartment are:

1) Glycolysis.
2) Glycogenesis.
3) Lipogenesis.

Thus there are many factors that affect blood glucose concentration of which hydrolysis of lactose is only one. These factors are of importance when evaluating results, for example of lactose loads, where the rise in blood glucose is used as a measure of the rate of hydrolysis of lactose by intestinal lactase.

7. SUMMARY

Lactose is the principal carbohydrate constituent of milk which is the sole nutrition of the suckling mammal. Lactase located on the small intestinal brush border is necessary for the hydrolysis of lactose into glucose and galactose which are then absorbed by a common sodium dependent active transporter embedded on the brush border. The beta-galactosidase complex with lactase activity that hydrolyses the milk sugar lactose, and phlorizin hydrolase (glycosylceramidase) active site, that aid in the digestion of milk fat, is called the lactase-phlorizin hydrolase (LPH). This enzyme has been characterised and cloned and the amino acid sequence deducted. It is a stalked protein of approximately 160,000 dalton anchored to the brush border membrane by a short C terminal sequence. Both hydrolase active sites are situated on the same polypeptide. The biosynthetic pathway is also characterised. The precursor is a large peptide of nearly two thousand amino acids which undergoes complex glycosylation and intracellular trimming by proteases to the mature enzyme complex (some 60% of the original molecular mass) which is inserted into the brush border membrane (Chapter 3, 3.3, 3.4).

The Na/glucose transporter is a smaller protein (72,000 dalton) embedded more deeply in the brush border membrane. This protein has also been cloned and the DNA and amino acid sequence deduced.

The kinetic mechanisms of glucose transport have been well studied and characterised (5.1).

CHAPTER 3

REVIEW OF LACTASE PHENOTYPES

1. INTRODUCTION

At the beginning of this century it was known that carbohydrates in food can cause diarrhoea (Schmidt and Strasburger 1901). In 1959 lactose malabsorption was described in two children (Holzel et al 1959). It was during this time that analytical procedures to measure intestinal enzyme activity were being developed (Dahlqvist 1961) and localisation of lactose hydrolysis to the brush border membrane of the small intestinal microvilli was being established (Dahlqvist and Borgstrom 1961, Miller and Crane 1961a, Miller and Crane 1961b).

In 1963 it was finally shown that lactase could be absent in histologically normal intestinal mucosa (Dahlqvist et al 1963, Auricchio et al 1963a).

2. PHENOTYPES

There are three phenotypes that can be recognised in humans. One phenotype with low or absent intestinal lactase presenting at birth, termed congenital lactase deficiency. The other two types refer to the intestinal lactase status in the adult. One with low intestinal lactase in adult life (lactase restriction phenotype) and the other with high intestinal lactase persisting into adult life (lactase persistence phenotype).

2.1 Congenital lactase deficiency

This condition was first described by Holzel et al (1959). Subsequent cases were described by Weijers and Van de Kamer (1962), Durand and Lamedica (1962), Thornton et al (1962), Sobel et al (1963) and Davidson et al (1964). These children present soon after birth. In all these cases the characteristic and outstanding clinical features were diarrhoea induced by milk which is sometimes severe enough to cause failure to thrive (Holzel et al 1959, 1962) and growth retardation (Davidson et al 1964). They thrive when sucrose is substituted for lactose in the diet. It is suggested that this is due to homozygosity for a gene with an autosomal recessive inheritance (Flatz 1987).

2.2 Adult lactase phenotypes

The adult population of the world can be divided into two groups: One group, the majority of the world population, has a high intestinal lactase at birth and during childhood and, like the rest of the animal kingdom (Mendel and Mitchell 1907, Blaxter 1961, Jonas et al 1985), demonstrate low lactase activity in adult life. In the second group, forming the minority, high intestinal lactase persists into adult life (Table 3.1).

Although the former group has been referred to as those with "lactase deficiency", "lactose malabsorption", "adult type lactase deficiency", "primary lactase deficiency" and "acquired lactase deficiency", these terms are misnomers as they suggest abnormality.

Originally when physiological studies were restricted to the white European races, lactase activity was found to persist in adult life, and hence it was considered that the human race was an exception to the rest of the animal kingdom and that it was the "normal" condition and the races that showed low lactase activity in adult life were "abnormal" and "lactase deficient". It is, however, unacceptable to classify two thirds of the world population as "abnormal".

Taking the above into consideration, the following terminology has been suggested (Flatz 1987); The world population of adults can be divided into two phenotypic groups based on the ability to hydrolyse lactose in the intestine:

1. High lactose digestion capacity (high LDC). Persistence of intestinal lactase (lactase persistence).

2. Low lactose digestion capacity (low LDC). Restriction of intestinal lactase (lactase restriction).

2.2.1 **Lactase restriction:** Although the first description of this condition is usually ascribed to Auricchio et al (1963a), the first adult cases were probably described over a quarter of a century earlier by Althausen et al (1935). These workers were among the first to recognise that dietary

carbohydrates could be the cause of intestinal symptoms. Although they failed to incriminate lactose, they made a passing comment on milk: "Most frequently the offending article of food is the potato, many patients also have a great deal of trouble with milk". In a study of 50 such patients they noticed that some of them had stools acid to litmus. They then suggested that large amounts of sugar or starch could enter the colon and that "the increased motility of the small intestine might be due directly to the formation of irritating acids by fermentation of the starch and sugars within the lumen". This was 30 years before Haemmerli et al (1965) proposed an identical explanation for lactose-induced diarrhoea in lactase deficient patients. Althausen and co-workers treated their patients with fermented milk. Although they wanted to alter the gut flora, it is interesting to note that fermented milk contains hydrolysed lactose.

The first definite cases of lactase restriction phenotype in normal adult subjects were documented simultaneously by Dahlqvist et al (1963) in the USA and Auricchio et al (1963a) in Switzerland. This was followed by a voluminous literature on "isolated lactase deficiency" in the adult. Cuatrecasas and others (Cuatrecasas et al 1965) established for the first time ethnic variability in the prevalence of the two lactase phenotypes. They studied 61 subjects and showed a significant difference in the number of malabsorbers among whites (27%) and negroes (73%).

Since then a high prevalence of lactase restriction has been established in the African races (Table 3.1). Cook and Kajubi (1966) studied several adults belonging to the agricultural and cattle rearing tribes, and found that the prevalence of lactose malabsorption in the agricultural tribe was significantly higher (89%) than that in the cattle rearing tribe (25%). This was later confirmed by Kretchmer (Kretchmer et al 1971).

Davis and Bolin (1967) first reported the high prevalence of lactose malabsorption among Asians. They also reported a correlation between milk consumption and lactose malabsorption (Bolin et al 1970). They analysed the milk intake of various ethnic groups, including Sri Lankans, and predicted that the prevalence of lactose malabsorption would be high among those with low milk intake.

Elliotte and co-workers (Elliotte et al 1967) established a high prevalence of the lactase malabsorption phenotype among the Australian aborigines.

In Europe the prevalence is highly variable and there seems to be a North-South gradient (Fig 3.1, Table 3.1): the prevalence of low lactase phenotype is lower among Northern Europeans than Southern Europeans.

In Britain the earliest report was in 1965 (McMichael et al 1965) when a prevalence of 32% of lactase restriction phenotype was found among a highly selected group with symptoms of "functional diarrhoea". A retrospective study of lactase activity on patients with histologically normal jejunal biopsies produced a figure of 3% (Ferguson et al 1984). Low prevalence of lactase restriction phenotype among the British adult population has been further confirmed (Pena et al 1973, Ho et al 1982). There are no British paediatric studies.

2.2.1.1 Age dependency of lactase restriction phenotype: As has been described in Chapter 2, in all mammals except man lactase activity is present only during the suckling period, and disappears during the post weaning period.

Man, in some parts of the world, is the only mammal in whom lactase activity is found in appreciable amounts throughout life (Auricchio et al 1963 b, Haemmerli et al 1965) and is the only mammal who (in some parts of the world) consumes milk even after weaning. Among those ethnic groups that show lactase restriction, the condition seems to be clinically expressed between the ages of two and five years (Kattamis et al 1973, Newcomer et al 1977, Keusch et al 1969(a), Elliotte 1967, Lisker et al 1974, Woteki et al 1976, Flatz et al 1982a) corresponding to the species specific post weaning age (Simoons 1980).

2.2.1.2 Global prevalence of lactase restriction: A summary of the distribution of adult lactase phenotypes in the world population and the relevant references are shown in Table 3.1 and Fig 3.1.

2.2.2 **Lactase Persistence:** Populations mainly living in Northern Europe and North America and some of the nomadic races in North Africa (Fig 3.1) have a high prevalence of lactase persistence phenotype. In these

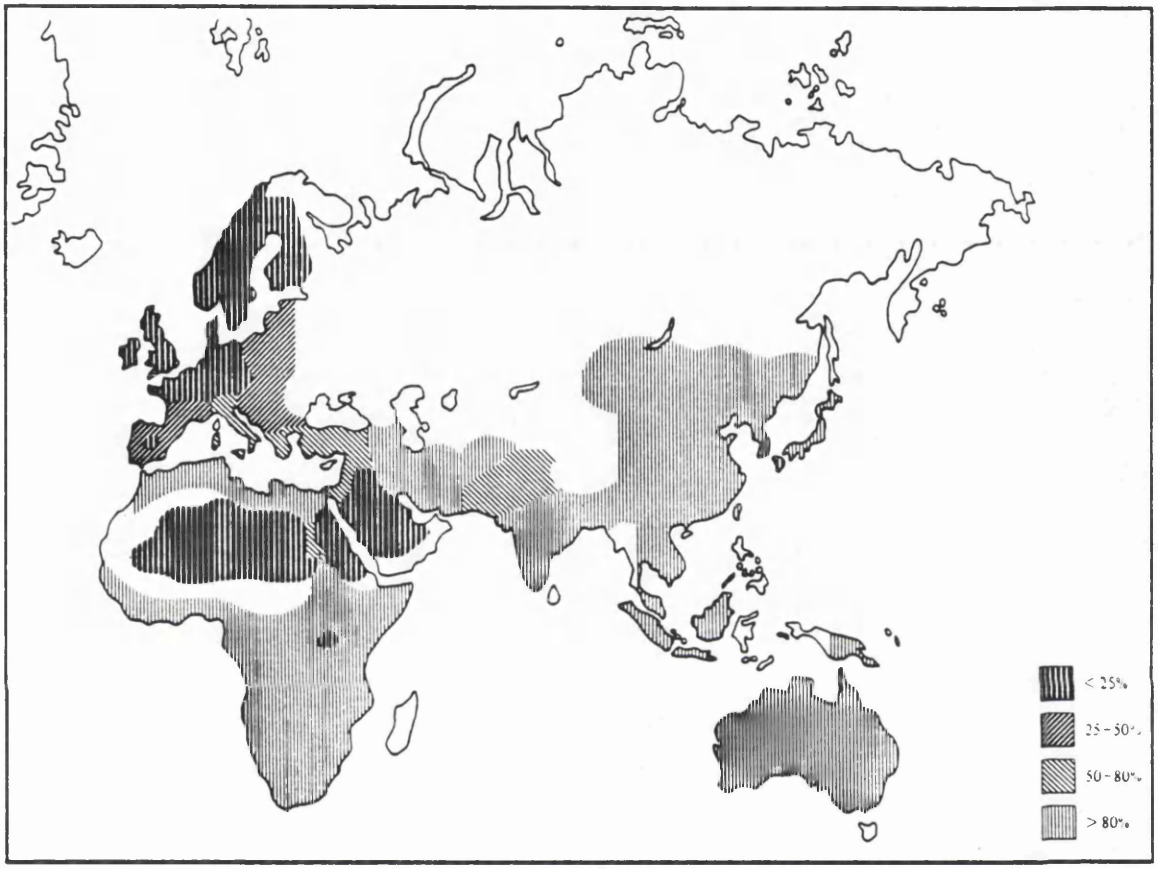
TABLE 3.1

DISTRIBUTION OF THE LACTASE PHENOTYPES IN DIFFERENT POPULATIONS OF THE WORLD (adapted from Flatz 1987)

Country	Population	Number of Subjects	Percent low Lactase	Reference
Populations with predominant lactase restriction				
Nigeria	Ibo, Yoruba	113	89	Kretchmer et al (1971)
Central Africa	Mostly Bantu	112	95	Elliott et al (1973)
Sudan	Blacks (farmers)	84	76	Bayoumi et al (1981)
	Nilotics	282	75	Bayoumi et al (1982)
Ethiopia	Ethiopian children (7 years)	58	90	Habte et al (1973)
Uganda	Bantu	114	99	Cook and Kajubi (1966); Cook et al (1967)
South Africa	Bantu	172	84	Cook et al (1973)
	Bushmen	65	95	Jenkins et al (1974); Nurse and Jenkins (1974)
	Herero	37	97	Currie et al (1978)
Morocco, Tunisia	Maghrebi	55	78	O'Morain et al (1978)
Egypt	Northern Egyptians	388	73	Halstead et al (1969);
	Central Egyptians	111	85	Hussein et al (1982)
Israel	Ashkenazic Jews	53	79	Gilat et al (1970)
Middle East	Jordanians	204	79	Snook et al (1976)
Iran	Iranians (12 years)	40	83	Sadre and Karbasi (1979)
Thailand	Thais	428	100	Flatz et al (1969); Keutsch et al (1969b); Flatz and Saengudom (1969)
China	Chinese	248	92	Wang et al (1984)
Taiwan	Chinese	71	100	Sung and Shih (1972)
Indonesia	Javanese	53	91	Surjono et al (1973)
Japan	Japanese	66	85	Shibuya et al (1970); Yoshida et al (1975); Nose et al (1979)
Pakistan	Pakistanis	414	60	Ahmad and Flatz 1984
Populations with predominant lactase persistance				
Subgroup I. Nomadic Pastoralists in North Africa and Arabia				
Sudan	Nomads	51	24	Bayoumi et al (1981)
Sudan	Beja, Nomads	303	17	Bayoumi et al (1982)
Uganda	Hima, Tussi	70	7	Cook and Kajubi (1966); Cook et al (1967).
Nigeria	Fulani	9	22	Kretchmer et al 1971)
Jordan	Bedouins	162	24	Hijazi et al (1983)
Saudi Arabia	Mainly Bedouins	22	23	Cook and Al-Torki (1975)

Country	Population	Number of Subjects	Percent low Lactase	Reference
Subgroup II. Northwestern Europeans				
Sweden	Swedes	400	1	Dahlqvist and Lindquist (1971)
Finland	Swedish-speaking	91	8	Sahi (1974b)
	Finnish-speaking	449	17	Sahi (1974b); Jusilla 1969b); Jusilla et al (1970)
Denmark	Danes	761	3	Gudmand-Hoyer et al (1969)
Britain	British	96	6	Pena et al (1973); Ho et al (1982)
			3	Ferguson et al 1984
Ireland	Irish	50	4	Fielding et al (1981)
France	Northern French	62	23	Cuddenec et al (1982)
Spain	Spaniards	265	15	Pena Yanez et al (1971b)
Germany	Germans	646	14	Rotthauwe et al (1972a); Flatz et al (1982b)
Switzerland	Swiss	64	16	Auricchio et al (1963b); Haemmerli et al (1965)
Austria	Austrians	528	20	Rosenkranz et al (1982)
Czechoslovakia	Czech	200	13	Madzarovova-Nohejlova (1982)
Soviet Union	Leningrad Russians	248	15	Valenkevich (1977)
Populations with intermediate lactase phenotype distribution				
Italy	Northern Italians	383	50	Marenco et al (1970)
Yugoslavia	Slovenians	153	35	Micetic-Turk (1982)
Hungary	Hungarians	535	37	Czeizel et al (1983)
	Matyo	172	37	Czeizel et al (1983)
Poland	Poles in Canada	21	29	Leichter (1972)
	Poles	275	37	Socha et al (1984)
Pakistan	Pandjab	53	45	Abbas and Ahmad (1983)
	Pandjab	322	59	Ahmad and Flatz (1984)
India	Northern Indians	264	27	Murthy and Haworth (1970)
Sudan	Nile Valley Sudanese	272	53	Bayoumi et al (1981)
	Nomads (cattle-owning)	115	53	Bayoumi et al (1981)
Countries with populations of high and low lactase phenotypes				
North America				
Greenland	Eskimos, "pure"	119	85	Gudmand Hoyer and Jarnum
	Eskimo/European	108	38	(1969); Asp et al (1975)
Canada	Whites	16	6	Leichter (1972)
	Amerindians	30	63	Leichter and Lee (1971)
United States	Alaskan, Eskimos and Indians	36	83	Duncan and Scott (1972)
	Amerindians	221	95	Bose and Welsh (1983); Newcomer et al (1977)

Country	Population	Number of Subjects	Percent low Lactase	Reference
	Northern European extraction	188	7	Cuatrecasas et al (1965); Welsh (1970); Duncan and Scott (1972)
	Whites	913	22	Sheehy and Anderson (1965);
	Blacks	390	65	Bayless and Rosensweig (1966); Gray and Santiago (1966); Welsh et al (1967); McGill and Newcomer (1967); Newcomer and McGill (1967); Page et al (1975)
	Mexican-Americans	305	52	Dill et al (1972)
Mexico	Mexicans	401	83	Lisker et al (1974)
South America				
Colombia	Mestizos	45	33	Alzate et al (1968)
	Chami Indians	24	100	Alzate et al (1969)
Peru	Mestizos	94	72	Figueroa et al (1971)
Bolivia	Aymara (10 years)	31	77	Balanza and Taboada (1985)
Brazil	Whites	53	49	Duarte and Dutra de Oliveira (1978)
	Nonwhites	31	74	Seva-Pereira et al (1983)
Australia and Oceania				
Australia	Whites	133	5	Bolin et al (1968)
	Aborigines	145	67	Bolin and Davis (1970); Brand et al (1983)
Papua	Tribal groups	123	90	Cook (1979)
Fiji	Fijians	12	100	Masarei et al (1972)



Adapted from Flatz 1986

Figure 3.1

Global prevalence of lactase restriction phenotype.

individuals the post weaning decline of intestinal lactase does not take place. These populations are also those who consume large amounts of milk in adult life. The suggested aetiology for lactase persistence in these populations is discussed below. There is some evidence of health hazards such as hyperlipidaemia (Sahi et al 1977), coronary heart disease (Segall 1980) and premature cataract among these populations (Simoons 1982) linked to high milk consumption.

3. AETIOLOGY OF THE ADULT LACTASE PHENOTYPES

The controversy over the aetiology of low intestinal activity in the adult human started in 1965 (Haemmerli et al 1965) and still continues. There are two theories. One is that the low intestinal lactase in the adults is an adaptive response to the lack of substrate (milk). Although there is general correlation among world populations that the non-milk drinking races are those with high prevalence of low intestinal lactase (Bolin and Davis 1969; Bolin et al 1970; Flatz 1987; Simoons 1970b), there is no correlation in individual cases. The alternative theory is that the adult lactase phenotypes are inherited.

3.1 **Adaptation of lactase to the lactose content of food as basis for aetiology:** Adaptation by animals to the lactose content of food has been studied widely (3.1.1), but evidence for this phenomenon in man is only circumstantial and experiments on lactose feeding in humans have not yielded conclusive results (3.1.2).

3.1.1 **Animal studies:** Adaptation of lactase activity of bacteria (E.coli) to the lactose content of the culture medium is well documented (Monod et al 1951). In pregnant mammals milk is produced in the mammary gland during the latter months of pregnancy, and this correlates closely with the appearance of lactase in the intestinal villi of the fetus (Mendel and Mitchell 1907). The earliest work, which claimed to support the ability of animals to adapt to lactose, is that of Fischer and Sutton (1953). They showed that after an oral dose of lactose, less lactose (61%) remained in the stomach and the intestine in rats pre-fed with lactose for six weeks as compared with a group of control animals who had 73% of the administered dose remaining in the stomach and the intestine. However, these experiments were unsatisfactory since most

(58%) of the "unabsorbed" lactose in the control group was still in the stomach. Plimmer (1906) fed adult rats with food containing 25% lactose and did not find an induction of lactase activity in the gut. He concluded (Plimmer 1906) that the rat intestine was incapable of adaptation to lactose and this was subsequently confirmed by others (Heilskov 1952, Alvarez and Sas 1961).

When intestinal lactase activity could be accurately assayed, more important evidence in favour of adaptation was reported. Girardet and his co-workers (1964) showed a four-fold rise in lactase activity in adult rats fed with 25% lactose and this was confirmed in several studies (Cain et al 1969, Bolin et al 1969). A more recent study (Tsuboi et al 1985) of rats on high carbohydrate diet showed that all disaccharidases including lactase increased and ^{3}H leucine incorporation indicated that the increase in enzyme activities were due to stimulation of their synthesis.

3.1.2 **Study in Humans:** The evidence in humans so far does not show that lactase is an adaptive enzyme. There was no change in the intestinal lactase activities after periods of lactose exclusion from the diet (Knudsen et al 1969). Similarly a high lactose diet did not affect the lactase levels in the intestine (Cuatrecasas et al 1965, Gilat et al 1972).

3.1.3 **Non-specificity of enzyme induction:** Although disaccharidases adapt to the sugar content of the diet (Rosensweig and Herman 1968, Deren et al 1967) the adaptation is non-specific. Various enzymes adapt to a variety of substances. Some adapt to the substrate, others to the end product and still others to substrates which are not related to the enzyme (Monod and Cohen 1952). For example, a fructose diet will increase sucrase activity more than glucose, and glucose more than a no-carbohydrate diet. Prolonged lactose feeding raised the lactase activity in rats (Bolin et al 1969). However, 30% glucose diet raised lactase in the rat more than a low carbohydrate diet, although not as high as a lactose diet. This suggest that, even in the absence of lactose, the presence of glucose (or possibly other carbohydrates) should be able to maintain lactase activity.

3.2 Genetic basis for aetiology

3.2.1 **Evidence for a genetic basis from dietary studies:** Several workers have described individuals who have consumed milk regularly but still have lactose malabsorption and others who have not consumed milk after weaning but their intestinal lactase was high (Flatz and Saengudom 1969, Flatz and Rotthauwe 1971, Sahi 1974a). In families that did not consume milk, some were lactose absorbers and others malabsorbers (Flatz and Saengudom 1969).

3.2.2 **Evidence for a genetic basis from population studies:** An autosomal recessive single gene inheritance has been proposed for the low lactase phenotype based on studies of populations of mixed ancestry with a high and low prevalence of low intestinal lactase (Bayless et al 1969, Flatz and Rotthauwe 1971, Gudmand-Hoyer et al 1973). For example the prevalence of lactose malabsorption among white North Americans was found to be 8.2%. Assuming 100% prevalence among African Negroes, the prevalence among American negroes was estimated to be 62%, which was similar to the actual prevalence of 70% found in that group (Bayless et al 1969).

3.2.3 **Evidence for a genetic basis from family studies:** Several family studies (Table 3.2) have indicated that lactose malabsorption could be inherited via a single autosomal recessive gene. This was conclusively shown by an extensive Finnish study (Sahi 1974a) of eleven probands and 226 relatives of the probands. A large study of 102 adult twins in Hungary also demonstrated a monogenic inheritance (Metneki et al 1984). Thus there is strong evidence that low and high human intestinal lactase in the adult are the phenotypic expression of two alleles (or group of alleles) at an autosomal locus.

3.2.4 **Proposed genotypes:** Based on family studies, a genetic model for the inheritance of intestinal lactase has been proposed (Ferguson and Maxwell 1967; Flatz 1987). The locus for lactase activity is termed LAC, the allele causing post-weaning lactase decline is LAC*R and that causing persistance of intestinal lactase is called LAC*P. The adults therefore will have the following phenotype/genotype relationship:

TABLE 3.2

List of reported family studies of lactase phenotypes
(Adapted from Sahi 1975(a)

	Author	Year of report	Country	No of families	Examined subjects	Examination method
I.	**Both parents and at least one child examined**					
1.	Fischer W Zapf J	1965	Germany	1	6	LTTE[a] (GGTTE)[b]
2.	Ferguson A, Maxwell J D	1967	England	1	8	LTT[c] biopsy[d]
3.	Welsh J D et al	1968	USA	3	16	LTT (GGTT)[e]
4.	Neale G	1968	England	1	9	
5.	Flatz G, Saengudom Ch	1969	Thailand	2	13	LTT (GGTT?)
6.	Welsh J D	1970	USA	5	23	LTT, (GGTT, biopsy in probands)
7.	Gudmand-Hoyer E et al	1970, 1973	Greenland	about 10		LTT, (GGTT, some biopsies)
8.	Ransome-Kuti O et al	1972	Nigeria	13	66	LTT
9.	Gilat T et al	1973	Israel	30	156	LTT, (some biopsies)
II.	**Incompletely examined families**					
1.	Fischer, W Zapf J	1965	Germany	1	3	LTTE (GGTTE)
2.	Siebner, H Klaus D	1966	Germany	2	7	LTT (GGTT)
3.	Fine A et al	1968	Ireland	1	8	LTT (3 biopsies
4.	Welsh J D et al	1968	USA	1	7	LTT
5.	Neale G	1968	England (Pakistan)	1	7	
6.	Gudmand-Hoyer E et al	1969	Denmark	1	3	LTT, (in two subjects GGTT and biopsy)
7.	Gilat T et al	1973	Israel	3	11	LTT

TABLE 3.2 (continued)

III. **Extensive family study**

1.	Sahi	1974(a)	Finland	22	226	LTTE

a Lactose tolerance test with ethanol administration

b Glucose-galactose tolerance test with ethanol administration

c Lactose tolerance test without ethanol

d Determination of histological structure and disaccharidase activities of a jejunal biopsy sample

e Glucose-galactose tolerance test without ethanol Parentheses mean that this examination method was not used on every person.

* From Sahi 1974(a)

Genotype	Enzyme phenotype
LAC*P/LAC*P	Lactase persistence
LAC*P/LAC*R	Lactase persistence
LAC*R/LAC*R	Lactase restriction

3.2.5 Molecular mechanism of the genetic aetiology

In the complex chain of events from coding for a polypeptide precursor of nearly two thousand amino acids, its glycosylation, its homing to the brush border and its membrane insertion, one or many steps could be affected by mutation. The result may be lack of lactase enzyme protein or the appearance of an abnormal enzyme. The beta-glycosidase complex present in the small intestine of individuals with lactase restriction and persistence phenotypes is identical with regard to electrophoretic mobility (Skovbjerg et al 1980, Crane et al 1976), immunological properties (Skovbjerg et al 1980, Potter et al 1985) and specific activity for lactase (Skovbjerg et al 1980, Potter et al 1985) and phlorizin hydrolase (Lorenz Meyer et al 1972).

There is no complete agreement on the mechanism of post weaning decline of intestinal lactase. Some workers have suggested accelerated cell kinetics (Tsuboi et al 1981, Tsuboi et al 1985). Others have proposed decreased rate of synthesis (Jonas et al 1985). Increased proteolytic degradation has been shown by yet others (Seetharam et al 1980). There has also been some evidence that the processing of the pro-beta-glycosidase to the mature enzyme complex would be altered (Nsi-Emvo et al 1987).

Recently the complete primary structure of human beta-galactosidase complex has been established by cloning and sequencing (Mantei et al 1988). The gene coding for the enzyme has been localised to the central part of the long arm of chromosome 2 (Kruse et al 1988, 1989). Levels of lactase mRNA have been measured in the suckling and adult mammals (rats and rabbits) and found to be the same in spite of low lactase activities in the adult (Freund et al 1989, Sebastio et al 1989). similar levels of mRNA were measured in human adults with lactase restriction and persistence phenotypes (Sebastio et al 1989). This suggests that the control of lactase gene expression is at a

post-transcriptional level. There is some evidence that the intracellular proteolytic processing of the lactase precursor is slower in organ culture of small intestinal biopsies from humans with low intestinal lactase activity (Sterchi et al 1989). This would lead to intracellular accumulation of the enzyme and probably to its subsequent degradation.

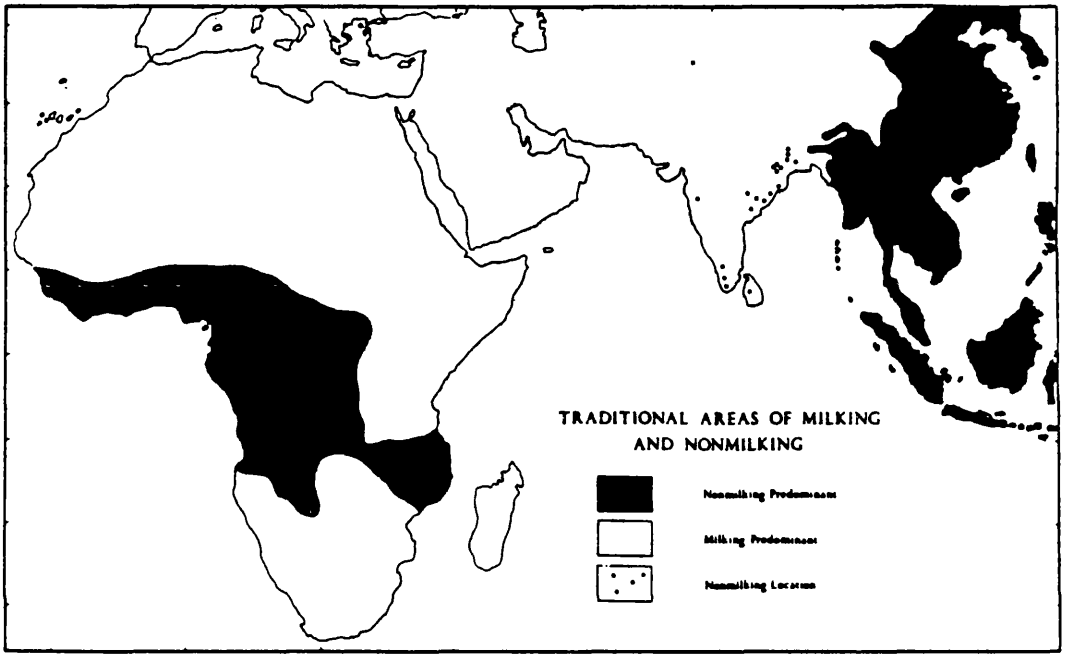
3.3 Effect of pastoralism on the prevalence of lactase phenotypes

When the different ethnic populations of the world are considered, those with high prevalence of lactose malabsorption are also those that do not consume milk after weaning (Fig 3.2, McCracken 1971, Simoons 1970b). These observations were used as evidence for the adaptive theory, that the enzyme loss was due to the absence of the substrate (Bolin and Davis 1970). However, the link between high prevalence of lactose malabsorption and milk drinking habits could also be explained by genetic factors, as such factors may enforce a consumption pattern based on adverse effects and natural selection.

In the biological history of man the use of milk by the adult is a comparatively recent event, dating back roughly to 10,000 years BC (Shatin 1966, Simoons 1970a), when domestication of cattle made this infant food available for consumption after weaning. This historical event evolved at "different times" among various Eurasian, African and Asian cultures.

It could be considered that, as in the rest of the animal kingdom, in humans the LAC*R with low lactase in adult life is the normal gene and that the LAC*P with high intestinal lactase in adult life is a mutation. Such an allele in some populations had a selective advantage and thus became more prevalent. Proposed factors that favoured such a selection are discussed below.

3.3.1 **Milk dependency:** In a study in Sudan among the Beja tribal group where the prevalence of lactase restriction phenotype is only 17%, the milk consumption was about 3 L/day (Bayoumi et al 1982). In their natural environment of barren desert, no other form of nutrition was available, and hence the individuals with the mutated LAC*P gene and high intestinal lactase had natural selection acting in their favour, resulting in a high prevalence of lactase persistence.



from Simoons 1970 (b)

Figure 3.2

Traditional areas of milking and non-milking.

3.3.2 **Lactose and calcium absorption:** In other groups (for example in Northern Europe) with a high prevalence of LAC*P gene, other selective factors such as calcium absorption association with milk consumption is thought to have acted in favour of LAC*P gene.

3.3.3 **Populations which were not affected by pastoralism:** The distribution of lactase restriction in the majority of the world population were not subjected to the selective factors described above. Hence the mutated lactase persistence gene did not have any selective advantage. The low prevalence of lactase persistence found in some of these countries could also be explained by migration of people with the lactase persistence gene.

4. SECONDARY LACTASE DEFICIENCY

This condition is mentioned here for completeness and is not the subject of this thesis. Lactase deficiency and lactose intolerance may occur as a transient phenomenon in a variety of small intestinal diseases (Walker-Smith 1986). In this condition the small intestinal histology is usually abnormal and other disaccharidase activity is also decreased. However, lactase seems to be more vulnerable than the others, and hence sometimes affected selectively. Secondary lactase deficiency is not genetically determined.

5. SUMMARY

Lactase is necessary for the hydrolysis of lactose. Intestinal lactase activity is high during infancy in all mammals when milk is the main nutrient. In all other mammals and about two thirds of humans the lactase activity declined after weaning (lactase restriction phenotype) In some human populations lactase activity persists in the adults. Family studies, population studies and a study of lactase phenotypes among twins have suggested that the phenotypes are genetically determined and that it fits an autosomal inheritance. The lactase restriction gene (LAC*R) is globally more prevalent and is the recessive allele. The lactase persistence gene (LAC*P) is considered the mutated, less prevalent allele which is dominant. There is similarity between the geographic areas of lactase persistence and traditional areas of milking. It is suggested that the mutated dominant gene carrying lactase persistence had a selective nutritional advantage in these areas where milking was established.

The gene coding for lactase has been cloned and localised to chromosome 2. There is some recent preliminary evidence that the molecular mechanism controlling the phenotypic expression include genetic control of post transcriptional processing of the lactase precursor.

CHAPTER 4

REVIEW OF INVESTIGATIVE PROCEDURES TO DETERMINE THE LACTASE PHENOTYPES

The different adult lactase phenotypes are usually not clinically apparent. Therefore direct and indirect methods of establishing the phenotype have been developed, which will be briefly described in this chapter.

1. ORAL LACTOSE LOAD TEST
   - 1.1 **Blood glucose after oral lactose**
   - 1.2 **Blood galactose after oral lactose**
   - 1.3 **Symptoms and stool pH after oral lactose**

2. INTESTINAL LACTASE
   - 2.1 **Intestinal variation of lactase activity**
   - 2.2 **Methodological consideration**
     - 2.2.1 **Choice of substrate**
     - 2.2.2 **Substrate concentration**
     - 2.2.3 pH
     - 2.2.4 **Time of incubation**
   - 2.3 **Definition of the lactase activity and reference values**
   - 2.4 **Improved modifications**
   - 2.5 **Use of oral lactose load and intestinal lactase in detecting lactase restriction phenotype**

3. ^{14} CARBON BREATH TEST

4. BREATH HYDROGEN TEST

5. LACTOSE - BARIUM MEAL

6. SUMMARY

1. ORAL LACTOSE LOAD

"Lactose intolerance" is implied by intestinal symptoms after the lactose load. However, all subjects who express the lactase restriction phenotype do not experience symptoms of intolerance after the lactose load and the diagnosis of a normal or abnormal test is based on the increment of blood glucose concentration after the load. Hence in this thesis the term **lactose load** is used in place of **lactose tolerance test.**

1.1 Blood glucose after oral lactose load

This is one of the indirect methods of assessing the lactase phenotype of an individual. In this test blood glucose is measured at varying intervals before and after an oral lactose load. Although it is the most widely used test, it has not been standardised.

Table 4.1 shows some of the variations in the procedure adopted by different research workers, including varying amounts of lactose, use of venous or capillary blood, varying sampling frequency and duration and different methods of analysing blood glucose.

The amount of the load has varied from 50g of lactose dissolved in 500ml water (10% solution, Newcomer and McGill 1967) to 100gm dissolved in 200ml of water (50% solution, Newcomer and McGill 1966). It is claimed (Newcomer and McGill 1967) that the dose is not critical if the peak rise of blood sugar is used. This claim has not been substantiated. Although different amounts of lactose may not affect the results of the test if there is complete absence of lactase, the result might differ if intestinal lactase is decreased, as the rate of hydrolysis is dependent on substrate concentration.

The time of blood sampling has been variable. Oral lactose has been shown to influence the rate of gastric emptying (Elias et al 1968) and hence the time at which the maximum rise in glucose concentration would occur. This effect might vary between subjects. Thus the frequency and duration of sampling is important in order to catch the maximum increase of blood glucose after the lactose load. Some

TABLE 4.1

REVIEW OF THE CONDITION USED FOR THE ORAL LACTOSE LOAD

Author	Year	Load	Sampling period (min)	Sampling frequencies (min)	Sample v = venous c = capillary
Peternel	1965	100g	120	15-30	V
Haemmerli et al	1965	50g	120	10-30	V
McMichael et al	1965	50g	?	30	V
Newcomer and McGill	1967	50g	120	15-30	V
Cook and Kajubi	1966	50g	150	30	C
McGill and Newcomer	1967	50g	120	15-30	V and C
Keusch et al	1969 a	2g/kg bw	90	15	V
Flatz et al	1969	0.8g/kgbw	30	1 point	?
Bolin et al	1970	2g/kgbw	90	15-30	C
Welsh	1970	50g/m bs^2	-	-	V
		1g/kg bw	-	-	C
Spanidou and Petrakis	1972	50g	90	15-30	C
Sahi et al	1972	1g/kgbw	40	2 samples 20, 40	C
Krasilnikoff et al	1975	2g/kgbw	120	15-30	?
Harrison and Walker-Smith	1977	2g/kgbw	120	30	C
Hijazi et al	1981	2g/kgbw	90	15-30	C
Ahmad and Flatz	1984	50	60	15-30	C
Metneki et al	1984	50g	60	15	C
Fielding et al	1980	50g	60	30	V

investigators (Dunphy et al 1965) sampled at 0, 30, 60, 90 and 120 minutes; others at 0, 15, 30, 45, 60, 90 and 120 minutes (Huang and Bayless 1967), or at 0, 15, 30, 45 and 90 minutes (Bolin et al 1970 a), still others as frequently as every ten minutes (Haemmerli et al 1965). In one population study (Flatz et al 1969) only one sample was taken after the load. It is likely that these differences in sampling procedure have introduced some degree of bias in the reported figures for lactase phenotypes (Ahmad and Flatz 1984).

Capillary or venous sample. Welsh (1970) has shown that the predictive value of the lactose load test is increased if capillary blood rather than venous blood is used for measuring glucose concentration. It was found that loads involving venous blood yielded 19% false results in individuals with normal lactase activity and 14% in those with isolated lactase deficiency. If capillary blood was used less than 1% false tests were obtained in subjects with normal lactase activity and 7% false tests in patients with isolated lactase deficiency.

In view of the many factors affecting the lactose load and the many ways in which it has been done, it is not surprising that there are different views on the value of this test as an index of intestinal lactase. Some authors (Welsh et al 1967; Welsh 1970, Peternel 1965, Bayless et al 1966) indicate that the lactose tolerance test is a good indicator of intestinal lactase while others (Gray 1971, Metneki et al 1984) feel that the test has a poor predictive value. Still others have suggested that the test is satisfactory provided certain precautions are taken (McGill and Newcomer 1967; Welsh et al 1967).

Optimum conditions for the performance of the lactose load test seems to be that (i) it should be carried out in the morning after an overnight fast, (ii) 50 g in adults (1 g/kg body weight in children) dissolved in 400 ml of water (or equivalent volume in children) is administered, (iii) capillary blood glucose is determined at least every 20 minutes for a period of 80-100 minutes (McGill and Newcomer 1967).

Reference value: A rise in blood glucose concentration of more than 1.1 mmol/L in a non-diabetic subject after a lactose load is considered normal by practically all investigators. The validity of this assumption has been further investigated in this thesis.

1.2 Blood galactose after oral lactose

This is a variant of the oral lactose load. The principle of the method is that orally administered lactose is hydrolysed into glucose and galactose which are both absorbed. The resultant rise in the blood galactose is normally not significant because of the increased rate at which it is metabolised by the liver. However, if the metabolism of galactose is inhibited by simultaneously administered ethanol, the rise in blood galactose concentration would reflect the intestinal lactase activity (Jussila 1969 a; Isokoski et al 1972).

The test is unsuitable in children and in subjects with fatty liver, as ethanol does not inhibit galactose metabolism sufficiently in such individuals (Salaspuro 1967). Also ethanol ingestion might not be socially acceptable to many patients (Taylor 1982).

1.3 Symptoms and stool pH after oral lactose

Some workers have attempted to use the symptomatic response to oral lactose to detect the absence of lactase in the intestine. When oral lactose is not hydrolysed it remains in the intestinal lumen where it attracts fluid into the bowel (Launiala 1968) by osmosis, resulting in increased motility. When lactose reaches the colon it is metabolised by bacteria into short-chain organic acids, which further increases the osmotic pressure and produces a watery acid stool with a characteristic odour. The clinical symptoms are abdominal colic, bloating, borborygmi, flatulence and frothy diarrhoea. Bayless and Rosensweig (1966), Haemmerli et al (1965) and Newcomer and McGill (1967) have given particular diagnostic importance to the occurence of symptoms during the oral lactose load. They feel that the measurement of blood glucose levels after lactose is really only a refinement. They argue that patients with lactose malabsorption will usually develop symptoms after the ingestion of such a large dose of lactose and a simple screening test would be to give 50g lactose on one day and 50g glucose the next. The patient is asked to report on any symptoms induced on the first which he did not experience on the second day. Newcomer and McGill (1967) found no correlation between symptoms after the oral

lactose load and lactase deficiency. Others (McMichael et al 1965, Taylor 1982) found that the approach was not helpful, especially in adults.

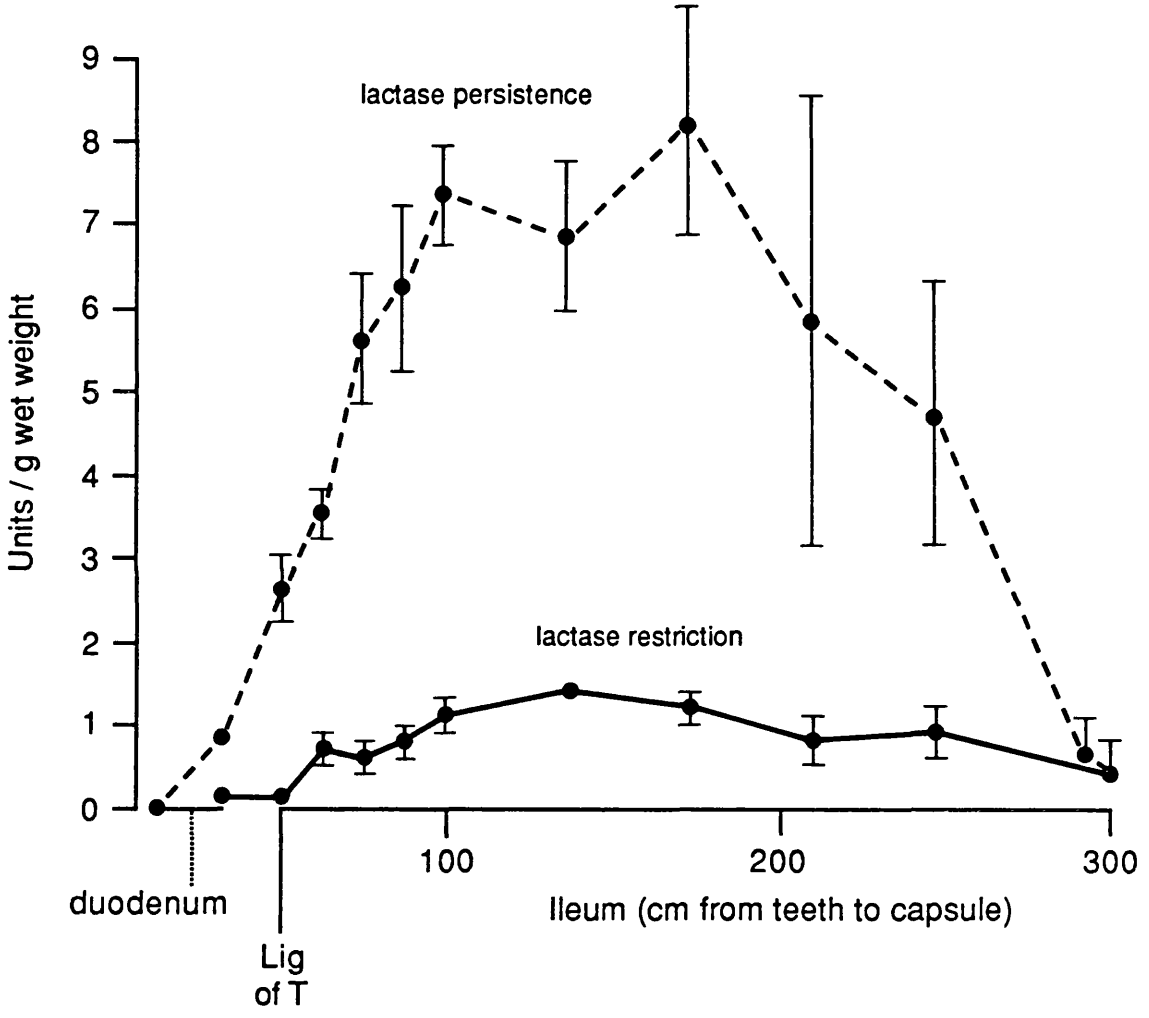
Many factors determine the symptomatic response to oral lactose. These include (i) the total lactase available for hydrolysis of lactose, in part a function of the length of the small bowel; (ii) the speed of gastric emptying; (iii) the presence of other food stuff; and (iv) the type of bacterial flora. Variability of these factors among the subjects investigated might well explain the variable outcome of the investigations.

2. INTESTINAL LACTASE

The most valuable test for the investigation of lactase deficiency should be the direct estimation of the enzyme in a biopsy from the small intestine. There are, however, several problems with the estimation of intestinal lactase activity.

2.1 Intestinal variation of lactase activity

One of the most serious objections to the enzyme assay as an index of lactase deficiency is the documented variation of enzyme activity along the length of the intestine (Newcomer and McGill 1966, Welsh et al 1966). Because of this gradient of enzyme activity, results obtained on specimens from different anatomical areas are not comparable. Most of the specimens for assay have been obtained near the duodeno-jejunal junction. However, some workers have obtained biopsies from more distant areas (McMichael et al 1965) or from unknown sites (Sheehy and Anderson 1965). Newcomer and McGill (1966) investigated seven subjects with normal lactase activity and seven with isolated lactase deficiency in order to study the distribution of lactase throughout the small intestine. In normal subjects a significant gradient of disaccharidase activity existed with low levels in the duodenum and ileum and peak activity in the jejunum. In subjects with lactase deficiency the enzyme activity remained low throughout the small intestine (Fig 4.1). The authors concluded that the lactase activity at the ligament of Trietz accurately reflects whether normal or low levels of enzyme would be found more distally, and that the presence of lactose and milk intolerance correlated reasonably well with lactase activity at this site.



From Newcomer and McGill 1966

Figure 4.1

Distribution of lactase activity in the small bowel of individuals of lactase restriction and lactase persistence phenotype.

2.2 Methodological consideration

2.2.1 Choice of substrate

The specificity of the different β-galactosidase to D lactose as well as synthetic substrates has been determined (Asp and Dahlqvist 1972). This shows that the transborder β-galactosidase that takes part in the hydrolysis of dietary lactose is more specific for lactose than the other substrates and hence the preferred substrate for lactase assay in humans is lactose (4-(β-D-galactapyranosyl)-D glucose) (Zapp et al 1966).

2.2.2 Substrate concentration

The effect of substrate concentration on the intestinal glycosidase has been studied in detail (Dahlqvist 1960). It showed "substrate inhibition" at higher concentration (>0.1 M) which was thought to be due to transglycosylation. A concentration of 0.028 M was recommended (Dahlqvist 1960). It was later standardised at 0.056 M (Dahlqvist 1964). However some workers have used 0.028 M lactose (Haemmerli et al 1965).

2.2.3 pH

The pH optima of the brush border β-galactosidase is 5.2, hence the substrate is prepared in 0.1 M maleate buffer at pH 5.6 (Dahlqvist 1964).

2.2.4 Time of incubation

The initial 15% of hydrolysis of lactose at 0.025 M by intestinal lactase follows zero-order kinetics (Dahlqvist 1960). during this phase of the reaction the amount of products formed during a certain period of time is proportional to the amount of enzyme. Incubation of 0.025 M lactose with the enzyme for 60 minutes, 5% of hydrolysis is equal to the formation of 0.5 mg of glucose. Thus 60 minutes is chosen as the incubation time (Dahlqvist 1964).

2.3 Definition of the unit of lactase activity and reference interval

In accordance with the recommendations by the joint sub-committee on clinical enzyme units of the international union of biochemistry and pure and applied chemistry (Dahlqvist 1964) one unit of disaccharidase activity is defined as the activity necessary to hydrolyse 1.0 µmole of the substrate per minute at 37°C (Dahlqvist 1964). Results have been expressed either in units per wet weight of the mucosa (U/gww) or as units per protein content of the mucosa (U/g protein).

The disadvantage of expressing the enzyme concentration as activity per gm wet weight of mucosa is the risk of obtaining falsely high values because of dehydration of the biopsy (Welsh 1970). To prevent this, the mucosa should be wrapped in foil before storage in the freezer. It has been shown that there is no added advantage in expressing results in terms of the protein content of the mucosa if precautions are taken to prevent dehydration (Sheehy and Anderson 1965).

The reported reference interval of lactase activities varies considerably in the literature as summarised in Table 4.2. This seems to be due to lack of standardisation of the investigative procedures used. The majority of the studies presented in this table are based either on the method of Dahlqvist (1964) or that of Auricchio et al (1963 a) with or without modifications.

There is some dispute as to the lowest normal level of lactase activity. Values in the literature vary from 0.5 U/gww (Newcomer and McGill 1966) to 1 U/gww (Littman et al 1968) or even 3 U/gww (McMichael et al 1965). Part of the discrepancy is explained by the use of slightly modified methods of assaying lactase activity and variations of the correlated lactose load.

2.4 Improved modifications

In the original method of Dahlqvist (Dahlqvist 1964) and the subsequent modifications (Miller 1981) o-dianisidine is used as the chromogen, which is a carcinogen. More recent modifications (Dahlqvist 1984) have used commercial glucose oxidase reagents which use safer chromogen.

TABLE 4.2

LACTASE ACTIVITY OF "NORMAL" SUBJECTS REPORTED IN THE LITERATURE

Author	Year	Biopsy site	Lactase Activity* (U/gww)	(U/g protein)
Auricchio et al	1963 b	J	-----	107 (39 - 258)
Dahlqvist	1964	J & I	0.2 - 19	
Townley et al	1965	D	2.9 (0.93 - 7.3)	-----
Newcomer and McGill	1967	L.T	3.5 (0.7 - 11.1)	30.9 (3 - 82.8)
McMichael et al	1965	J	- (3.0 - 24.4)	-----
Littman et al	1968	L.T	5.9 (1.1 - 18.6)	40.2
Welsh	1970	L.T	3.21 (1.02 - 11.48)	35.8 (12.5 - 93.7)
Desai et al	1967	J	----	14.6 (1.6 - 33.0)
Ferguson et al	1984	J	(0.9 - 9.2)	-----
Newcomer et al	1975	L.T	3.6 (1.1 - 9.5)	-----
Forget et al	1985	D	(0.8 - 4.5)	-----
Peters et al	1976	J		95.9±11.9
Dahlqvist	1984	J		41 (21 - 80)

I - Ileum

J - Jejunum

D - Duodenum

L.T - Ligament of Trietz

* mean and range

Fluorimetric assays (Peters et al 1976) in addition to the advantage of using a safer detecting agent has also increased the sensitivity of the method ten-fold. This is of particular value in assaying the lactase activity of subcellular fractions of human tissue.

2.5 Use of the oral lactose load and intestinal lactase in detecting lactase restriction phenotype

There are conflicting reports concerning the discriminatory power of the lactose load test (Newcomer and McGill 1966, Friedland 1965) and intestinal lactase activity (Sheehy and Anderson 1965) in detecting lactase phenotypes. Abnormal oral lactose loads have been reported in supposedly normal subjects (Friedland 1965) and in persons with normal lactase activity (Weser et al 1965, Newcomer and McGill 1966). However, an individual without a history of milk intolerance may have low intestinal lactase. Falsely abnormal lactose load tests have been attributed to delayed gastric emptying (Kern and Struthers 1966, Newcomer and McGill 1966) or because of inadequate sampling frequency of blood (Newcomer and McGill 1966). Further aspects of the discriminatory power of the two investigations, singly or in combination, in detecting low lactase phenotype is dealt with in this thesis (Chapter 7, 2.1.2).

3. ^{14} CARBON BREATH TEST

In this test carbon labelled lactose (^{14}C - lactose) is ingested by the subject and expired ^{14}carbon dioxide is collected over a period of time. The amount of lactose hydrolysed is inferred from the percentage of radioactive carbon dioxide excreted from the metabolism of glucose and galactose. Though it is recommended as a screening test for lactase deficiency (Salmon et al 1969), the specialised apparatus involved makes it inapplicable in many developing countries, especially as a screening procedure. It is less sensitive and specific than the breath hydrogen test (Levitt and Donaldson 1970, Newcomer et al 1975), see below. The use of radioactive isotopes is not recommended especially in the paediatric population.

4. BREATH HYDROGEN TEST

Breath hydrogen analysis after oral lactose load has been shown to identify lactose malabsorption (Calloway et al 1969, Levitt 1969, Levitt and Donaldson 1970). In lactase deficient subjects unabsorbed lactose will reach the colon where it is metabolised by the colonic bacteria producing hydrogen which is absorbed and exhaled by the subject. As no mammalian cells produce hydrogen, breath hydrogen indicates the presence of lactose in the colon. This method requires the use of gas chromatography, which is not available in developing countries like Sri Lanka. Recently relatively easy and inexpensive apparatus using electrochemical sensors have proved useful (Bartlett et al 1980, Berg et al 1985).

False negative results may be obtained in patients receiving antibiotics (Davidson and Robb 1985, Gilat et al 1978). Up to 5-10% of normal children do not possess hydrogen producing bacteria in their colon (Bond and Levitt 1977, Gilat et al 1978) and may produce false negative results. Dietary fibre also gives low results (Nguyen et al 1982). Bacterial colonisation of the small intestine and gastrectomy give high values and do not indicate lactose malabsorption (Welsh and Griffiths 1980, Armbrecht et al 1985, Forgert et al 1985).

In the diagnosis of lactase restriction phenotype the breath hydrogen test is shown to have a sensitivity and specificity of 100% and 87% respectively whereas the oral lactose load with glucose estimation is said to have a sensitivity and specificity of 70% and 100% respectively (Crochet et al 1981). Thus the breath hydrogen test is more sensitive and the lactose load test with glucose estimation is more specific. However in the absence of a reference test to detect lactase restriction both tests have their place in the investigation of lactase phenotypes and should be interpreted with care (Cooper 1986). The breath hydrogen test may have a valuable place in the clinical investigation of children (Hyams et al 1980) because of its non-invasive nature.

5. LACTOSE - BARIUM MEAL

25g of lactose in 120ml of barium sulphate contrast medium for radiographic examination of the small intestine is given orally (Laws et al 1967). In subjects with low intestinal lactase activity there is a rapid transit time, dilution of the contract medium and a dilution of the small intestines due to the osmotic effect of non-hydrolysed lactose.

The lactose-barium meal has been advocated as a screening test for lactase deficiency (Laws et al 1967, Preger and Amberg 1967, Neale 1968). However other workers have found a poor predictive value compared with lactose load test and enzyme assays both in adults and children (Bolin et al 1970).

6. SUMMARY

The oral lactose load test is poorly standardised (Table 4.1). The optimum conditions for the performance of the test to determine lactase phenotypes seems to be that 1) it be carried out in the morning after an overnight fast; 2) 50g of lactose in adults (1g/kg body weight in children) dissolved on 400ml of water (or equivalent volume in children) is administered; 3) capillary blood glucose is determined at least every 20 minutes for a period of 80-100 minutes.

The conditions for the assay of intestinal lactase activity are better standardised. However there is poor agreement about the cut-off limit for the determination of the phenotypes (this is discussed further in chapter 8).

Breath hydrogen test has its limitations although it has a place in the clinical investigation of children because of its non-invasive nature.

CHAPTER 5

AIM AND STRATEGY OF PRESENT INVESTIGATION

1. INTRODUCTION

2. SELECTION OF PATIENTS
   - 2.1 **Adults in Sri Lanka**
   - 2.2 **Children in Sri Lanka**
     - 2.2.1 Infants
     - 2.2.2 Children

3. DIAGNOSTIC METHODS

4. CLINICAL IMPORTANCE OF LACTASE RESTRICTION PHENOTYPE IN SRI LANKA
   - 4.1 **Functional diarrhoea in Sri Lankan adults**
   - 4.2 **Study of milk intake**
     - 4.2.1 In adults
     - 4.2.2 In children

5. INVESTIGATION OF A BRITISH PAEDIATRIC HOSPITAL POPULATION
   - 5.1 **Determining the prevalence rate of lactase restriction phenotype**
   - 5.2 **Determining the reference interval for lactase activity among British children**
   - 5.3 **Determining the age dependency of the lactase phenotypes**

1. INTRODUCTION

The present study was carried out to establish the prevalence rate of the lactase phenotypes in Sri Lanka, with the aim of answering the following questions:

I. What is the prevalence rate of the lactase restriction phenotypes in the Sri Lankan adult population?
II. What is the age of appearance of the lactase restriction phenotype?
III. What is the clinical importance of lactase restriction to the population?

The above data was compared with data obtained from a British paediatric hospital based population.

2. SELECTION OF PATIENTS

2.1 Adults in Sri Lanka

Patients who were admitted for trivial or unrelated conditions with no intestinal symptoms were chosen for the study. Ideally these investigations should have been done on healthy non-hospitalised normal people to determine the prevalence rate in the general population. However, it was difficult to persuade completely normal subjects to sacrifice half a day in the laboratory. Fortunately in Sri Lanka, because of the lack of an adequate screening of outpatients, there are a number of unnecessary admissions of patients with trivial complaints such as non-specific chest pain, headache, coryza etc or with unrelated clinical conditions. These subjects were chosen as a reference population for normal adults, having ensured that they did not have a past or present history of abdominal complaints. The presenting complaints and disorders of the 197 adult subjects studied are indicated in Table 5.1. The racial composition of the subjects studied was representative of that of the surrounding geographical area (Table 5.2).

TABLE 5.1

PRESENTING COMPLAINTS AND DISORDERS OF THE SUBJECTS STUDIED

(n = 197)

Main disorder/presenting complaint			%
Nil wrong	(chest pain Ischaemic heart disease excluded) (leg pain) (headache)	)	44.3
Urinary tract infection			11.4
Respiratory tract infaction			10.0
Joint problems			7.1
Influenza			5.7
Gynaecological problems			4.3
Poisoning (after recovery)			4.3
Psychiatric disorder			2.9
Cirrhosis			2.9
Anaemia			4.3
Hypertension			1.4
Mitral valve disease			1.4
			100%

TABLE 5.2

ETHNIC COMPOSITION OF THE SAMPLE STUDIED COMPARED WITH THAT OF THE AREA

Race	Sample Studied		Population in Kandy*
	(n)	%	%
Sinhalese	158	80.2	71.9
Tamil	30	15.2	20.5
Moor	9	4.6	7.0
Others	0	0.0	0.6
Total	197	100.0	100.0

* 1971 Census

2.2 Children in Sri Lanka

2.2.1 **Infants:** In order to determine the age at which subjects became unable to hydrolyse lactose, lactose loads were initially carried out on a small group of infants (n = 24, aged 0 - 1 year). It was found that all had a normal lactose load test (see results), indicating the need for an extended study in different age groups from one year to adulthood.

2.2.2 **Children:** The children were selected on the same basis as adults, ie those without intestinal symptoms. The study was conducted in stages with results evaluated at each stage. Initially subjects in the following age groups were studied.

Age (years)	Number
1 - 2	18
2 - 3	10
3 - 5	7
5 - 10	10
10 - 15	15

Analysis of preliminary results showed that there was a sudden rise in the prevalence rate of abnormal oral lactose loads in the 5 - 10 year group. Additional children (n = 10) were therefore studied in the age group 5 - 10 years.

3. DIAGNOSTIC METHODS

Oral lactose load and intestinal lactase estimation were used to diagnose the phenotype. Oral glucose and sucrose loads and xylose absorption test were used to exclude generalised malabsorption. These are described in Chapter 6.

4. CLINICAL IMPORTANCE OF LACTASE RESTRICTION PHENOTYPE IN SRI LANKA

4.1 Functional diarrhoea in Sri Lankan adults

In order to determine the significance of the lactase restriction phenotype as a cause of functional intestinal symptoms, two studies were carried out. In the first study an attempt was made to assess the prevalence rate of functional diarrhoea in the population by a detailed study of the patients presenting in the outpatient department.

A detailed report was kept of the presenting symptoms of consecutive patients seen by the author over a period of two weeks, in the general outpatient department of the General Hospital, Kandy.

In the second study a specific search was made for patients with diarrhoea or chronic functional abdominal problems by direct questioning of all the patients attending the medical follow-up clinic.

Finally, oral lactose loads were carried out on patients who had chronic abdominal problems, ie patients who had a chronic history of diarrhoea, abdominal pain or borborygmi for which no other cause was found.

4.2 Study of milk intake

4.2.1 **In Adults:** A careful history of milk intake was obtained from all the subjects on whom oral lactose load was performed. Questions were asked to determine the amount and frequency of milk intake. Based on this history the subjects were classified as milk drinkers or non-milk drinkers. A subject was considered a "milk drinker" if the equivalent of at least a glass of milk per day was consumed regularly.

4.2.2 **In Children:** This study was done in the "well-baby" clinic at the General Hospital, Kandy. The mothers interviewed were each asked about the type and duration of milk intake of her previous child and the age at which she stopped giving breast milk to that child.

5. INVESTIGATIONS OF A BRITISH PAEDIATRIC HOSPITAL POPULATION

5.1 Determining the prevalence rate of lactase restriction phenotype

A retrospective study was carried out on children referred to a gastro-enterological unit in a paediatric hospital in London (Queen Elizabeth Hospital, Hackney).

These children were referred either for chronic intestinal symptoms or for failure to grow. Their ages ranged from 3 months to 15 years. They had jejunal biopsies as part of their routine investigation. The biopsies were obtained after an overnight fast. A double port paediatric capsule (Kilby 1976) was used to obtain a biopsy at the duodeno-jejunal flexure. Histopathological examination was carried out on one part of the biopsy and the other was immediately frozen, wrapped in aluminiun foil and stored at -20^{o}C, and used for the assay of maltase, sucrase and lactase according to Dahlqvist 1964. A detailed study of case notes was carried out on all the children who were found to have lactase values below the lower end of the reference interval. The small intestinal histology and in some cases electron microscopy on these children were reviewed to confirm that they were normal.

5.2 Determining the reference interval for lactase activity among British children

Lactase activities in histologically normal samples with normal maltase and sucrase activities were used to determine the reference interval for lactase.

5.3 Determining the age dependency of the lactase phenotypes

Two thirds of the children referred to this London paediatric hospital were below the age of 5 years. Hence a more extensive study was carried out for a further 2 years of all children over 5 years who demonstrated a normal intestinal mucosa on histological examination. The case notes of those who had lactase activities less than 1U/gww were reviewed. The histology and elctron microscopy were reviewed to confirm that they were normal.

CHAPTER 6

METHODS

1. ORAL LACTOSE LOAD
   - 1.1 **Dose and administration of lactose, blood sampling**
   - 1.2 **Blood glucose estimation**
   - 1.3 **Mode of expressing results of the load**

2. TESTS TO EXCLUDE GENERALISED MALABSORPTION
   - 2.1 **Oral glucose load**
   - 2.2 **Xylose absorption test**
   - 2.3 **Oral sucrose load**

3. CHROMATOGRAPHY OF LACTOSE IN URINE AND STOOL

4. INTESTINAL LACTASE
   - 4.1 **Jejunal biopsy**
     - 4.1.1 Surgical biopsy
     - 4.1.2 Peroral biopsy
   - 4.2 **Estimation of lactase activity**
     - 4.2.1 Reagents
     - 4.2.2 Method

5. STATISTICAL METHODS

1. THE LACTOSE LOAD

1.1 Dose and administration of lactose and blood sampling

50g of lactose dissolved in 400ml of water in adults and 1.0g/kg body weight of lactose as a 15% solution in children was used as the lactose load, (McGill and Newcomer 1967). This quantity of lactose does not easily go into solution but requires warming to about 80°C. Since there was a possibility that the warming might produce hydrolysis (eg from bacterial activity or from chemical decomposition) chromatography was carried out on the solution after warming to see whether there was any glucose in the solution. The results indicate that, firstly, the lactose was not contaminated, and secondly, that there was no hydrolysis; that what was being administered was pure lactose.

The lactose solution was drunk over a period of five minutes and capillary blood samples were obtained before and at 20, 40, 60, 80 and, in most cases, 100 minutes after the administration of the lactose. If the subject vomited, as happened infrequently, the test was abandoned and repeated on another day.

Subjects were questioned every 30 minutes for six hours after the load for intestinal symptoms: diarrhoea, abdominal pain, flatulence and borborygmi. If the subject had diarrhoea the pH of the stool was determined and in some instances sugars in stool were analysed by chromatography.

400 ml of water without lactose was administered to 33 subjects. This group served as a control group to measure the effect of water alone on blood glucose concentration during the test.

1.2 Blood glucose estimation

Blood glucose was determined by the Nelson-Somogyi method (Somogyi 1945(a), 1945(b), 1952). A neutral solution of zinc sulphate and barium hydroxide was used to precipitate the proteins. This was followed by reduction of an alkaline copper tartrate solution (Somogyi 1952).

The amount of cuprous copper formed was estimated colorimetrically by reacting it with arsenotungstate acid which produces a yellow colour. Thus this method estimates the total reducing substances (including glucose, lactose and galactose, if any).

In some patients who excreted lactose in the urine, duplicate analysis by the Nelson Somogyi method and glucose oxidase method (Huggett and Nixon 1957) was carried out before and at 20, 40, 80 and 100 minutes after lactose in order to exclude the possibility that the rise in blood "glucose" was due in part to intact lactose being absorbed.

In some instances urine and stool were collected for chromatography.

1.3 Mode of expressing results of the load

The following parameters have been used to characterise the outcome of the different loads (cf. Geisler et al 1985).

Maximum post load increment in blood glucose concentration = $\hat{g}$
Maximum glucose increment after lactose load = $\hat{g}$ (lactose)
Maximum glucose increment after water = $\hat{g}$ (water)
Maximum glucose increment after glucose = $\hat{g}$ (glucose)
Maximum glucose increment after sucrose = $\hat{g}$ (sucrose)
Time at which the maximum increment of blood glucose occurred = $\hat{t}$

2. TESTS TO EXCLUDE A GENERALISED MALABSORPTION

2.1 Oral glucose load

In patients who have an abnormal lactose load, the standard procedure to exclude monosaccharide malabsorption is to repeat the test with equal amounts of glucose and galactose (the monosaccharides obtained from the hydrolysis of lactose) and show that these are absorbed. Galactose is expensive and its use greatly increases the cost of the total investigation. It had been shown by Bolin et al 1970 that there was no difference in the blood glucose levels after an oral glucose load and a mixture of glucose and galactose load. Therefore on patients who had an abnormal lactose load, the test was repeated the next day using

25g glucose in 200ml of water and the blood glucose was estimated at 20, 40, 60, 80 and 100 minutes.

After a review of the results of the first 80 patients, testing of monosaccharide absorption was abandoned since a defect in monosaccharide absorption was not found in any of the subjects. Others (McMichael et al 1965; Welsh 1970) had the same experience and, after reviewing the lactose loads in 100 patients, suggested that there was no further diagnostic information obtained from this part of the investigation.

2.2 Xylose absorption test

This test was carried out on 14 patients who demonstrated an abnormal oral lactose load to exclude the presence of a generalised malabsorption. After an overnight fast, the subject was asked to empty the bladder and 5g of xylose in 250ml water was administered orally. Urine was collected during the next five hours and the bladder emptied at the end of the period. Xylose in the urine was estimated by the method of Roe and Rice (1948).

2.3 Oral sucrose load

Twenty three subjects who demonstrated an abnormal oral lactose load were subjected to a sucrose load. 50g of sucrose in 400ml water was administered orally. Capillary samples for blood glucose were collected after the load.

3. CHROMATOGRAPHY OF LACTOSE IN URINE AND STOOL

Collection and preservation of specimens

Urine: Care was taken not to contaminate the urine with faeces because of the possible breakdown of lactose by bacterial enzyme. A tiny crystal of thymol was added and the urine stored in the refrigerator immediately after voiding.

Stool: Stool were immediately frozen.

Treatment of the specimens before chromatography

Urine: Desalting was found to be unnecessary since salts added to a solution of lactose and glucose did not alter the mobility of the sugars. 100 µL of urine was applied with a micro pipette at the top of a Whatman No 4 paper 18 cm by 19 cm, the bottom of which was serrated.

Faeces: Approximately 5g faeces was homogenised with an equal amount (w/v) of acetone and centrifuged. 100 µL of the extract was applied with a micro pipette without desalting.

Chromatography of lactose in urine and stool

Solvent: Butanol, acetic acid and water (120: 30: 50) were used as solvent and descending chromatography overnight (16 hours) was carried out, without prior equilibration of the paper. The next morning the chromatogram was dried, dipped in the locating reagent (aniline-diphenylamine phosphate reagent) and dried in an oven at 80°C.

4. INTESTINAL LACTASE

4.1 Jejunal biopsy

4.1.1 **Surgical biopsy:** Surgical biopsies of intestinal mucosa were obtained at the ligament of Trietz in nine subjects who had abdominal operations for gastro-intestinal disease not associated with small intestinal disease. The biopsy was wrapped in aluminium foil and immediately stored at -20°C for no longer than three days.

4.1.2 **Peroral biopsy:** Jejunal biopsies were obtained by Crosby capsule from 32 hospitalised patients with trivial complaints not related to the gastro-intestinal tract. The loaded capsule was passed up to the 90cm mark on the tube and the subject was asked to lie on his right side. A sedative was given for the night and 1ml of neostigmine injected intramuscularly. (This facilitates relaxation of the pyloric sphincter.) The next morning a straight X-ray of the abdomen was taken to confirm the position of the capsule at ligament of Trietz. If it had not

reached the jejunum, the tube was taken out and the test abandoned. If it had reached the jejunum, the capsule was fired after injecting a few millilitres of normal saline gently down the tube. The capsule was withdrawn and the mucosa biopsy wrapped in foil and immediately stored at -20°C for no longer than three days.

4.2 **Estimation of lactase activity** (according to Dahlqvist 1964, Huggett and Nixon 1957)

4.2.1 **Reagents**

Maleate buffer (0.1M maleate buffer, pH 5.6)
1.16 g of maleic acid was dissolved in 13.2 ml of 1 N NaOH and made up to 100 ml with water.

Substrate solution
0.056 M solution of lactose in the maleate buffer was prepared. Toluene 1 ml/100 ml was added as a preservative.

Tris-Glucose Oxidase (TGO) Reagent: Following stock solutions were prepared.

Tris Buffer: 61.0 g of Tris in 85 ml of 4 N HCl, made up to 1 L with distilled water. pH of the solution was adjsuted to 7.

Peroxidase solution: 1 mg/1 ml in distilled water. Stored at -20°C.

O-Dianisidine solution: 10 mg/ml in 95% ethanol. This was kept in the dark and was discarded if it turned brown.

Triton X-100: 10 ml in 40 ml of 95% ethanol.

To prepare the TGO reagent, 125 mg of glucose oxidase was placed in a 100 ml volumetric flask and about 50 ml of the Tris buffer was added. The concents were shaken for 0.5 minute. 0.5 ml of the peroxidase solution, 0.5 ml of O-dianisidine solution and 1 ml of Triton X-100 solution were added. Tris buffer was added to make up to 100 ml. This solution was filtered and stored in the refrigerator.

4.2.2 Method

The biopsy was thawed in a chilled beaker, taken out of the foil, washed in chilled physiological saline, dried on a filter paper and weighed. It was transferred into a glass homogeniser. Homogenisation was carried out with 4 parts (w/v) of chilled physiological saline while the homogeniser was placed in a beaker with crushed ice. Cell debris was removed by centrifugation in an ordinary laboratory centrifuge for 10 mins. 0.1 ml of the homogenate was incubated with an equal volume of the substrate solution (56 mmol/L of lactose in maleate buffer of pH 5.6) for 1 hour at 37°C. 0.8 ml of distilled water was added and the tube immediately placed in boiling water. A control solution was also incubated after boiling the mixture at the beginning of incubation. At the end of the incubation period glucose was assayed by the glucose oxidase method (Huggett and Nixon 1957). The lactase activity was calculated by the following formula:

$$\text{Lactase activity (U/gww)} = \frac{(a-b)\ d}{540 \times ww}$$

a = glucose content in test solution (µg)

b = glucose content in control solution (µg)

d = dilution factor

ww = wet weight of biopsy (g)

1 unit of lactase activity = 1µ mol/min

5. STATISTICAL METHODS

Simple regression analysis by sums of square was used if one of the variables was considered error-free. If both variables were not error-free orthogonal analysis was carried out (Wakkers et al 1975).

Comparison of samples: X^2 test was used where appropriate.

CHAPTER 7

RESULTS

1. PERFORMANCE CHARACTERISTICS OF THE INVESTIGATIONS USED

1.1 **Blood glucose**

1.2 **Oral lactose load**

1.2.1 Reproducibility of the oral lactose load

1.2.2 Effect of water intake on the concentration of blood glucose

1.3 **The assay of intestinal lactase**

2. INVESTIGATIONS OF SRI LANKAN ADULTS

2.1 **Oral lactose load**

2.1.1 Prevalence rate of the lactase restriction phenotype and estimates of its uncertainty

2.1.2 Effect of sampling on prevalence rate

2.1.3 A fall in blood glucose concentration after lactose load.

2.1.4 Prevalence rate of abnormal oral lactose load in the different ethnic groups

2.1.5 Symptomatic response to oral lactose load

2.1.6 Lactosuria

2.1.7 Chromatography of stool

2.1.8 Simultaneous determination of blood sugar and blood glucose after oral lactose load

2.2 **Oral glucose load**

2.3 **Xylose absorption test**

2.4 **Oral sucrose load**

2.5 **Intestinal lactase activity**

2.5.1 Prevalence rate of low intestinal lactase among Sri Lankan adults

2.5.2 Uncertainty of the prevalence rate estimates

2.5.3 Correlation of intestinal lactase and g (lactose)

3. INVESTIGATIONS OF SRI LANKAN CHILDREN

3.1 **Oral lactose load**

3.2 **Age dependency of abnormal oral lactose load**

4. FUNCTIONAL DIARRHOEA IN SRI LANKAN PATIENTS

4.1 **Incidence in a random hospital population**

4.1.1 Study in the outpatient department

4.1.2 Study in the medical clinic

4.2 **Oral lactose load on adults with functional diarrhoea**

4.3 **Case report of a symptomatic patient with lactase restriction phenotype**

4.4 **Milk Drinking Habits**

5. INVESTIGATIONS OF A BRITISH PAEDIATRIC HOSPITAL POPULATION

5.1 **Reference Interval for intestinal lactase**

5.1.1 Based on histology

5.1.2 Based on maltase and sucrase activities

5.1.3 Based on comparison with another population (Sri Lankan) who

demonstrate predominantly lactase restriction phenotype.

5.2 **Prevalence rate of lactase restriction phenotype**

5.3 **Age dependency of intestinal lactase**

6. SUMMARY

1. PERFORMANCE CHARACTERISTICS OF THE INVESTIGATIONS USED

1.1 Blood glucose

Imprecision of the blood glucose method was determined by performing five blood glucose estimations each on six different samples of blood (ie 30 estimations), the sampling being done as follows:

Venous blood from six patients (A,B,C,D,E and F) was collected in six fluoride bottles. Blood from each patient was put into five tubes selected at random (random sampling numbers) from a series of thirty tubes, numbered from 1 to 30.

Thus patient A - tubes 1, 6, 10, 11, 14
patient B - tubes 2, 5, 7, 12, 13 etc.

The results appear in Appendix I. The coefficient of variation (CV) of the replicate analysis on each sample ranged from 0.8% to 1.5% over the range of blood glucose of 3.3 to 6.1 mmol/L. In addition 30 duplicate blood glucose estimations were performed. The CV was 0.9% within a blood glucose range of 3.6 to 6.5 mmol/L (Appendix II).

1.2 Oral lactose load

1.2.1 **Reproducibility of the oral lactose load:** Since several factors (see Ch.2,6.) influence the rise in blood glucose concentration after the oral lactose load, it was important to establish the reproducibility of the test. Thus the oral lactose load was repeated in 13 subjects at two separate occasions, one to 51 days apart. The results are shown in Table 7.1 and Fig 7.1. A statistically significant, positive correlation could be established between $\hat{g}$ at the first (x) and second (y) investigation ($y = 0.33 + 0.87x$) by orthogonal regression analysis;

TABLE 7.1

WITHIN-SUBJECT VARIABILITY OF $\hat{g}$ (mmol/L) AND SYMPTOMS AFTER ORAL LACTOSE LOAD (50g)

Subject No	Interval between the two tests (days)		$\hat{g}$ 1st test	$\hat{g}$ 2nd test	Interpretation 1st test	Interpretation 2nd test	Symptoms 1st test	Symptoms 2nd test
1	6		0.3	0.5	A	A	*0	+
2	47		0.3	0.7	A	A	*0	+
3	5		0.3	0.9	A	A	*0	+
4	2		0.6	0.7	A	A	0	0
5	15	*	0.7	1.1	A	N	0	0
6	29	*	0.8	1.4	A	N	0	0
7	1	*	1.1	0.7	N	A	+	+
8	21		1.1	2.2	N	N	+	+
9	41	*	1.2	0.9	N	A	+	+
10	5		1.3	1.5	N	N	+	+
11	9		1.4	1.7	N	N	*+	0
12	10		2.8	3.2	N	N	0	0
13	51		2.8	1.5	N	N	0	0

A/N = Abnormal or normal result using $\hat{g}$ = 1.1 mmol/L as the cut-off point

* indicates results, where the outcome is different for the first and second test

CV of duplicate $\hat{g}$ = 20%

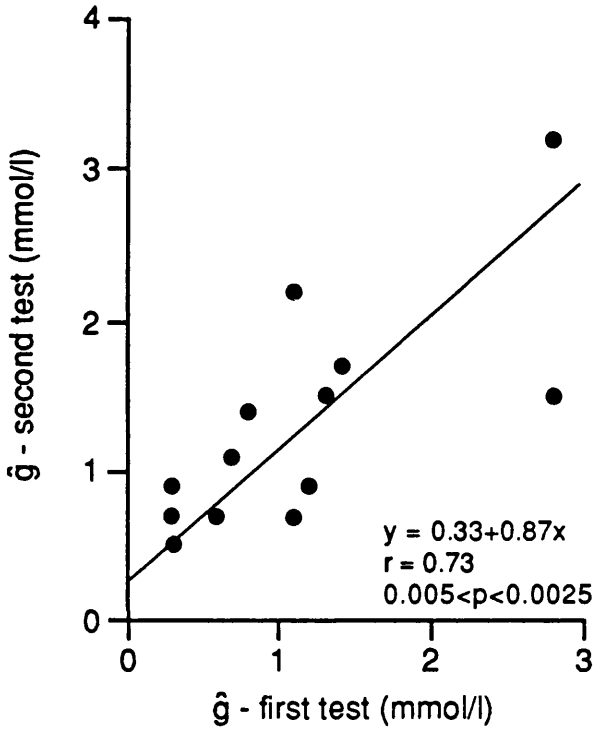


Figure 7.1

Reproducubility of oral lactose loads.

$r = 0.73$, $n = 13$, $0.005 < p < 0.0025$. There is however a considerable variability around the regressive line which will influence the predictive value of a single $\hat{g}$ value. This is also illustrated in Table 7.1, where it can be seen that misclassification would occur within an interval of approximately $\pm 20\%$ around a $\hat{g}$ value of 1.1 m mol/L. The mean and SD of the difference between individual $\hat{g}$ values at the first and second occasion was -0.18 ± 0.59 mmol/L which is not different from zero. There was no correlation between the difference in $\hat{g}$ at the two occasions and the time interval between the tests ($r = 0.35$; $0.15 < p < 0.10$), indicating that the within subject variability of $\hat{g}$ (lactose) is not time dependent. (Fig 7.2)

The symptoms after the lactose load were reproducible only in eight out of 13 subjects (Table 7.1). The reproducibility of symptoms was not statistically significant.

1.2.2 **Effect of water intake on the concentration of blood glucose:** Since the interpretation of the lactose load depends on the increase in the blood glucose concentration after oral lactose, it was important to determine how blood glucose varies, after the ingestion of the same volume of water as used for the lactose load. Thirty-three subjects took part in this control experiment. Individual results are shown in Appendix III.

Mean $\hat{g}$(water) $\pm$ SD was 0.04 ± 0.3 mmol/L range -0.7 to +0.6 mmol/L. The mean value was not significantly different from zero ($p < 0.05$). However, the variation around the mean value is quite considerable and will influence the interpretation of the results of lactose load, as is discussed later (Ch.8, 1.2).

1.3 The assay of intestinal lactase

Replicate analyses were carried out on a single piece of mucosal tissue in order to determine the analytical impression of the lactase assay and the intra-individual variability of lactase activity. Three series of experiments were done, the first to determine the variability of the estimates using the same homogenate, the second to study the variation in lactase activity in different biopsies from the same site and the third to study the stability of mucosal biopsies and homogenates at $-20^{\circ}C$.

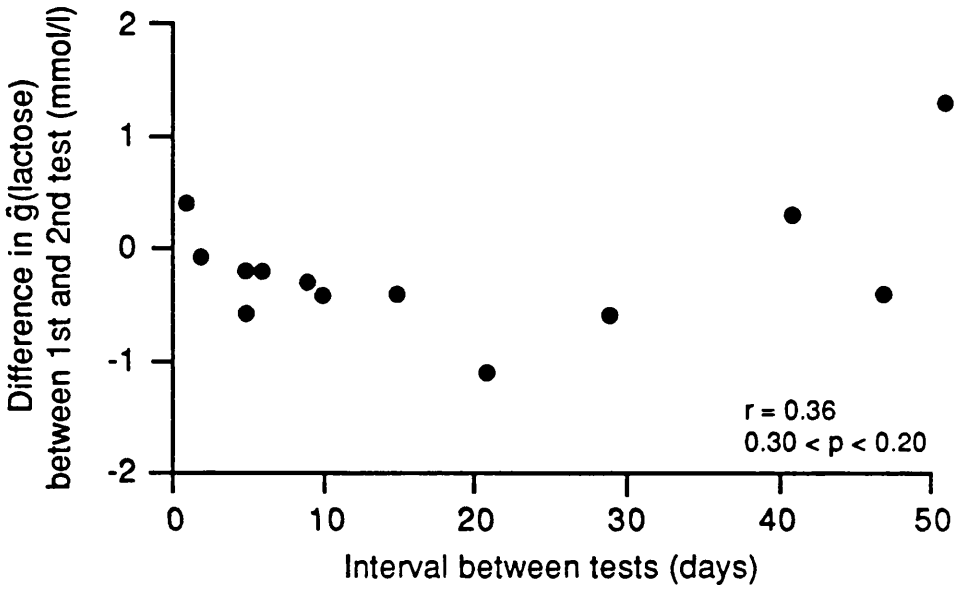


Figure 7.2

Correlation of the difference in $\hat{g}$ between two tests in the same individuals and varying time interval between the tests.

In the first series duplicate and triplicate assays of lactase activity were carried out on homogenates of random biopsies (n = 7). The mean coefficient of variation was 6.8% (Table 7.2). This gives a measure of the analytical variability.

In the second series the variation of lactase activity in different samples obtained at the same site were studied. A specimen of gut resected at laparotomy was subjected to repeated biopsies by a Crosby capsule and two to six samples from the same intestinal site were obtained from six patients. The lactase activity was determined in each of them. The coefficient of variation of lactase activity from the same jejunal site was 2.7% (Table 7.3). This indicates the intra-individual variability of lactose activity at a particular jejunal site.

Further experiments were carried out to study the stability of the lactase in homogenates and in intact biopsies by keeping samples frozen at -20°C and repeating the analysis of the enzyme activity at intervals. The results (Table 7.4) indicate that the enzyme was stable for four days when the intact mucosa was stored at -20°C. If the homogenate was stored at -20°C the activity decreased by 6-8% per day.

2. INVESTIGATIONS OF SRI LANKAN ADULTS

2.1 Oral lactose loads

2.1.1 **Prevalence rate of the lactase restriction phenotype and estimates of its uncertainty:** Individual data for the oral lactose load are shown in Appendix IV and summarised in Table 7.5. A cumulative frequency distribution, generated from data in Table 7.5, is shown in Fig 7.3. If a value of $\hat{g}$ (lactose) of 1.1 mmol/L is considered the upper limit for subjects with a lactase restriction phenotype, the prevalence rate of this group is 73%. This increased to 85% if the cut-off point for $\hat{g}$ (lactose) is increased to 1.4 mmol/L (Fig 7.3).

However the imprecision of $\hat{g}$(lactase) is 20% (Table 7.1). If a 20% variability is considered at each $\hat{g}$(lactose) cut-off point of 1.1 and 1.4mmol/L, the prevalence rate would vary between 60-80% and

TABLE 7.2

WITHIN-BIOPSY VARIABILITY OF MUCOSAL LACTASE ACTIVITY

Biopsy	Lactase activity (U/gww)		
	assay 1	assay 2	assay 3
1	0.30	0.37	-
2	0.42	0.44	-
3	0.42	0.41	0.39
4	0.58	0.59	-
5	2.18	2.11	-
6	2.66	2.44	-
7	2.66	2.89	2.96

Coefficient of variation = 6.8%

$$CV = \frac{\text{overall SD}}{\text{overall mean}}$$

$$\text{overall SD} = \frac{\text{Sums of square of difference from group mean}}{\text{Total number of values - number of groups}}$$

TABLE 7.3

VARIABILITY OF LACTASE ACTIVITY IN MUCOSAL BIOPSIES FROM THE SAME JEJUNAL SURGICAL SPECIMENS

Subject	Lactase activity (U/gww) in biopsy number					
	1	2	3	4	5	6
1	0.16	0.17	----	----	----	----
2	0.30	0.30	0.30	0.30	0.33	0.33
3	0.35	0.31	0.31	0.35	0.29	0.29
4	0.56	0.58	0.59	----	----	----
5	2.08	2.07	----	----	----	----
6	2.51	2.49	----	----	----	----

CV = 2.7%

(calculation of CV p.89)

TABLE 7.4

STABILITY OF LACTASE IN INTACT MUCOSA AND IN A HOMOGENATE AT -20°C

	Lactase activity (U/gww)						
Mucosal Biopsy	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	0.16	----	----	0.17	----	----	----
2	0.35	----	----	----	----	----	0.30
3	0.58	----	----	0.59	----	----	0.42
Homogenate							
1	0.58	----	0.51	----	----	----	----
2	0.30	----	0.43	----	----	----	----
3	0.58	----	----	----	----	0.40	----
4	0.59	----	----	----	----	0.41	----

TABLE 7.5

$\hat{g}$ (lactose) AFTER ORAL LACTOSE LOAD (50g)

$\hat{g}$ (mmol/L)	Number of Subjects	%
<0-0.0	21	10.7
0.1 - 0.2	17	8.6
0.3 - 0.4	37	18.8
0.5 - 0.6	15	7.6
0.7 - 0.8	27	13.7
0.9 - 1.0	26	13.2
1.1 - 1.4	23	11.7
1.4	31	15.7
Total	197	100.0

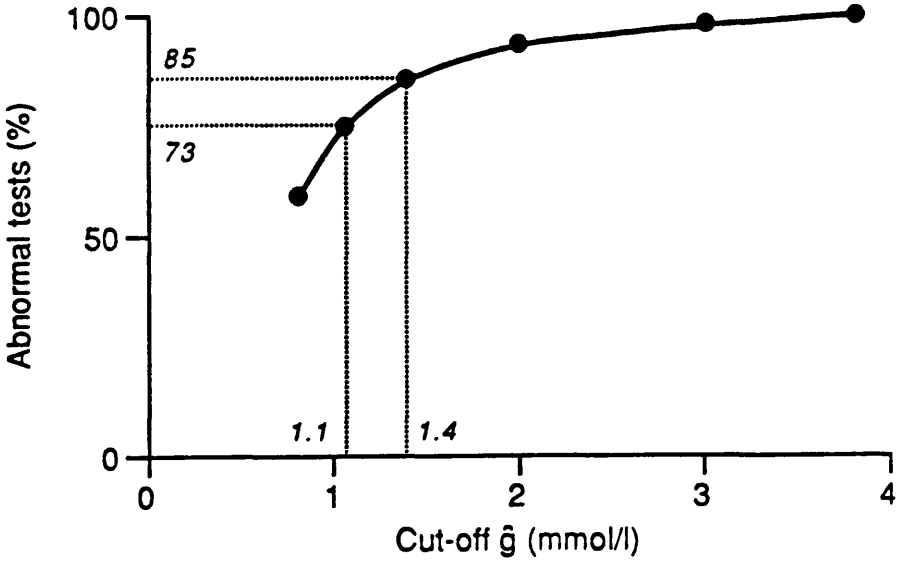


Figure 7.3

Percentage abnormal tests with different ĝ cut-off points in SriLankan adults.

73-92% respectively. Thus the prevalence rate of abnormal lactose loads in this population lay between 60 and 92%.

2.1.2 **Effect of sampling on prevalence rate:** There were six recognisable patterns of changes of blood glucose concentration after oral lactose (Fig 7.4).

a) **Early rise and a gradual fall** such that the blood glucose was still more than 1.1 mmol/L above the baseline even at 80 minutes.

b) **Early rise and a prompt fall** where after an initial normal rise, the blood glucose was down to the baseline as early as 40 minutes after the lactose.

c) **Middle-rise** where the blood glucose rose to more than 1.1 mmol/L at 60 minutes.

d) **A late rise** where a normal rise was not found until 80 minutes.

e) **A flat curve** where the maximum rise was less than 1.1 mmol/L.

f) **A fall** below the fasting level.

The percentage distribution of these types of curves are given in Table 7.6. These different patterns will introduce bias in the estimates of prevalence rate for the lactase restriction phenotype if a less rigorous sampling frequency is followed than in the present study. This is illustrated in Table 7.7 where it can be seen that if only one sampling point was used the prevalence rate would be overestimated by 10-18% independent of the chosen time of sampling.

2.1.3 **A fall in blood glucose concentration after lactose load:** 8.6% of the subjects (Table 7.6) demonstrated a fall in blood glucose concentration. The mean and S.D of $\hat{g}$(lactose) in this group is -0.8 $\pm$ 0.5mmol/L, which is significantly different ($p < 0.05$) to that of $\hat{g}$(water), (0.04 $\pm$ 0.3mmol/L, Appendix III).

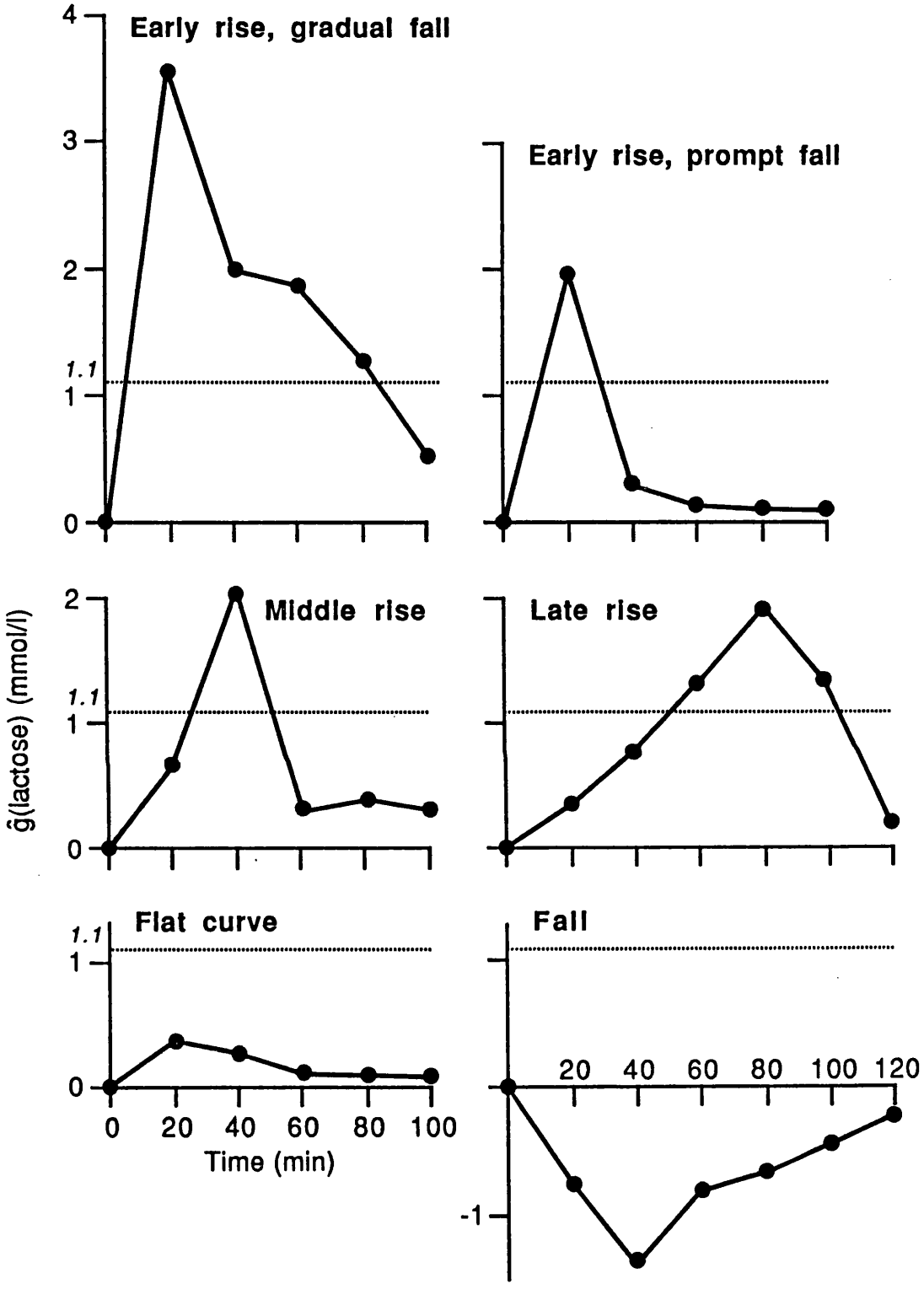


Figure 7.4

Patterns of blood glucose response after oral lactose load.

TABLE 7.6

TYPES OF CHANGE OF BLOOD GLUCOSE CONCENTRATION AFTER ORAL LACTOSE LOAD

	n	%
a) Early rise ($\hat{g} \geq 1.1$ mmol/L), gradual fall	9	4.6
b) Early rise ($\hat{g} \geq 1.1$ mmol/L), prompt fall	4	2.0
c) Middle - rise ($\hat{g} \geq 1.1$ mmol/L)	32	16.2
d) Late rise ($\hat{g} \geq 1.1$ mmol/L)	9	4.6
e) Flat curve ($\hat{g} < 1.1$ mmol/L)	126	64.0
f) Fall below basal level	17	8.6
Total	197	100.0

TABLE 7.7

VARIATION IN PREVALENCE RATE OF ABNORMAL ORAL LACTOSE LOAD IF A SINGLE BLOOD SAMPLING TIME IS USED TO DETERMINE $\hat{g}$ (197 SUBJECTS, $\hat{g}$ < 1.1 mmol/L)

Time of blood sampling (minutes after lactose)	Number with $\hat{g}$<1.1mmol/L		Difference from overall prevalence of 72.6%	Significance level
	n	%		p
20	178	90.4	+17.8	0.01
40	171	86.8	+14.2	0.01
60	163	82.7	+10.1	0.05
80	172	87.3	+14.7	0.01
100	*79	83.2	+10.6	0.001

* 100 minute samples were collected in 79 subjects

2.1.4 **Prevalence rate of abnormal oral lactose load in the different ethnic groups:** The prevalence rate of abnormal oral lactose load among the Sinhalese was 73% (n = 158) and among the Tamils was 73% (n = 30), using a $\hat{g}$(lactose) of less than 1.1 mmol/L as the cut-off point (Table 7.8). The number of Moors studied was too small to allow statistical analysis.

2.1.5 **Symptomatic response to oral lactose load:** Four groups of subjects could be recognised:

i) Those with abnormal $\hat{g}$ and symptoms (the abnormal test)
ii) Those with abnormal $\hat{g}$ without symptoms
iii) Those with a normal $\hat{g}$ with symptoms
iv) Those with a normal $\hat{g}$ and no symptoms (the normal test).

The symptoms usually consisted of diarrhoea and abdominal pain (96.7%) and in some instances (3.3%) only of abdominal pain. In the majority (93.5%) the symptoms occurred within three hours. However, in 6.5% of the subjects the symptoms did not occur until six hours after the lactose load was administered.

The relative frequency of these four groups is shown in Table 7.9. If the presence of symptoms after oral lactose load is used to indicate lactose restriction then the prevalence rate would be 30.9%. Twelve of the 61 lactose intolerant subjects (20%) absorbed lactose normally (ie $\hat{g} > 1.1$ mmol/L). Twelve of the 54 lactose absorbers classified on the basis of $\hat{g} > 1.1$ mmol/L (22.2%) had symptoms, hence were lactose intolerant on the basis of symptoms after oral lactose. This confirms that there is a poor correlation between lactose intolerance and lactose malabsorption ($X^2 = 2.63$, $0.50 < p < 0.10$).

2.1.6 **Lactosuria** Urine was examined for reducing substances in 31 adult subjects who also had oral lactose loads. Twenty-five had an abnormal test ($\hat{g} < 1.1$ mmol/L), three demonstrated a $\hat{g}$-value between 1.1-1.4 mmol/L, and a further three a $\hat{g}$ value of > 1.1 mmol/L (Table 7.10). Twenty of the twenty five subjects who had abnormal lactose loads showed the presence of increased amounts of a reducing substance in the urine. The reducing substance was found to be lactose on chromatography. Presence of lactose in the urine was a non-specific feature as three out of three subjects with a normal $\hat{g}$ and three out of three with borderline $\hat{g}$ also demonstrated lactosuria.

TABLE 7.8

PREVALENCE RATE OF ABNORMAL ORAL LACTOSE LOAD IN THE DIFFERENT ETHNIC GROUPS

($\hat{g}$ (lactose) < 1.1 mmol/L

	Sinhalese	Tamil	Moor
Normal	43 (27.2%)	8 (26.7%)	3 (33.3%)
Abnormal	115 (72.8%)	22 (73.3%)	6 (66.7%)
Total number	158	30	9

TABLE 7.9

THE CORRELATION OF SYMPTOMS WITH $\hat{g}$ AFTER ORAL LACTOSE LOAD

$\hat{g}$ (lactose)		Symptoms present	Symptoms absent	Total
Normal	1.1 mmol/L	12 (22.2%)	42 (77.8%)	54
Abnormal	1.1 mmol/L	49 (34.3%)	94 (65.7%)	143

TABLE 7.10

BENEDICT'S TEST ON URINE FROM SUBJECTS WITH LOW $\hat{g}$ (lactose) AFTER ORAL LACTOSE

Oral Lactose Load testtested	Number	Results of Benedict's test			
		0	+	++	+++
Abnormal	25	5	4	13	3
Borderline rise	3	-	1	2	-
Normal rise	3	-	2	1	-

Urine was collected over a period of seven hours after oral lactose in eight subjects and the total amount of reducing substances was estimated. The amount corresponded to 0.2-2.6 percent of the lactose load (Table 7.11). The purpose of this investigation was to determine if lactose was being removed in appreciable amounts from the gut, which could explain the absence of symptoms in patients who demonstrated a low $\hat{g}$ value after the lactose load. The results indicate, that although there was lactose in the urine, the quantity was too small to be of significance in this respect.

2.1.7 **Chromatography of stool:** Paper chromatography of stool from subjects with diarrhoea after oral lactose showed the presence of lactose, indicating that diarrhoea was caused by unabsorbed lactose reaching the colon.

2.1.8 **Simultaneous determination of blood sugar (including lactose) and blood glucose after oral lactose load:** In two subjects who demonstrated a normal $\hat{g}$ and lactosuria, blood sugar and blood glucose were determined using Somogyi Nelson (Somogyi et al 1952) and glucose oxidase methods (Hugget and Nixon 1957) respectively at 20, 40, 60, 80 and 100 minutes after oral lactose. There was no significant difference in the blood sugar and blood glucose values. Thus the minute amount of lactose absorbed does not contribute to the blood glucose concentration measured by the non-specific Somogyi Nelson method.

2.2 Oral glucose load

Twenty-two subjects who demonstrated an abnormal lactose tolerance test, and who received an oral glucose load (25 g) on a subsequent day, all showed a $\hat{g}$(glucose) of more than 1.1 mmol/L (Table 7.12). The mean $\hat{g}$(glucose) was substantial (3.4 ± 1.4 mmol/L), indicating that glucose absorption was normal.

TABLE 7.11

URINARY LACTOSE IN SUBJECTS
WITH A LOW $\hat{g}$ (lactose) AFTER ORAL LACTOSE LOAD

Subject *	Urinary lactose	$\frac{\text{Urinary lactose}}{\text{lactose load}} \times 100$
	(mg/7 hours)	%
1	1072	2.1
2	479	0.9
3	627	1.3
4	1300	2.6
5	199	0.4
6	894	1.8
7	134	0.3
8	78	0.2

* arbitrary subject number

TABLE 7.12

$\hat{g}$ AFTER ORAL GLUCOSE (25g) AND ORAL LACTOSE (50g) IN SUBJECTS WITH INITIALLY ABNORMAL ORAL LACTOSE LOAD

Subject *	$\hat{g}$ (lactose) mmol/L)	$\hat{g}$ (glucose) mmol/L)
1	0.1	3.3
2	0.1	2.8
3	2.2	2.1
4	0.2	1.5
5	0.2	5.1
6	0.2	3.7
7	0.2	3.7
8	0.3	5.9
9	0.3	1.8
10	0.6	3.3
11	0.6	2.2
12	0.7	2.3
13	0.7	2.8
14	0.8	4.3
15	0.8	3.7
16	0.8	2.6
17	0.8	3.1
18	0.9	2.1
19	1.0	5.1
20	1.0	5.1
21	1.0	6.0
22	1.0	4.8
Mean $\pm$ SD	0.6 $\pm$ 0.3	3.4 $\pm$ 1.4

* random number

2.3. Xylose absorption tests

In 14 subjects who had low values of g after oral lactose a xylose absorption test was performed. The absorption of xylose was normal in all subjects as judged by the urinary excretion of the carbohydrate, indicating that there was no generalised jejunal malabsorption (Table 7.13).

2.4. Oral sucrose load

In 18 subjects who had low values of g after oral lactose, a sucrose load was carried out. Mean $\hat{g}$(sucrose) was 3.5 mmol/L (range 1.7 to 6.8 mmol/L (Table 7.14). This is further evidence that an abnormal lactose tolerance test is due to a selective abnormality of lactose hydrolysis.

2.5 Intestinal lactase activity

Intestinal lactase activity was carried out on 41 subjects, 32 of whom had jejunal biopsies using a Crosby capsule. In the remaining subjects a jejunal biopsy was obtained during laparotomy for unrelated conditions. The weights of the biopsies and their lactase activities are shown in Appendix V. In biopsies by Crosby capsule the mean and SD of lactase activity was 0.52 ± 0.46 U/gww and in the nine surgical specimens 0.61 ± 0.74 U/gww. Since the difference between mean values is not significant the two sets of biopsies have been combined for further statistical analysis. The distribution of lactase activities within the combined group is shown in Table 7.15.

2.5.1 **Prevalence rate of low intestinal lactase among Sri Lankan adults:** A frequency distribution histogram of the lactase activities is shown in Fig 7.5. It is evident that the values do not fit a normal distribution. The probability plots are, however, consistent with two subgroups; one group with low lactose activities and an upper limit of lactase activity of between 0.8 and 1.0 U/gww, and another group with values above that limit (Figs 7.6, 7.7).

TABLE 7.13

XYLOSE ABSORPTION TESTS IN SUBJECTS
WITH $\hat{g}$ (lactose) < 1.1 mmol/L

Subject *	$\hat{g}$ (lactose) (mmol/L)	Urinary xylose ** (% of oral dose)
1	-0.5	42
2	0.2	29
3	0.2	24
4	0.2	36
5	0.3	23
6	0.3	37
7	0.4	27
8	0.5	20
9	0.7	29
10	0.8	26
11	0.8	31
12	0.8	31
13	1.0	37
14	1.0	37
mean $\pm$ SD	0.5 $\pm$ 0.4	30 $\pm$ 6

* arbitrary subject number

** Reference value: > 12%

TABLE 7.14

ORAL SUCROSE LOAD (50g) IN SUBJECTS
WITH $\hat{g}$ (lactose) < 1.1 mmol/L

Subject*	$\hat{g}$ (sucrose) mmol/L	$\hat{g}$ (lactose) mmol/L
1	5.8	-0.8
2	3.6	-0.6
3	3.3	-0.3
4	4.1	0.3
5	2.7	0.3
6	3.7	0.3
11	1.9	0.3
12	3.1	0.2
13	4.1	0.4
14	2.7	0.5
15	3.1	0.6
16	3.2	0.7
17	2.0	0.8
18	1.7	0.9
19	3.9	1.0
20	2.7	1.0
21	6.8	1.0
22	4.3	1.0
mean $\pm$ SD	3.5 ± 1.3	0.5 ± 0.6

* arbitrary subject number

TABLE 7.15

FREQUENCY DISTRIBUTION OF INTESTINAL LACTASE ACTIVITY IN SRI LANKAN ADULTS

Lactase activity U/gww Class Interval	Frequency	Cumulative Frequency	Cumulative Frequency %
0.00 - 0.01	3	3	7.3
0.11 - 0.20	5	8	19.5
0.21 - 0.30	8	16	39.0
0.31 - 0.40	6	22	53.7
0.41 - 0.50	4	26	63.4
0.51 - 0.60	4	30	73.2
0.61 - 0.70	3	33	80.5
0.71 - 0.80	1	34	82.9
0.81 - 0.90	1	35	85.3
0.91 - 1.00	1	36	87.8
1.01 - 1.10	2	38	92.7
1.11 - 1.20	0	38	92.7
1.21 - 1.30	0	38	92.7
1.31 - 1.40	1	39	95.1
1.41 - 1.50	0	39	95.1
1.51 - 1.60	0	39	95.1
1.61 - 1.70	0	39	95.1
1.71 - 1.80	0	39	95.1
1.81 - 1.90	0	39	95.1
1.91 - 2.00	0	39	95.1
2.01 - 2.10	0	39	95.1
2.11 - 2.20	0	39	95.1
2.21 - 2.30	1	40	97.6
2.31 - 2.40	0	40	97.6
2.41 - 2.50	1	41	100.0
Total	41	41	100.0

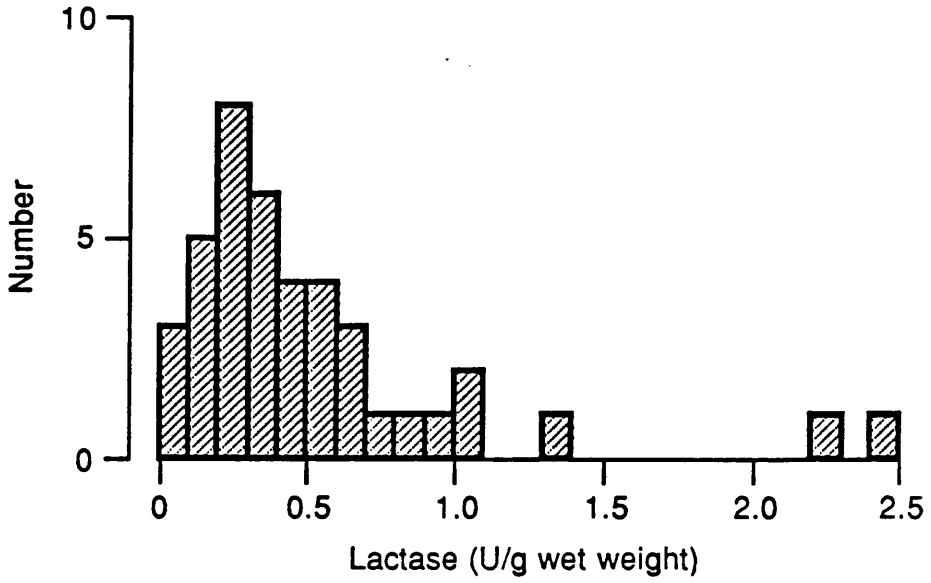


Figure 7.5

Frequency distribution of intestinal lactase activity in SriLankan adults.

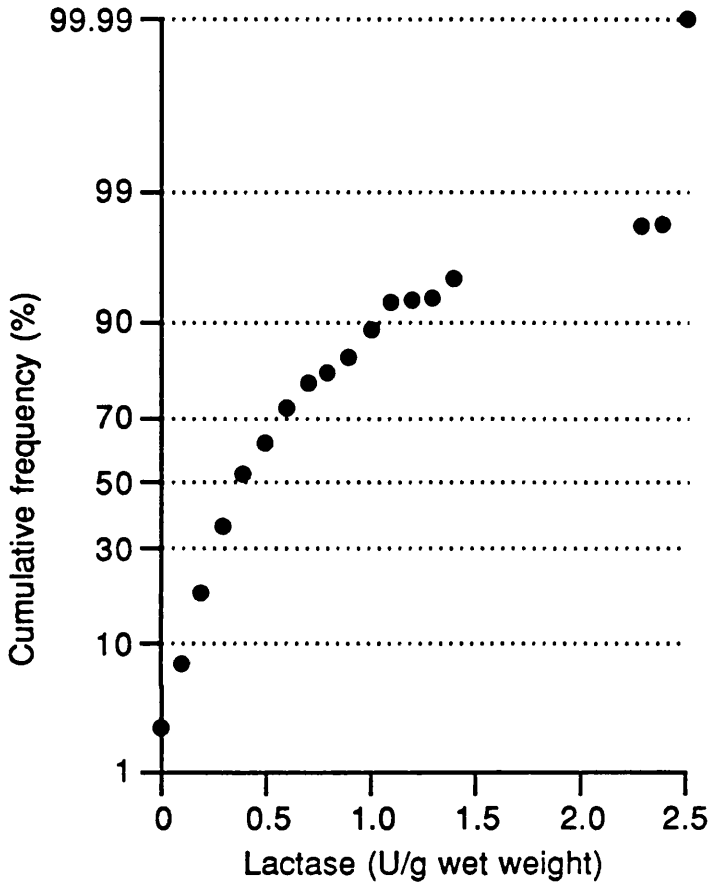


Figure 7.6

Probability plot of intestinal lactase activity of all values in SriLankan adults.

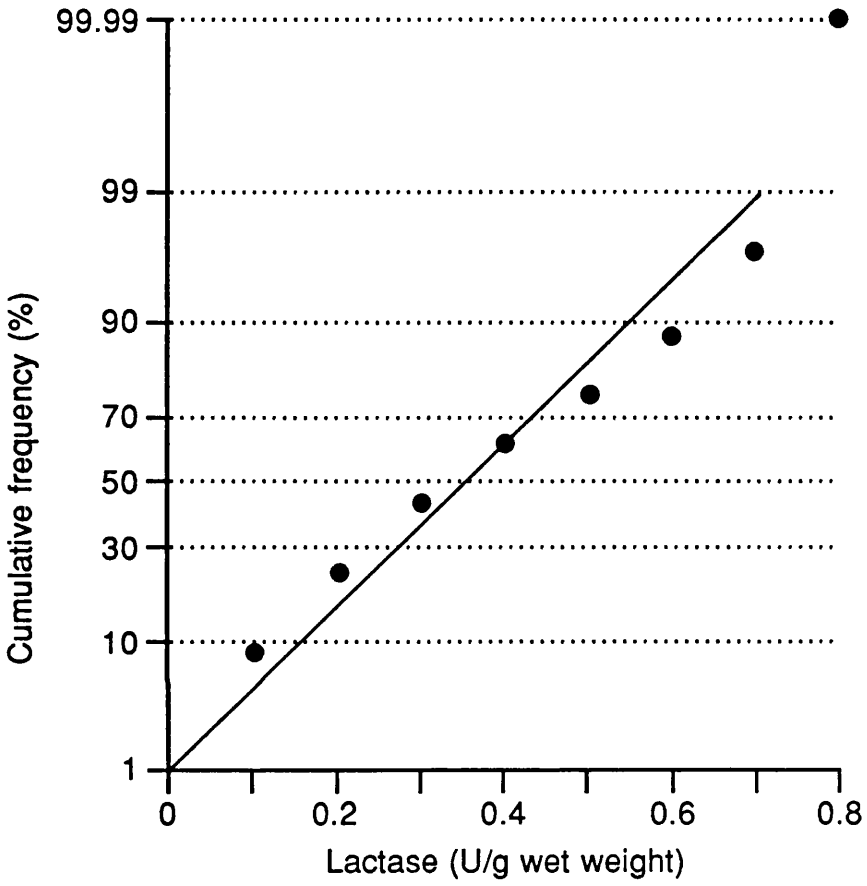


Figure 7.7

Probability plot of intestinal lactase activity of values up to 0.8 U/g wet weight.

2.5.2 **Uncertainty of the prevalence rate estimate** Fig 7.8 shows a cumulative frequency diagram of intestinal lactase activities constructed from data in Table 7.15. The graph demonstrates that the prevalence rate of lactase restriction phenotype in the population varies between 83% and 88% if lactase activities of 0.8 and 1.0 U/gww respectively are considered as the upper limit of reference interval of that population. Thus the figure for the prevalence rate is not too seriously affected by the variability of the discriminatory value of (cf. Biological and analytical variability, section 1.3., Chapter 7)

2.5.3 **Correlation of intestinal lactase and $\hat{g}$ (lactose)** The lactase activities and the corresponding values for $\hat{g}$ (lactose) carried out in 33 subjects have been correlated in Figure 7.9.

As can be seen there are both positive and negative $\hat{g}$ values correlated with lactase activities. There are several ways this correlation can be treated statistically.

- Orthogonal regression analysis, assuming a linear relationship between the variables. A statistically significant but moderate direct correlation can be established (Figure 7.9a).

- Orthogonal regression analysis excluding the three negative values of $\hat{g}$ and assuming that these values represent outliers. This approach increases the correlation coefficient (Figure 7.9b).

- Fitting a low degree polynom to all values, assuming that the correlation between all $\hat{g}$ values and lactase activities is a continuous function. The second degree polynom $y = 0.41 - 0.23x + 0.18x^2$ represents a statistically significant fit to the values (Figure 7.9c).

- Orthogonal regression analysis of all values below and above a defined common cut-off point. (In Figure 7.9d the common cut-off point is 1.0mmol/L). A statistically significant inverse correlation can be established between lactase activities and $\hat{g}$ values in the interval - 1.3 and 1mmol/L and a statistically significant positive correlation between the enzyme activity and $\hat{g}$ values between 1 and 3.8mmol/L.

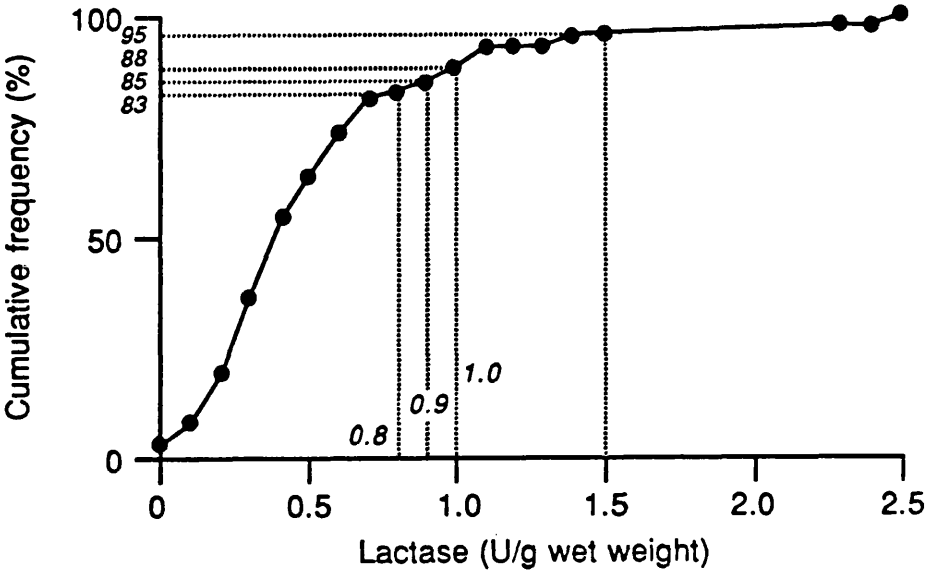


Figure 7.8

Cumulative frequency diagram of intestinal lactase activities in SriLankan adults.

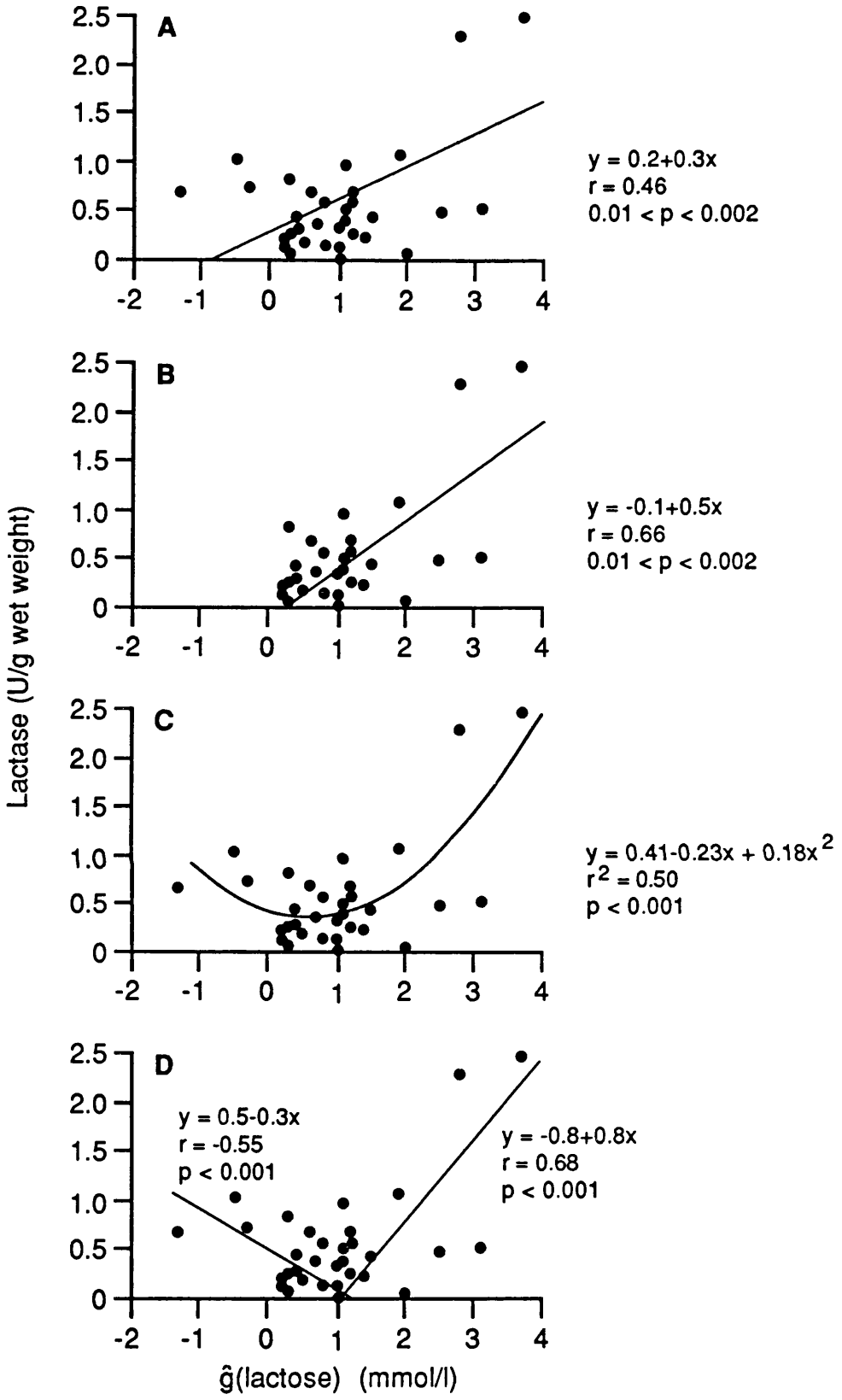


Figure 7.9

Correlation of ĝ(lactose) with lactase activities in SriLankan adults.

The outcome of the statistical analysis indicates that there is a highly significant direct correlation between intestinal lactase and positive values of $\hat{g}$(lactose). The correlation is surprisingly close (highest r-value = 0.6) for two biological parameters controlled by two different organs, the gut and the liver. It cannot be concluded with certainty from the present data if a rectilinear or a curvilinear function is the best fit.

The statistical analysis also supports the concept that negative values of $\hat{g}$(lactose) could be considered outliers from a statistical or biological point of view. Alternatively, all values of $\hat{g}$(lactose) might relate to intestinal lactase in a u-shaped fashion. Again the present data are too restricted and supplementary information (for example, about blood galactose levels) is necessary in order to decide what alternative might be the more accurate one.

The physiological implication of the correlation are further discussed in Chapter 8, 1·4, 7·1

3. INVESTIGATIONS OF SRI LANKAN CHILDREN

3.1 Oral lactose load

Oral lactose load (1 gm per kg body weight) was performed on 94 children aged one day to 15 years. Individual results are shown in Appendix VII.

3.2 Age dependency of abnormal oral lactose load

The results of lactose loads in the different age groups are shown in Table 7.16 and Fig 7.10. The prevalence rate of abnormal tests in the age groups 5 - 10 years (10.0%) is significantly lower ($p < 0.001$) than that in the group 11 - 15 years (66.7%).

Cumulative frequency plots of $\hat{g}$ values after oral lactose load in different age groups are shown in Fig 7.11. The median $\hat{g}$ (lactose) in the age groups 9 - 15 years and over 16 years is comparable, whereas that in the age group 0 - 8 years is significantly lower ($p < 0.001$). No abnormal results were observed before the age of about eight years. This indicates that the loss of intestinal lactase activity reaches a

TABLE 7.16

PREVALENCE RATE OF ABNORMAL Lactose Loads ($g < 1.1$ mmol/L) AND MEAN $\hat{g}$ (lactose) IN THE DIFFERENT AGE GROUPS

Age groups (years)	Number studied	Abnormal lactose loads		Mean $\hat{g}$
		n	%	
New born	2	0	0.0	5.9
0 - 0.5	7	0	0.0	3.9
0.5 - 1	15	0	0.0	2.6
1 - 2	18	0	0.0	3.6
2 - 3	10	0	0.0	3.2
3 - 5	7	0	0.0	2.2
5 - 10	20	2	10.0	2.1
11 - 15	15	10	66.7*	0.6
16 - 20	34	25	73.5	0.8
21 - 25	38	25	65.8	0.9
26 - 30	26	17	65.4	1.0
31 - 35	20	18	90.0	0.6
36 - 40	15	12	80.0	0.7
41 - 45	10	7	70.0	0.9
46 - 50	7	5	71.4	1.1
51 - 60	14	8	57.1	1.1
61 - 70	5	4	80.0	0.2
Total	266			

* Significantly different $p < 0.001$ from previous group

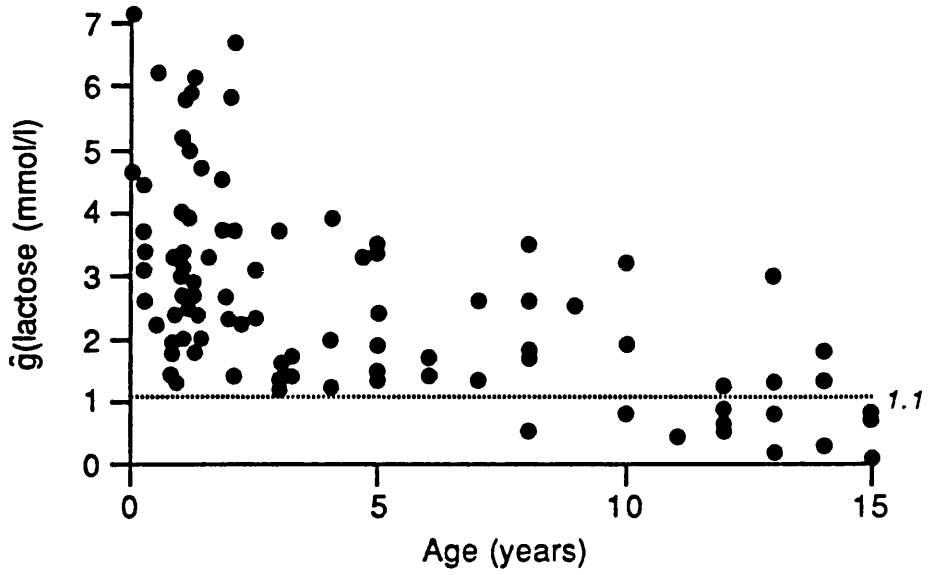


Figure 7.10

Correlation of ĝ(lactose) with age in SriLankan children.

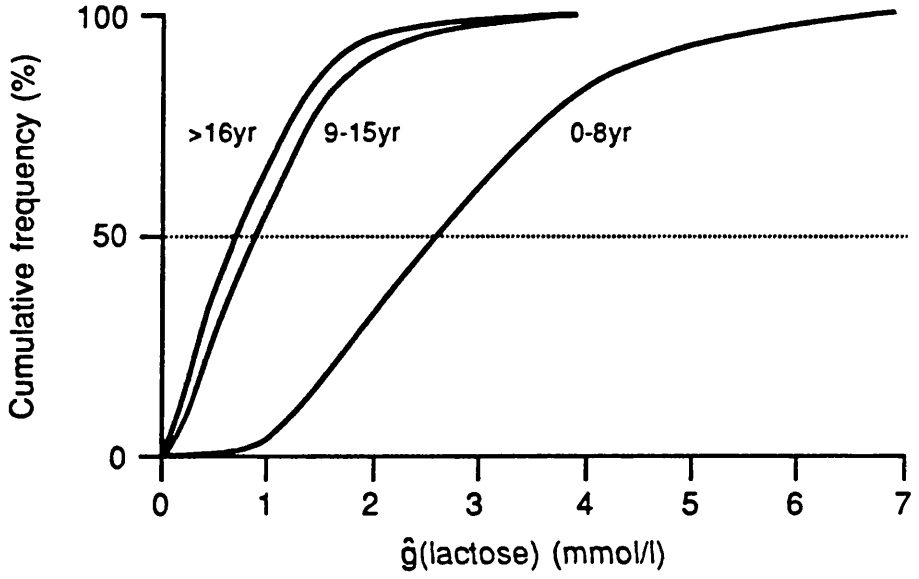


Figure 7.11

Cumulative frequency of ĝ(lactose) in SriLankan subjects of different age groups.

physiologically critical level around the age of eight years in the Sri Lankan population. In Fig 7.12 the $\hat{t}$ values are correlated with age. The correlation is poor, partly because $\hat{t}$ is a step function ($r = 0.2$, $0.05 < p < 0.02$). However, if cumulative frequency plots of $\hat{t}$ values are constructed below and above the age of eight years (Fig 7.13), it can be seen that the median values for $\hat{t}$ (27 and 42 minutes) in these groups differ significantly ($p < 0.05$).

Fig 7.14 shows a three dimensional scatter plot of $\hat{g}$ and $\hat{t}$ vs age in the Sri Lankan subjects. This confirms that in the younger children the maximum rise of glucose concentration after a lactose load is higher and occurs sooner, and that with increasing age the maximum rise of glucose concentration after lactose is lower and occurs later.

4. FUNCTIONAL DIARRHOEA IN SRI LANKAN PATIENTS

4.1 Incidence in a random hospital population

4.1.1 **Study in the outpatient department** Five hundred and twenty two consecutive patients who visited the general outpatient department in a general hospital were questioned about the presenting symptoms.

Only 12 of the patients (2.3%) complained of chronic intestinal symptoms (Table 7.17).

4.1.2 **Study in the medical clinic** A further 500 patients attending the medical follow-up clinic were asked about symptoms of functional diarrhoea or chronic intestinal symptoms. Only five (1.0%) complained of such symptoms.

4.2 Oral lactose load on adults with functional diarrhoea

The results of an oral lactose load on the five patients with symptoms of functional diarrhoea and in two members of the staff with similar symptoms are shown in Table 7.18. All the subjects had a low $\hat{g}$ value and developed symptoms after oral lactose.

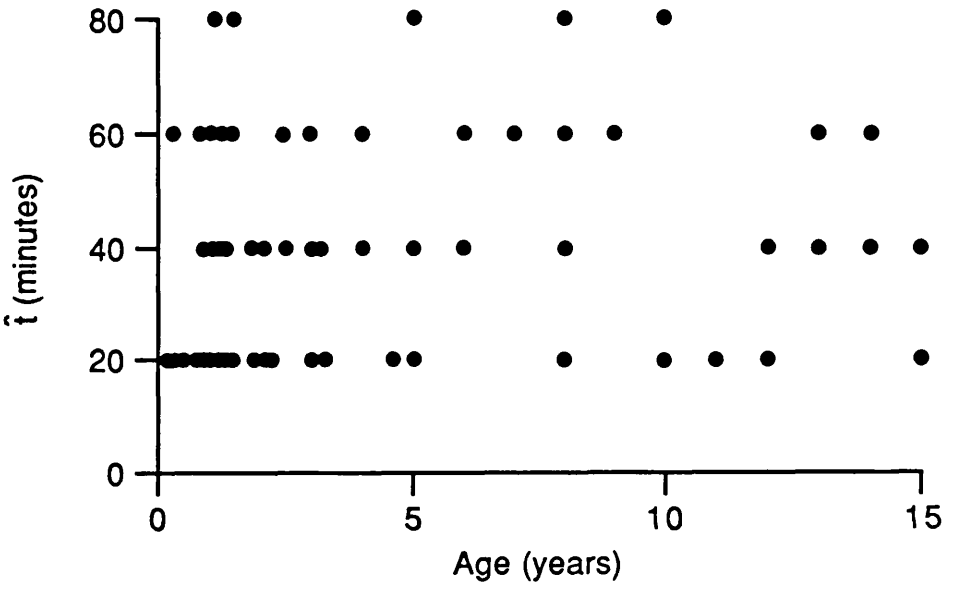


Figure 7.12

Correlation of $\hat{t}$ with age in children living in SriLanka.

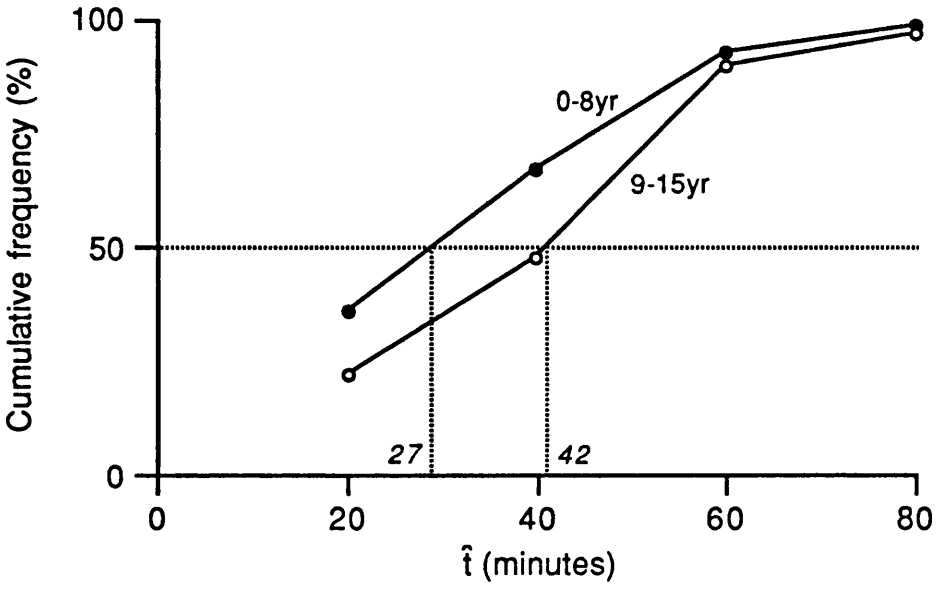


Figure 7.13

Cumulative frequency diagram of $\hat{t}$ of SriLankan children in age groups 0-8 and 9-15 years.

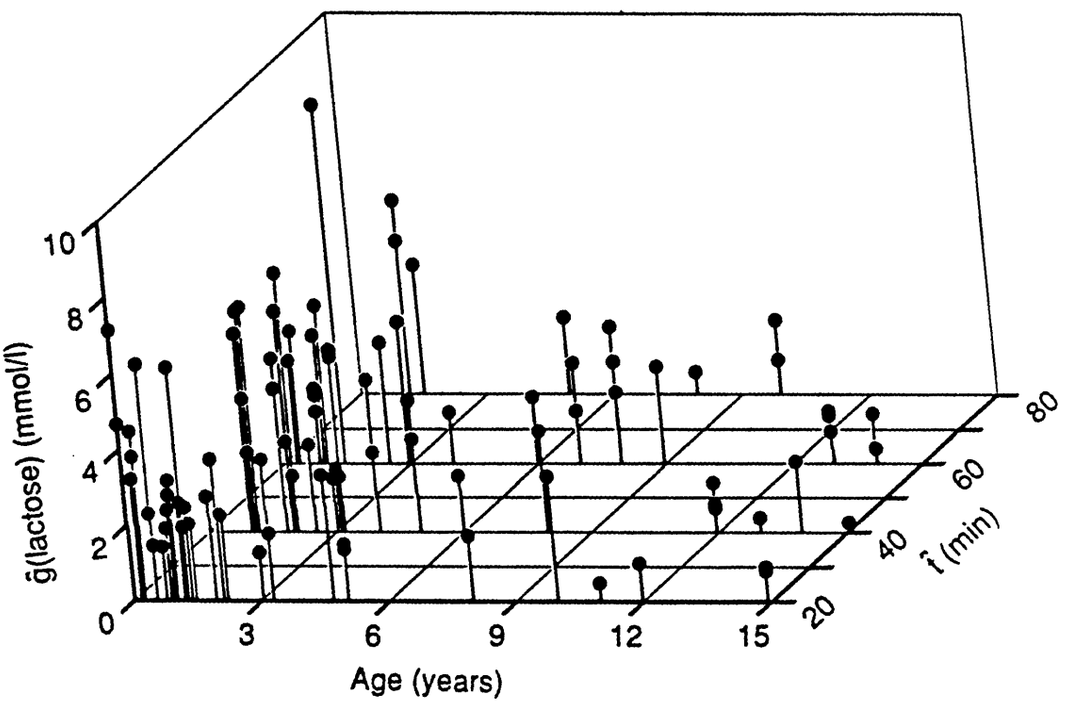


Figure 7.14

Three dimensional plot of $\hat{g}$(lactose) and $\hat{t}$(lactose) versus age in SriLankan children.

TABLE 7.17

PRESENTING COMPLAINTS IN SRI LANKAN ADULTS SEEN AT THE OUTPATIENT DEPARTMENT

Presenting complaint	Number	Percentage %
Acute diarrhoea	18	3.4
Chronic abdominal pain, distension	12	2.3
Constipation	1	0.2
Asthma, cough	180	34.5
Aches and pain, headache	66	12.6
Cardiovascular symptoms	5	1.0
ENT, Eye, Skin, Surgical and gynaecological complaints	57	10.9
Wounds	138	26.4
Administrative problems	30	5.8
Symptoms of urinary tract infection	9	1.7
Others	6	1.1
Total	522	100.0

TABLE 7.18

ORAL LACTOSE LOAD IN SEVEN SUBJECTS WITH FUNCTIONAL DIARRHOEA

Subject *	Age	Sex	Symptoms	$\hat{g}$ (lactose) (mmol/L)
1	23	M	D	1.0
2	23	F	D	1.0
3	18	F	D	0.3
4	27	F	D	1.0
5	43	M	D + A	0.1
6	28	F	D + A	1.0
7	18	F	A + F	0.3

D = Diarrhoea

A = Abdominal colic

F = Flatulence

* random numbers

4.3 Case report of a symptomatic patient with lactase restriction phenotype

A woman of 40 years presented at the outpatient clinic with diarrhoea of two years' duration. She passed a watery diarrhoea three to four times per day with no blood or mucous. At first she could not associate drinking milk or milk products with the occurrence of diarrhoea. However, on keeping a detailed record of the times she took various food items and the occurrence of diarrhoea, the relation to ingestion of milk products was found. Even milk added to tea produced diarrhoea. The oral lactose load was found to be abnormal ($\hat{g}$ = 0.5 mmol/L); the oral sucrose load was normal ($\hat{g}$ = 3.0 mmol/L). Milk was withdrawn from the diet for one year and she reported a dramatic improvement in symptoms. She then started taking small amounts of milk without developing diarrhoea and a year later a cup of milk, again without effects. At this time the oral lactose load was repeated, still found to be abnormal ($\hat{g}$ = 0.7 mmol/L). The load evoked acid diarrhoea and abdominal pains. However, the symptoms were much less severe than at the first test. This case illustrates that despite a low intestinal lactase and an abnormal oral lactose load, adaptation to the symptoms produced by milk may occur.

4.4 Milk drinking habits

Detailed history of milk intake was obtained from 50 consecutive patients attending the outpatient department. Only one (2%) consumed more than a glass of milk (approximately 200 ml = 10 gm lactose) per day. The average daily milk intake was 0.02 L/day with a range of 0 - 0.2 L/day.

5. INVESTIGATIONS OF A BRITISH PAEDIATRIC HOSPITAL POPULATION

5.1 Reference interval for intestinal lactase

5.1.1 **Based on histology:** A frequency distribution histogram of lactase values in all jejunal biopsy specimens obtained over a period of 12 months is shown in Fig 7.15. Histologically abnormal biopsy specimens demonstrated a wide scatter of values (range 0 - 6.0 U/gww). In specimens that were histologically normal, the lactase activity was more than 1 U/gww except in four children who had values of 0 and 0.9 U/gww. Their maltase and sucrase activities were normal.

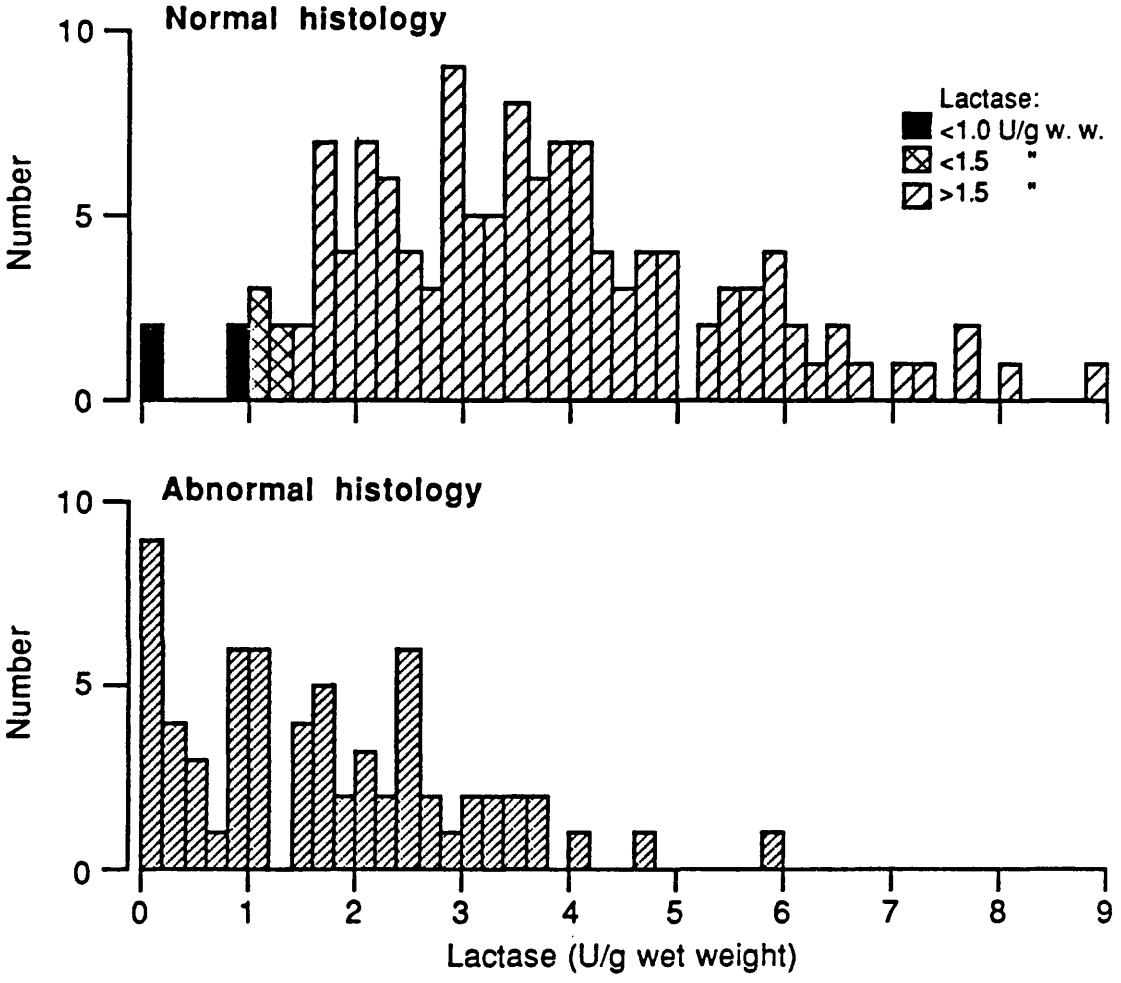


Figure 7.15

Frequency distribution of intestinal lactase activity of histologically normal and abnormal biopsies of children living in London.

(Maltase > 8 U/gww and sucrase > 3 U/gww were considered normal, Dahlqvist 1970). Detailed examination of the case notes revealed that, although these four children were referred for failure to grow (non-intestinal cause), they would fit into the category of lactase restriction phenotype (Table 7.19, Subjects 1 - 4).

5.1.2 **Based on maltase and sucrase activities:** The frequency distribution histograms of Maltase/Lactase ratios and Sucrase/Lactase ratios related to the mean lactase activities of the group with histologically normal specimens are shown in Figs 7.16 and 7.17 respectively.

Maltase/Lactase ratios seem to demonstrate a bimodal distribution with one group with values less than 10 that correspond to mean lactase activities of more than 1.5 U/gww. The second group with maltase/lactase values more than 10 correspond to mean lactase values of less than 1.5 U/gww.

Similarly sucrase/lactase ratios of less than 2.5 represent the group with lactase values of 1.5 or more U/gww.

Although the clinical histories and frequency distribution of histologically normal biopsies indicate a lower cut-off value of 1 U/gww, when maltase/lactase and sucrase/lactase ratios are considered there seems to be a group with lactase values up to 1.5 U/gww, who show selective lowering of lactase activity relative to the other disaccharidases. Hence the lower limit for the population with lactase persistence phenotype seems to lie between 1 and 1.5 U/gww.

5.1.3 **Based on comparison with another population (Sri Lanka) who predominantly demonstrate lactase restriction phenotype:** The cumulative frequency distribution of lactose values in histologically normal biopsies on the British children is shown in Fig 7.18 with a similar diagram for Sri Lankan adults for comparison. The analytical methods are comparable. If a cut-off point at 1 U/gww of lactase activity is considered, 88% of the Sri Lankan adult population have values less than 1 U/gww. On the other hand only 3% of the British children have values less than 1 U/gww. Since 88% of the Sri Lankan adults can be considered to

TABLE 7.19

DETAILS OF CHILDREN LIVING IN BRITAIN
WHOSE INTESTINAL LACTASE ACTIVITY IS LESS THAN 1.5U/gww

Subject	Age (years)	Lactase (U/gww)	Maltase (U/gww)	Maltase/ Lactase	Sucrase (U/gww)	Sucrase/ Lactase	Ethnic origin
1	12	0	17.1		4.1		Asian
2	11	0.9	9.9	11	2.7	3	British
3	3	0.9	11.7	13	3.0	3	Asian
4	9	0.9	27.3	30	7.5	8	African
5	11	1.2	30.9	28	8.7	8	Asian
6	13	1.1	22.5	21	6.8	6	British
7	4	1.2	15.3	13	4.8	4	British
8	11	1.2	16.8	14	4.4	4	British
9*	4	1.1	17.1	16	4.6	4	Asian
10	12	1.2	21.3	18	5.0	4	Asian

* child with coeliac disease after 1 year of gluten free diet

Reference values;	Maltase	> 8U/gww
	Sucrase	> 3U/gww
	Maltase/Lactase	< 10
	Sucrase/Lactase	< 2.5

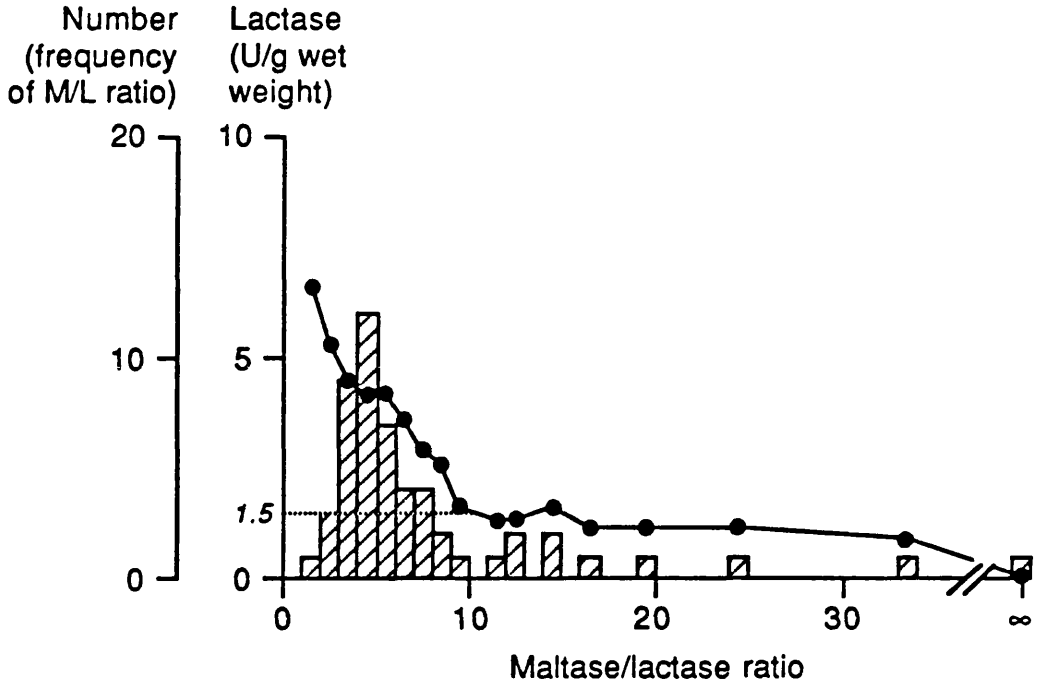


Figure 7.16

Frequency distribution histogram of maltase/lactase ratio and the mean lactase activity of the class interval (●) of histologically normal biopsies from children living in London.

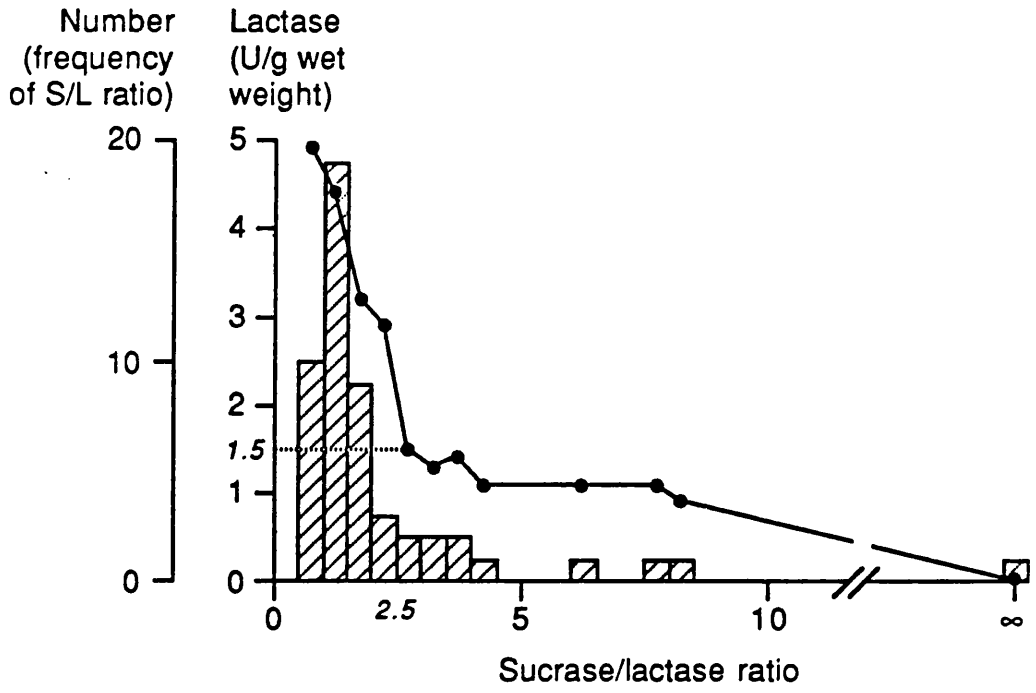


Figure 7.17

Frequency distribution histogram of sucrase/lactase ratio and the mean lactase activity of the class interval (•) of histologically normal biopsies from children living in London.

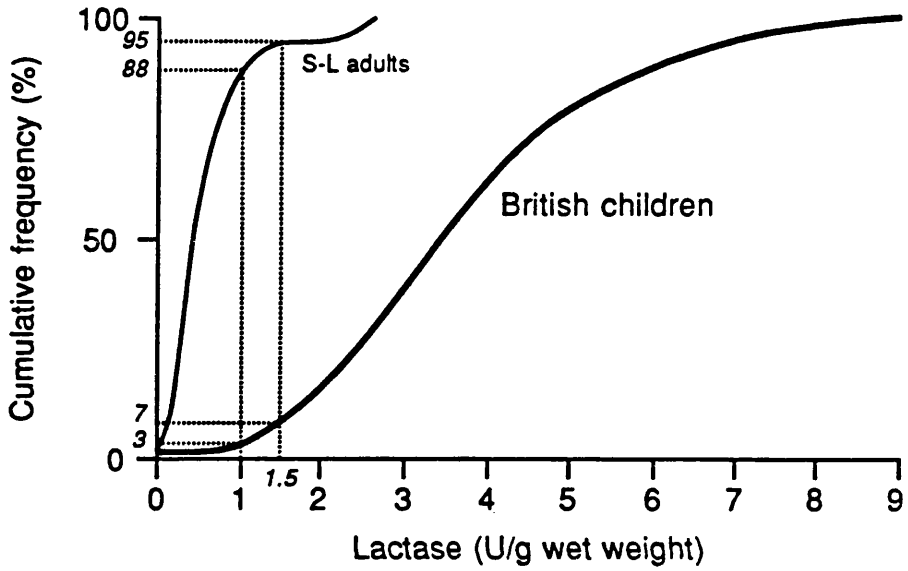


Figure 7.18

Cumulative frequency diagram of lactase activities among British children living in London compared with a similar diagram of adults in SriLanka.

belong to the lactase restriction phenotype, it is reasonable to assume that the 3% of the British children too represent this phenotype.

The upper limit of lactase activity (1 U/gww) of the Sri Lankan group whose majority (88%) would represent homozygous lactase restriction phenotype, corresponds to the lower end of the reference interval (1 U/gww) of a group whose majority (97%) constitute the homozygous and heterozygous individuals for lactase persistence gene (ch. 3, 3.2.2).

5.2 Prevalence rate of lactase restriction phenotype

Over a two year period 128 biopsies were investigated where histology and maltase and sucrase values were normal. Of these, four had a lactase value of less than 1 U/gww, and nine had a lactase value of less than 1.5 U/gww. Details of the four children with a lactase of less than 1 U/gww are given in Table 7.19 (No. 1-4). There were a further six children who had a lactase value between 1 and 1.5 U/gww and maltase/lactase and sucrase/lactase ratios of >10 and >2.5 respectively (Table 7.19).

Closer scrutiny of the case notes of these children revealed the following:

All four children (numbers 1 - 4) with a lactase activity of less than 1 U/gww did not reveal any small intestinal disease clinically and their jejunal biopsy was normal histologically. Apart from low lactase activity, activities of the other disaccharidases were normal. These could represent the group with lactase restriction phenotype. Two were of Asian origin and one African and one white British. Three of the four children were over nine years of age. One of the Asian children was three years old.

Of the six with lactase values between 1 and 1.5 U/gww (Table 7.19) one (number 9) should be classified as secondary lactase deficiency. This child's biopsy, although it revealed normal histology, had coeliac disease and he was on a gluten free diet at the time of this biopsy. The rest (numbers 5, 6, 7, 8, 10) were aged 11, 13, 4, 11 and 12 years, they were referred for short stature. No small intestinal disease was established in this group. These five children could also be classified as lactase restriction phenotype.

Thus the prevalence rate of lactase restriction phenotype among the British children living in London is four out of 128 (3.1%) (if 1 U/gww is considered the lower reference interval) and nine out of 128 (7.0%) (if 1.5 U/gww is taken as the lower reference interval).

5.3 Age dependency of intestinal lactase

An additional 42 children over the age of 5 years were included in the subsequent study. Thus the total number investigated were 170. Figs 7.19 and 7.20 show lactase activities and maltase/lactase ratios correlated with age in these children. Correlation of lactase activity with age does not show a statistically significant trend. Figure 7.21 shows the percentage cumulative frequency plots of the lactase activities in the British children above and below the age of 5 years, with an inset of Figure 7.11 of a similar diagram for the Sri Lankan children. This indicates that the median values for the lactase do not change significantly with age. This is in contrast to the situation in Sri Lanka where the median $\hat{g}$ (lactose), (which is an indirect measure of lactase activity) changes significantly with age. The correlation of maltase/lactase ratios with age (Figure 7.20) suggests that as the age increases there are two groups, one with high ratios (> 10) and another with low ratios. This indicates that the decrease in lactase activity with age seen in a minority of the children is selective, affecting only lactase and not maltase activity.

Age related prevalence rate of lactase restriction phenotype of the Sri Lankan children and the British children living in London is shown in Table 7.20. After the age of 9 years, there is a significantly higher prevalence rate ($p < 0.05$) among the Sri Lankan than the British children.

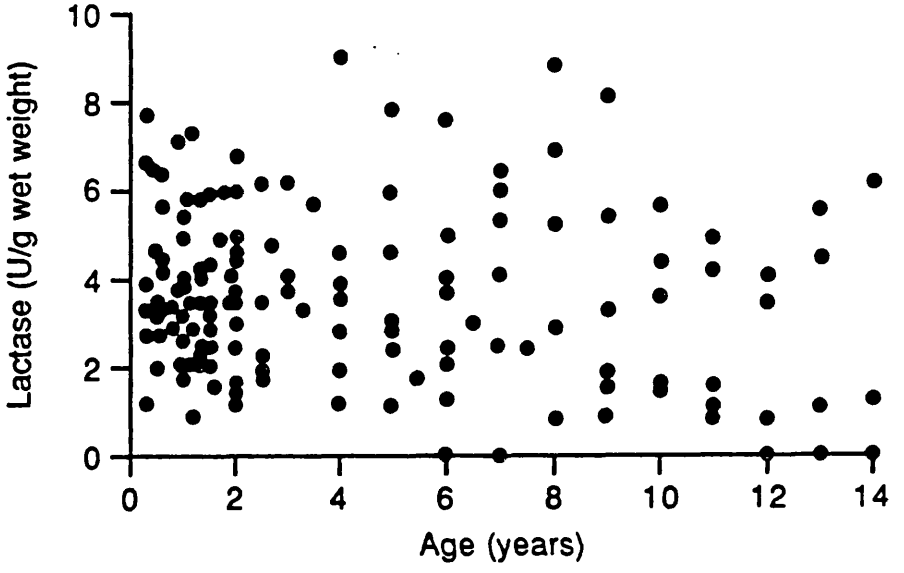


Figure 7.19

Correlation of lactase activities with age in children living in London.

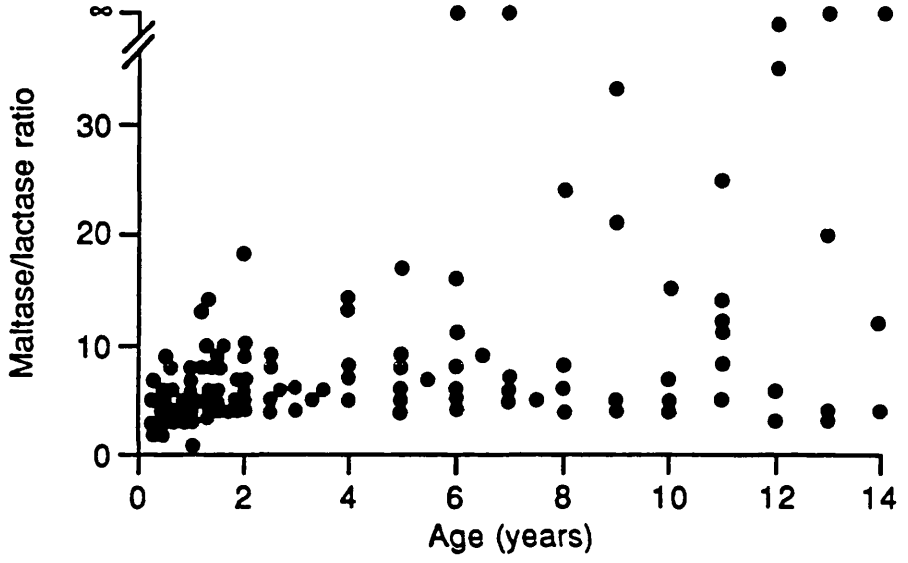
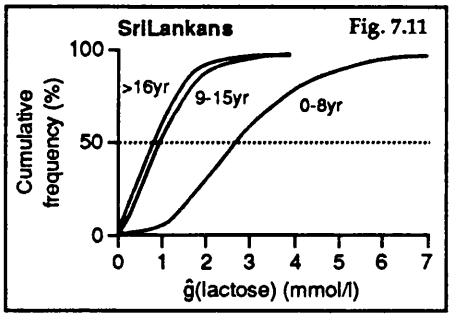


Figure 7.20

Correlation of maltase/lactase ratios with age in children living in London.



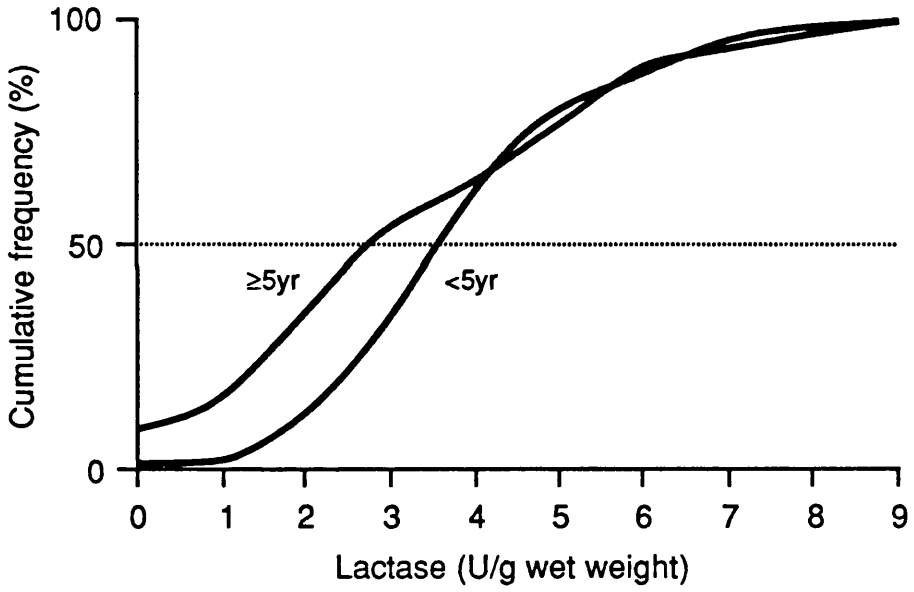


Figure 7.21

Cumulative frequency plot of lactase activity in British children above and below 5 years of age.

TABLE 7.20

COMPARISON OF AGE RELATED PREVALENCE RATE OF LOW LACTASE PHENOTYPE AMONG CHILDREN LIVING IN SRI LANKA AND BRITAIN

AGE GROUP	SRILANKA		BRITAIN	
(years)	no. studied	no. (%) $\hat{g}$<1.1mmolL	no. studied	no. (%) lactase<IU/gww
0 - 3	52	0(0)	87	1(1)
4 - 6	16	0(0)	31	1(3)
7 - 9	9	1(11)	20	3(15)
10 - 12	9	5(56)	17	3(18)*
13 - 15	18	11(61)	7	1(14)*
Total 0 - 1	104	17(16)	162	9(5)

* Significant difference between Sri Lankan vs British figures $p < 0.05$

6. SUMMARY

There was a good reproducibility of the oral lactose load test (Fig 7.1). Coefficient of variation of lactase activity from different biopsies at the same site was 2.7% (Table 7.3). The stability of the enzyme activity of biopsy stored at -20°C was acceptable up to four days (Table 7.4).

The cut-off value of the glucose increment after the lactose load to define lactase phenotypes is between 1.1-1.4 mmol/l (Fig 7.3). For the purpose of defining the lactase phenotypes blood sampling after the lactose load should be carried out at 20 minute intervals up to 80-100 minutes. Less frequent blood sampling would overestimate the prevalence rate by about 13.5% (Table 7.7). Symptoms after a lactose load has a poor discriminatory power (Table 7.9).

The cut-off value of intestinal lactase activity was between 1.0-1.5 u/gww (Fig 7.5, 7.8, 7.15, Fig 7.16, 7.17, 7.18).

There was a good correlation of intestinal lactase activity and the increment of glucose concentration after the lactose load (Fig 7.9).

The prevalence rate of lactase restriction phenotype among the Sri Lankan adults using the lactose load and intestinal lactase activity is between 73% and 88% (Fig 7.3, Fig 7.8). The prevalence rate is not significantly different in the different ethnic groups in Sri Lanka (Table 7.8).

The lactase restriction among the Sri Lankan children is expressed around the age of eight years (Fig 7.10, 7.11).

The predominant phenotype among the British children is lactase persistence (Fig 7.15 top, 7.18). The lactase activity does not significantly change with age (contrast Sri Lankan children (Fig 7.21)). The children in Britain who demonstrate lactase

restriction phenotype are mostly of Asian and African origin (Table 7.19). The age of expression of the restriction phenotype is not different in the two populations studied (Table 7.20). It is significant that after the age of nine years the prevalence of lactase restriction among children living in London is between 14-18%.

CHAPTER 8

DISCUSSION

1. DIAGNOSTIC PROCEDURES FOR LACTASE RESTRICTION PHENOTYPE
   - 1.1 **Intestinal Lactase**
   - 1.2 **Oral lactose load**
   - 1.3 **Sampling frequency of oral lactose load**
   - 1.4 **Correlation of oral lactose load with intestinal lactase**

2. LACTASE RESTRICTION IN SRI LANKAN ADULTS
   - 2.1 **Selection of Subjects**
   - 2.2 **Prevalence rate of lactase restriction**
   - 2.3 **Lactose intolerance**
     - 2.3.1 Absence of symptoms in subjects with the lactase restriction phenotype
     - 2.3.2 Presence of symptoms in the lactase persistence phenotype
   - 2.4 **Lactosuria**

3. LACTASE RESTRICTION AMONG A SRI LANKAN AND BRITISH PAEDIATRIC HOSPITAL POPULATION

4. AGE DEPENDENCY OF LACTASE RESTRICTION PHENOTYPE

5. CLINICAL SIGNIFICANCE OF LACTASE RESTRICTION PHENOTYPE AMONG SRI LANKAN ADULTS

6. MILK FEEDING PROGRAMMES IN SRI LANKA

7. CLINICAL SIGNIFICANCE OF LACTASE PERSISTENCE
   - 7.1 **Occurrence of a population subgroup with a possible alteration in galactose metabolism**

8. AETIOLOGY OF LACTASE RESTRICTION

9. SUMMARY

1. DIAGNOSTIC PROCEDURES FOR LACTASE RESTRICTION PHENOTYPE

1.1 Intestinal lactase

Although the determination of lactase is the definitive method for establishing the lactase restriction phenotype, this procedure is less suitable for population studies. This is because of the technical difficulty of obtaining the biopsy and the need for the biopsy to be transported at -20°C. Thus intestinal lactase has been used mainly as a reference method in this study with the aim of testing the performance of the oral lactose load.

There is no established quality control system for lactase assays as there is no reference enzyme preparation of known activity available. There is also very little work on the reliability of the method reported in the literature (Townley et al 1965, Welsh et al 1967, Ferguson et al 1984). Hence each laboratory has to determine its own reproducibility of estimations and establish their own reference intervals. In this study a value of 0.8 to 1.0 U/gww was obtained as the lower reference value for normal subjects, which is in agreement with values reported by other workers (Table 4.2). This cut-off point is also in agreement with the value used to classify patients in the British paediatric study. The imprecision of the estimation of lactase activity on the same biopsy sample was low (CV 7%), indicating good reproducibility of the established method. The variability of lactase activity on multiple biopsies on six patients was also low (CV = 2.7%). Ferguson et al 1984 reported a wider variability on multiple biopsies. However, most of their patients had high lactase activity, whereas the patients in the present study demonstrated low lactase activity. This indicates that the variability of lactase activity in the intestine is higher in those with lactase persistence than those with lactase restriction.

1.2 Oral lactose load

There are many factors, other than the presence of intestinal lactase, which can affect the change in blood glucose after an oral lactose load and consequently the parameters used to characterise such a change ($\hat{g}$

and $\hat{t}$ in this study), for example the dose of lactose given, gastric emptying, the rate of hydrolysis, the rate of absorption of glucose into the circulation and the peripheral uptake and the intermediary metabolism of glucose.

Despite all these limitations the performance characteristics of the oral lactose load used in this study was good. The $\hat{g}$ (lactose) of 1.1 mmol/L, taken as the lower reference value, was significantly higher than $\hat{g}$ (water) which on the average was 0.04mmol/L. This result indicates that blood glucose changed as a result of increased input of glucose into the blood circulation and not as a result of an altered plasma volume due to the water included in the oral lactose load (Appendix III). There was also good reproducibility of the load in the same individual (Table 7.1, Fig 7.1). The overall uncertainty of a single $\hat{g}$ value, taking analytical and biological factors into account, was approximately $\pm$ 20% (Table 7.1).

1.3 **Sampling frequency of oral lactose load** The kinetics of the blood glucose rise after oral lactose load will influence the outcome if only one blood sample is drawn at a defined time after the load. The effect is that the prevalence of an abnormal oral lactose load will be overestimated, as has been shown in this thesis (Table 7.7). Others have shown that 3-5% will be misclassified if only one sample at 30 minutes is taken (Fielding 1980). However if, as they suggest, it is used as a preliminary screening test for clinical purpose it would be sufficient. However in population studies (Flatz and Saengudom 1969, Flatz et al 1969), using a one-point oral lactose load, may have overestimated the prevalence of lactase restriction, which was reported to amount to 100% among the Thais.

1.4 **Correlation of oral lactose load with intestinal lactase** The prevalence rate of lactase restriction based on the oral lactose load was similar to that based on the enzyme estimation (Ch 7, 2.1.1 and 2.5.2). There was a statistically highly significant correlation between $\hat{g}$(lactose) and intestinal lactase. This correlation would mean that the lactase activity is a fairly good indicator of the total amount of the enzyme in the gut.

2. LACTASE RESTRICTION IN SRI LANKAN ADULTS

2.1 Selection of subjects

The subjects for this study were chosen among hospitalised individuals who were relatively healthy (Table 5.1) and who did not have any disease affecting the small intestine, in order to exclude patients with a pathological secondary disaccharidase deficiency. However, this approach excluded those with lactase restriction phenotype and symptoms of milk intolerance. Hence the prevalence figure in this thesis will be an underestimate of the true prevalence, as those with milk intolerance would have been excluded. Since this study also indicated that the incidence of symptoms that can be attributed to milk intolerance in the Sri Lankan population is low (4.1, Chapter 7), the error in the obtained prevalence rate figures is likely to be small.

2.2 Prevalence rate of lactase restriction

The prevalence rate of the lactase restriction phenotype, based on oral lactose load (73 - 85%) and intestinal lactase (88-95%) indicates that the lactase restriction gene is common in Sri Lanka.

Similarly high figures have been reported in other populations in Asia (Table 3.1).

2.3 Lactose intolerance

2.3.1 **Absence of symptoms in subjects with the lactase restriction phenotype:** Only 34% of the subjects who had an abnormal oral lactose load had symptoms after the load. This result is in agreement with previous reports that subjects with low intestinal lactase may remain asymptomatic after ingestion of large amounts of milk or 50 g of lactose (Welsh et al 1967; Bedine and Bayless 1973; Cook 1973; Stephenson and Latham 1974; Lisker et al 1978; Porro et al 1981). The absence of symptoms suggests one of several possibilities:

- o A low value of $\hat{g}$(lactose) is due to factors other than a low or absent intestinal lactase (for example, slow gastric emptying or rapid glucose utilisation). However, in the 16 subjects who had low $\hat{g}$ values and no symptoms (Appendix VI), all except one had a lactase activity of less than 0.8 U/gww.

o Lactose is absorbed without hydrolysis, and either excreted by the kidney or metabolised in the body. Renal excretion of lactose is quantitatively small, as confirmed in this thesis, and is the experience of others (Bickel 1961, Welsh et al 1967, Weser and Sleisenger 1965). Thus absorbed lactose would not be excreted by the kidney to any significant extent. It was also shown in this thesis that in the subjects who demonstrated lactosuria the blood did not contain any significant amounts of reducing substances other than glucose.

Lactose is unlikely to be metabolised in the rest of the body. Lactase has not been demonstrated in human organs other than the intestine. A minimal lactase activity has been demonstrated in rat kidney (Dahlqvist and Brun 1962), but no information is available on human kidney. The major part (85-90%) of intravenously administered lactose is excreted in the urine within 24 hours (Stuhlfauth et al 1962, Koehler et al 1935). In rats, 83% of intravenous ^{14}C labelled lactose is excreted intact in the urine; by contrast 64% of orally fed ^{14}C labelled lactose appears as expired $^{14}CO_2$ within the first hour (Carleton et al 1955). This shows that lactose is metabolised only if it is hydrolysed to its monosaccharides in the intestinal mucosa.

o The gut adapts to the presence of unabsorbed lactose. This has been positively documented (Haemmerli et al 1965, Cuatrecasas et al 1965, Keusch et al 1969b) and is supported by the case report in this thesis (Ch 7, 4.3) where the patient was able to consume considerable amounts of milk without developing symptoms. Colonic salvage of unabsorbed lactose by bacteria has been demonstrated in rats and humans (Bond and Levitt 1976). Such salvage of unabsorbed lactose by colonic bacteria may account for the lack of symptoms in the majority of human subjects with low intestinal lactase. The variability of the occurrence of symptoms may be due to the variability of the composition of gut flora.

2.3.2 **Presence of symptoms in the lactase persistence phenotype:** A fifth of the subjects with normal $\hat{g}$ values after oral lactose, implying normal intestinal lactase, had symptoms. This finding could imply that the lactase activity was insufficient to hydrolyse all lactose, leaving unhydrolysed lactose in the gut. Presence of symptoms following ingestion of 0.5 - 1 L of milk or 50 g of lactose among individuals with

normal intestinal lactase has been reported by others (Flatz et al 1982b; Czeizel et al 1983). Thus, it is likely that unhydrolysed lactose might be left in the gut despite a normal lactase activity in some individuals. If microbiological salvage is missing in such a situation, free lactose will become osmotically active, which might induce symptoms.

2.4 Lactosuria

Although most of the subjects (80%) who had an abnormal lactose load ($\hat{g} < 1.1$ mmol/L) demonstrated lactosuria, the degree of lactosuria was usually mild. Two of the eight subjects on whom lactosuria was quantitated, excreted 1.1 and 1.3 G in the seven hours after the 50 G lactose load. These also had low intestinal lactase activity. Other workers have documented lactosuria which was more pronounced in those with low lactase activity (Weser and Sleisenger 1965, Bickel 1961, Haworth 1960, Dahlqvist et al 1963, Welsh et al 1967).

Lactose is normally not absorbed intact by the small intestinal mucosa. The passage of lactose across the mucosa is due to the permeability of the mucosa, which is defined as the property of the membrane which facilitates the unmediated diffusion of a solute across the membrane. There has been great interest recently on this property of the intestinal brush border in understanding the aetiology of some intestinal disease as well as designing tests which would discriminate between them (Menzies 1972 and 1974, Menzies et al 1979, Bjarnason et al 1983, Cook and Menzies 1986, Bjarnason and Peters 1984, Maxton et al 1986, Bjarnason et al 1986).

The qualitative change in the membrane lipid composition could have opposing changes in permeability to lipophilic and lipophobic substances (Bjarnason et al 1986). This has resulted in tests that measure the excretion ratios of two substances which increases the discriminating power of the test (Menzies et al 1979, Cook and Menzies 1986).

In the two subjects excreting 1.1 and 1.3 G of lactose, the intestinal permeability could have been altered due to unrecognised subclinical intestinal disease.

This study has confirmed that lactosuria is a non-specific finding as it was demonstrated in subjects with both normal and abnormal lactose loads (Table 7.10).

3. LACTASE RESTRICTION AMONG A SRI LANKAN AND BRITISH PAEDIATRIC HOSPITAL POPULATION

It is evident from the present study that lactase restriction is common among Sri Lanken children after the age of eight years, and that the lactase persistence is the commoner phenotype among the British children of mixed origin (Figure 8.1). The prevalence rate of lactase restriction is likely to be even lower among white British children if it is taken into consideration that children of Asian and African origin constituted about half the group of lactase restriction phenotype (Table 7.19).

It is, however, of considerable clinical significance that about 18% of the investigated paediatric population over the age of 8 years in London demonstrated lactase restriction. This finding makes measurement of intestinal disaccharidases important in British children of this age group presenting with intestinal symptoms, especially in areas where there are ethnic minorities from Asia and Africa.

4. AGE DEPENDENCY OF LACTASE RESTRICTION PHENOTYPE

As has been indicated in Chapter 2, there is a post-weaning decrease in the activity of the intestinal lactase in all mammals. The results in this thesis show that the majority of Sri Lankan adults, like the majority of the world population, lack lactase in adult life, that the loss of lactase is selective (sucrose loading was normal) and that the decline of lactase activity is age dependent. In Sri Lankan children the decrease in lactase activity seems to reach a critical level around the age of eight years, unlike other ethnic groups where this occurs earlier, sometimes as early as at one year of age (Cook 1967; Keusch et al 1969a; Elliotte et al 1967; Flatz 1982a, b; Hijazi et al 1981). It has been suggested (Keusch et al 1969a) that the more rapid decline reported by some investigators could be explained by the inclusion of secondary lactase deficiency. However, a Finnish study of adolescents (Sahi and Launiala 1978; Sahi et al 1983) has also documented a late expression of the lactase restriction phenotype. It was suggested that environmental

factors such as high milk consumption might maintain intestinal lactase. However, this hypothesis cannot explain the late presentation of the lactase restriction phenotype in Sri Lanka, as milk consumption there is low.

The social implication of the finding of a late onset of the lactase restriction phenotype among Sri Lankan children is that foreign aid in the form of milk to pre-school children in Sri Lanka is appropriate, contrary to an earlier suggestion (Bolin et al 1970). Cow's milk protein intolerance has not been studied in Sri Lanka. Children with this disorder are symptomatic and usually present within the first six months of life (Walker-Smith 1988). Hence it is unlikely to be a significant clinical problem among normal school children.

In the British paediatric study the ages of the children who demonstrated lactase restriction phenotype ranged from 3-12 years (Table 7.19). The three year old child was of Asian origin. The number of children studied is too small for any firm conclusions about the age dependency of expression of the restriction phenotype in Britain irrespective of origin. However, there seem to be no major differences in the pattern of expression of the lactase restriction phenotype between the Sri Lankan and the British populations (Figure 8.1).

5. CLINICAL SIGNIFICANCE OF LACTASE RESTRICTION PHENOTYPE AMONG SRI LANKAN ADULTS

In this study the majority of the adult subjects (66%) with low levels of lactase activity or a low value of $\hat{g}$(lactose) did not experience any symptoms on 50 g of lactose. This suggests that there is an adaptive response to unhydrolysed lactose in the gut, as has been discussed previously (Ch 8, 2.3.1). The absence of a functional expression of lactase restriction in adult Sri Lankans is further supported by the fact that "functional diarrhoea", or "irritable colon" is not a clinically prominent problem in Sri Lanka. Only 2.3% of patients visiting a general outpatient department complained of symptoms that could be attributed to "functional diarrhoea" or "irritable colon". This is an insignificant figure considering that nearly 85% of the Sri Lankan adult

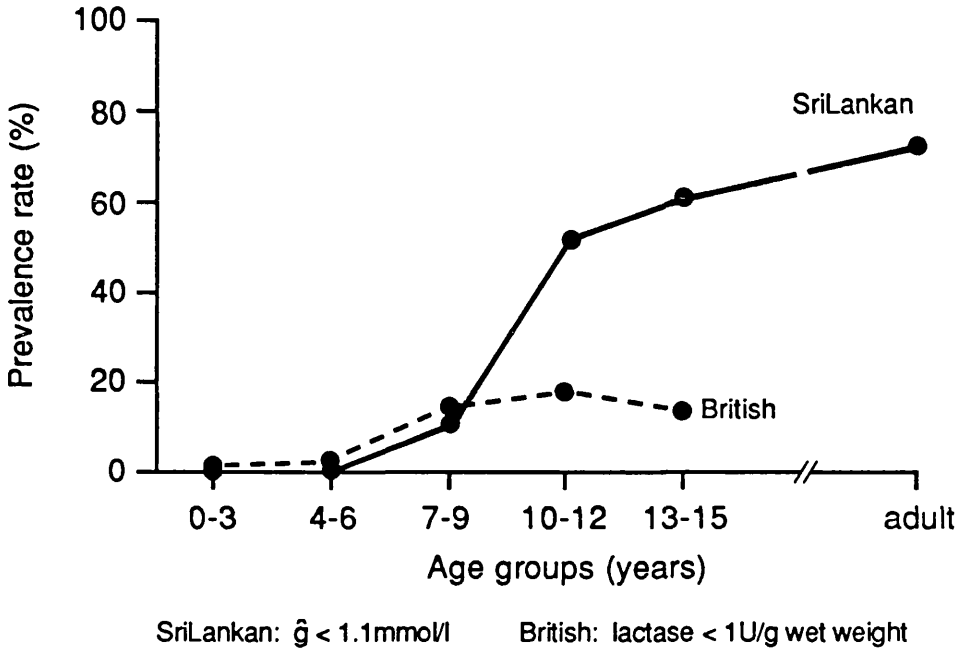


Figure 8.1

Prevalence rate of lactase restriction among SriLankans and a British paediatric hospital population in London.

population have a substantially reduced activity of intestinal lactase. A main contributing factor towards this outcome could be that the majority of Sri Lankan adults do not drink milk (Ch 7, 4.4). A study in the United States provides evidence that milk intolerance is dose dependent (Bedine and Bayless 1973). 240 ml milk (10 g of lactose) produced symptoms in 75% of the subjects, but 60 ml (3 g of lactose) only occasionally caused diarrhoea and abdominal pain. Several workers (Klotz and Lubos 1967, Welsh 1970, Dunphy et al 1965, McMichael et al 1965, Newcomer and McGill 1967) have also demonstrated that "isolated lactase deficiency" can exist in asymptomatic individuals.

Only a small number of Sri Lankan patients presented with symptoms suggestive of "functional diarrhoea" and an abnormal oral lactose load (Case Report, Table 7.21). Symptoms occurred after the oral lactose load and declined on milk withdrawal (as in the case report in Chapter 7, 4.3).

In Britain, one investigation (Pena and Truelove 1973) suggested that patients with "irritable colon" do not have a higher incidence of lactose malabsorption than the normal population. However, McMichael et al (1965) reported a higher frequency of lactose intolerance in patients with an "irritable colon". A Danish study (Gudmand-Hoyer et al 1973) came to the same conclusion. Although the Danish patients did not have a history of milk intolerance, elimination of milk from their diet often improved their symptoms. A Finnish study has also shown that there was improvement on a lactose free diet in patients with non-specific abdominal symptoms (Jussila et al 1970). The oral lactose load is therefore worthwhile in patients with an "irritable colon" and the effect of withdrawal of milk should be tried if the oral lactose load is abnormal. Conversely, chronic abdominal symptoms should not be attributed to lactose intolerance even in the presence of an abnormal oral lactose load if these symptoms do not respond to milk withdrawal, and other causes must be sought (BMJ editorial 1975).

In conclusion this study shows that "functional diarrhoea" (and the irritable colon syndrome) is rare in Sri Lanka, although the majority of the population belongs to the lactase restriction phenotype. This outcome is most probably due to a low milk intake and colonic salvage of unhydrolysed lactose.

6. MILK FEEDING PROGRAMMES IN SRI LANKA

It has been claimed (Davis and Bolin 1967) that milk feeding programmes are of little value in the developing countries because of the high prevalence of "lactase deficiency". This claim was based on the finding of early age of expression of the lactase restriction phenotype in some ethnic groups such as the Thais (Keusch et al 1969 a). The results in this thesis imply that these conclusions are not applicable to Sri Lanka because of the relatively late age of expression of lactase restriction (eight years). Milk feeding programmes among pre-school and primary school children should therefore be encouraged in Sri Lanka.

7. CLINICAL SIGNIFICANCE OF LACTASE PERSISTENCE

It is not clear if consumption of lactose-rich diet in adult life exposes the individual to any health risks. There has been some evidence that high milk consumption in subjects with lactase persistence was a contributory cause of hyperlipidaemia (Sahi et al 1977) and coronary heart disease (Segall 1980). Simoons has noted a high incidence of senile cataract in some groups of lactase persistent adults consuming large amounts of lactose rich food (Simoons 1982). In a population that is predominantly lactase restricted, the prevalence of cataract was more in the minority with lactase persistence (Rinaldi et al 1984). A similar study in Sweden has not confirmed this (Bengtson et al 1984). The work in this thesis indicates that there could be a sub-population showing biochemical polymorphism affecting the galactose clearance. This subgroup would be at a risk if exposed to prolonged lactose consumption in adult life (see below).

7.1 Occurrence of a population subgroup with possible alteration in galactose metabolism

A decrease in the blood glucose concentration below the basal value after an oral lactose load occurred in 8.6% of subjects studied in this thesis (Chapter 7, Table 7.8). A decrease in blood glucose concentration after oral galactose or milk has previously been observed in galactosaemic patients (Komrower et al 1956) and was thought to be due to the inhibition of glycogenolysis by the accumulation of

galactose-1- phosphate in the liver. In case reports, Thornton et al (1962) and Evans (1965) had also noticed that there was a considerable fall in the blood glucose after a lactose or galactose load, which did not occur after glucose or sucrose loads. They too suggested that galactose-1-phosphate might inhibit phosphoglucomutase in glycogenolysis.

A decrease in blood glucose concentration after a lactose load in adults is less well documented. It has been noted by others (Bryant et al 1970, Spanidou and Petrakis 1972) but not commented on. In this study, some of the adult subjects investigated, whose blood glucose concentration fell after oral lactose, had normal lactase activity. It is possible that absorbed galactose reached a significantly high enough blood concentration in the portal blood in these cases to inhibit the glycogenolytic pathway. This possibility is also indicated by a negative correlation between lactase and low or negative values of $\hat{g}$ (lactose) observed after some of the lactose loads (Chapter 7, Figure 7.9). This correlation would suggest that in this subgroup, the higher the lactase activity, the higher the portal blood galactose concentration and the more pronounced the inhibition of glycogenolysis. It is possible that this subgroup has decreased galactose clearance which would be expected in those individuals heterozygous for galactosaemia. However, the subgroup studied in this thesis would be different from such heterozygotes for galactosaemia because of the high prevalence (almost 1:10 of the subjects investigated). Population studies have indicated that from 0.9 to 1.25 percent are heterozygous for the classic galactosaemia (Beutler et al 1966). However variant enzymes of the galactose metabolism have been described (eg Duarte, Los Angeles). Heterogygotes for these variants are estimated to be 8 to 13 percent (Mellman et al 1968). Hypothetically a modified enzyme involved in the metabolism of galactose with changed kinetic properties might be the molecular cause of the decrease in blood glucose level seen after a lactose load. To confirm this hypothesis further investigations measuring the kinetic properties of the enzymes involved in galactose metabolism and study of galactose tolerance of subjects with senile cataract are necessary.

Another explanation for negative and low values of $\hat{g}$(lactose) after lactose load could be an increased glucose utilisation due to increased insulin release. This mechanism has been suggested (Evans 1965) but not confirmed.

8. AETIOLOGY OF LACTASE RESTRICTION

There is strong evidence (Chapter 3, 3.2) that the different lactase phenotypes are genetically determined and that the more prevalent or "normal" genotype is the lactase restriction gene resulting in a decreased intestinal lactase during the post-weaning period, as in the Sri Lankan population. The high prevalence of the mutated lactase persistence gene in certain ethnic groups, for example in Northern Europe, could be due to natural selection. Factors that may have promoted such a selection are milk dependency for survival (as North Africa Chapter 3, 3.3.1) or facilitated calcium absorption (as in Scandinavia, Chapter 3, 3.3.2). These factors do not apply to the Sri Lankan population. Sri Lanka is mainly an agricultural country, and the population did not depend on milk for survival. There is plenty of sunlight and hence decreased calcium absorption due to lack of vitamin D would not have contributed to the selection of the lactase persistence gene. The low prevalence of the lactase persistence phenotype in Sri Lanka (20-25%) is possibly due to inter-mixing with the Caucasian race, which would have occurred during the many invasions of the country by Caucasian colonial powers in the past.

The present study has confirmed that the decline in lactase activity occurs about the same time in the two populations studied (Fig 8.1, Table 7.20), one with predominantly lactase persistence and the other with predominantly lactase restriction phenotype. The study has also confirmed that children of Asian and African origin, who are genetically destined to be lactase restricted, and living in Britain in an environment where people are predominantly lactase persistent, demonstrate a decline in lactase activity about the same time as in their countries of origin. This finding suggests that lactase restriction is not affected by environmental factors and is in support of a genetically determined "biological clock".

The precise molecular mechanism of the age dependent decline of lactase activity in the majority of the Sri Lankan population and the persistence of the same enzyme activity in the majority of the British population remains to be elucidated. Possible mechanisms include age dependent differences in transcription, synthesis rate, intracellular processing or degradation.

In the past, difficulty with purification of stable lactase with an adequate yield has made characterisation of each of these processes difficult. However recently purification of the enzyme was accomplished (Skovbjerg 1981), which has resulted in the production of polyclonal and monoclonal antibodies (Skovbjerg et al 1981, Potter et al 1985, Sjostrom et al 1983, Danielsen et al 1981a). This has made it possible to characterise the biosynthetic forms of lactase (Danielsen et al 1984b, Skovbjerg et al 1984). The DNA coding for the enzyme complex has recently been cloned (Mantei et al 1988) and the gene localised to the long arm of chromosome 2 (Kruse et al 1988 and 1989). Evidence for the possible molecular mechanism controlling the phenotypic expression of the genetic control of lactase has emerged from both animal and human studies.

Animal studies: Intra-luminal instillation of ^{3}H-leucine and pulse-chase experiments in suckling and adult rats showed that the fractional incorporation of the label into lactase compared to total protein in the suckling rats was higher (Jonas et al 1985) and characterization of the enzyme on SDS-polyacrylamide gel electrophoresis was the same in the rats at both ages. Another group studying the incorporation of injected ^{3}H-leucine demonstrated that lactase synthesis takes place at a constant rate throughout development and interpreted that the net fall in lactase activity results from a more rapid enterocyte replacement (Tsuboi et al 1981 and 1985). Cytochemical studies on the rate of migration of the enterocyte and lactase activity of cells along the villi suggests that the fall in lactase activity with age is associated with a shortening of the time available for lactase expression in the brush border and that this takes place at two different rates during the post-weaning decline (Smith and Jones 1987). Others have suggested that the decline in lactase at weaning is associated with changes at the level of intracellular glycosylation of the enzyme protein leading to the formation of inactive enzyme protein

(Nsi-Emvo et al 1987). The same group of workers have also shown that the expression of the brush border lactase activity is under hormonal control, ie cortisol and/or thyroxin (Raul et al 1983, Mahmood and Torres-Pinedo 1985, Nsi-Emvo et al 1986, Henning 1986, Nsi-Emvo et al 1987). Recent cloning of the cDNA for lactase has led to some further proposals regarding the genetic control of lactase expression (Mantei et al 1988, Kruse et al 1988 and 1989). The level of mRNA in the adult animals (rats and rabbits) remains the same as that in the suckling animals even though the intestinal lactase activity had declined (Sebastio et al 1989, Freund et al 1989). This suggests that the control is post transcriptional.

Human studies: Density gradient ultracentrifugation studies of biopsy specimens from infants and adults with lactase restriction or persistent phenotypes have shown that the physical and chemical properties of the enzymes from these three groups (substrate specificity, kinetic properties, pH optimum curves) are identical (Lebenthal et al 1974).

Using the technique of separating the brush border membrane proteins by polyacrylamide gel and determining the disaccharidase activities of the different sections, it was shown that although there was a reduction of lactase activity in individuals with lactase restriction, qualitatively there was no difference in the composition of the brush border proteins (Freiburghaus et al 1976).

Using an alternative technique of immunoelectrophoresis which separates the enzyme proteins according to their charge and antigenicity, it has been shown that there is a constant correlation between the amount of lactase activity and the immunologically active enzyme protein in individuals with lactase restriction and lactase persistence (Skovbjerg et al 1978 and 1980). This suggests that the decline in activity is not due to the formation of inactive form of the enzyme. It has also been shown by studies using lactase purified by monoclonal immunoadsorbent chromatography that the human adult and infant lactases are indistinguishable immunologically (Potter et al 1985).

A recent report (abstract) has demonstrated that the intracellular proteolytic processing of the lactase precursor is delayed in organ culture of small intestinal biopsies from lactase restricted individuals (Sterchi et al 1989). Levels of mRNA remain the same in individuals with lactase restriction and lactase persistence (Sebastio et al 1989 (abstract)). The details of these studies are not available. However they suggest that the control of the genetic expression in humans too is post transcriptional. A further step in defining the molecular mechanism for lactase decline would be to study the different biosynthetic forms of lactase in the human biopsy specimens in the subcellular fractions of the human intestinal mucosa. The paediatric population in Britain investigated in this thesis would form the ideal material as it consists of both phenotypes. Biopsy material obtained from ages between three and twelve years should be subjected to subcellular fractionation and immunoprecipitation with monoclonal antibodies (Danielsen 1982, Bolton et al 1984). Electrophoresing the immunoprecipitates on SDS/polyacrylamide gel and using the staining technique with different lactins will enable the identification of the different biosynthetic forms and their mannose content and the relative position of these forms in the subcellular compartments. In parallel the mRNA levels could be determined at the different ages and compared with the biosynthetic forms.

9. SUMMARY

The performance of the lactose load test has been critically evaluated. There was good reproducibility of the test and the overall uncertainty of the increment of blood glucose taking analytical and biological factors into account was approximately $\pm$20% (Chapter 7, Table 7.1, Fig 7.1). There was good correlation between the lactose load test and the intestinal lactase activity.

The prevalence rate of lactase restriction among Sri Lankan adults is between 75% and 95%. The children in Sri Lanka have high intestinal lactase activity at birth and show a decline in the enzyme activity at around eight years of age. In addition although the majority of adult Sri Lankans demonstrate lactase restriction phenotype, milk intolerance is not a significant clinical problem. Therefore milk feeding programmes, especially amongst pre-school and primary school children, should not be discouraged.

The commoner phenotype among the British children is lactase persistence. The restriction phenotype seen amongst these children occurred in the children of Asian and African origin. It is however significant that 18% of the children in London between the ages of eight and fifteen years show decreased intestinal lactase and this diagnosis should be considered in the relevant clinical situation (Chapter 8, 3 and 4).

The molecular mechanism for the aetiology of the lactase phenotypes is discussed in the light of recent findings (Chapter 8, 8), and it has been suggested that the low intestinal lactase activity is a result of genetic control which include post transcriptional processing of the Pre-Pro form of the enzyme complex.

New avenues of research are suggested based on the findings in the thesis and recent developments in the characterisation of the lactase enzyme (Chapter 8, 8). These would involve the study of the different biosynthetic forms and their intracellular localisation in the intestinal mucosa of children from the age of three years to twelve years.

There is some evidence of a subpopulation in whom galactose metabolism may be different (Chapter 8, 7). The risks of increased exposure to galactose may be aggravated among those with lactase persistence phenotype. Ways of elucidating this in future research is suggested.

CONCLUSIONS

Lactase restriction phenotype is common in Sri Lanka affecting the majority of the population. The intestinal enzyme is present at birth but disappears about the age of eight years. The age of expression of the phenotype is later than in some other populations. Although lactase restriction is widely prevalent in this country, clinical symptoms as a result of this are rare. This is not entirely because of the low milk intake. The adaptive mechanisms that are responsible for the lack of symptoms are discussed. It is important not to attribute low lactase to chronic intestinal symptoms unless they are alleviated by milk withdrawal.

In contrast, lactase restriction is uncommon in Britain. The low prevalence rate of lactase restriction was contributed to a large extent by Asian and African ethnic minorities living in Britain.

The expression of the lactase restriction phenotype at around the age of eight years and the lack of significant milk intolerance amongst the Sri Lankan population indicate that milk feeding programmes especially amongst pre-school and primary school children should not be discouraged.

APPENDIX I

THE PRECISION OF BLOOD GLUCOSE ESTIMATIONS

Patient	Random No	Blood Glucose (mmol/L)	Coefficient of variation (%)
A	1	3.9	
	6	3.8	
	10	3.8	1.2
	11	3.8	
	14	3.9	
B	2	3.4	
	5	3.3	
	7	3.4	1.5
	12	3.3	
	13	3.4	
C	3	3.4	
	4	3.3	
	8	3.4	1.5
	9	3.3	
	15	3.3	
D	16	6.0	
	19	6.1	
	21	6.1	0.8
	27	6.0	
	30	6.1	
E	17	3.7	
	23	3.7	
	25	3.8	1.1
	26	3.7	
	28	3.7	
F	18	4.6	
	20	4.7	
	22	4.7	1.1
	24	4.7	
	29	4.6	

APPENDIX II

DUPLICATE ESTIMATIONS OF BLOOD GLUCOSE (mmol/L)

Sample	Run 1	Run 2	Difference
1	3.6	3.6	0.0
2	3.8	3.8	0.0
3	4.0	4.0	0.0
4	4.1	4.1	0.0
5	4.1	4.1	0.0
6	4.1	4.1	0.0
7	4.1	4.1	0.0
8	4.3	4.3	0.0
9	4.3	4.3	0.0
10	4.4	4.4	0.0
11	4.4	4.4	0.0
12	4.5	4.4	0.1
13	4.5	4.6	-0.1
14	4.6	4.6	0.0
15	4.7	4.7	0.0
16	4.7	4.5	0.2
17	4.8	4.9	-0.1
18	4.9	4.9	0.0
19	5.2	5.2	0.0
20	5.2	5.2	0.0
21	5.2	5.2	0.0
22	5.2	5.3	0.0
23	5.4	5.4	-0.1
24	5.5	5.5	0.0
25	5.6	5.6	0.0
26	5.7	5.7	0.0
27	5.8	5.7	0.1
28	5.9	5.8	0.1
29	6.0	6.0	0.0
30	6.4	6.5	-0.1

Coefficient of variation = 0.9%
Range 3.6 - 6.5 mmol/L

APPENDIX III

BLOOD GLUCOSE CONCENTRATION AT DIFFERENT TIMES AFTER INGESTION OF WATER (400ml)

Number	Blood Glucose Concentration (mmol/L) time (min)						$\hat{g}$ (water) (mmol/L)
	0	20	40	60	80	100	
1	3.5	3.5	3.7	3.6	3.5	-	+0.2
2	3.0	3.1	3.0	3.0	3.0		+0.1
3	4.1	4.0	3.5	3.5	3.5		-0.6
4	4.9	4.5	4.3	4.7	4.7	-	-0.6
5	3.4	3.8	3.8	3.6	3.7	-	+0.4
6	3.5	3.7	3.6	3.8	3.8	-	+0.3
7	4.8	4.6	4.5	4.6	4.6	-	-0.3
8	3.0	3.1	3.2	3.3	3.1	-	+0.3
9	3.9	3.9	4.2	4.3	4.3	-	+0.4
10	4.3	4.1	4.2	4.2	4.2	-	-0.2
11	4.2	4.2	4.2	4.1	3.7	-	-0.5
12	3.8	3.3	3.6	3.6	3.4	-	-0.5
13	4.1	4.1	3.9	4.0	4.2	-	-0.1
14	3.5	3.4	3.3	3.2	3.2	-	-0.3
15	3.6	3.5	3.5	3.6	3.5	-	-0.1
16	3.1	3.2	3.3	3.5	3.5	-	+0.4
17	3.8	4.0	3.5	3.9	4.2	-	+0.4
18	5.1	5.2	4.0	5.2	4.6	-	+0.1
19	3.4	3.4	3.3	3.4	3.5	-	+0.3
20	3.5	3.5	3.2	3.3	3.5	-	+0.3
21	4.3	4.4	4.5	4.7	4.5	4.2	+0.4
22	4.2	4.4	4.4	4.3	4.2	4.2	+0.2
23	3.8	4.1	4.0	3.8	3.8	3.7	+0.3
24	4.6	4.4	4.2	3.7	4.8	4.4	-0.6
25	4.2	4.1	4.6	4.3	4.2	4.2	+0.4
26	3.1	3.5	3.5	3.7	3.3	3.1	+0.6
27	5.4	5.1	5.6	5.6	5.6	5.7	+0.3
28	5.8	5.8	5.8	6.0	5.6	5.6	+0.2
29	5.1	4.7	4.9	5.0	4.5	4.4	-0.6
30	4.6	4.3	4.3	4.5	4.5	4.0	-0.3
31	3.8	4.1	3.8	4.3	4.3	3.9	+0.6
32	4.1	4.1	4.2	3.8	4.1	4.1	-0.3
33	5.3	5.4	5.5	5.4	5.4	5.5	+0.2

g Mean = +0.04 mmol/L

SD = 0.3 mmol/L

g Range +0.6 to -0.7 mmol/L

Appendix IV

ORAL LACTOSE LOADS IN SRI LANKAN ADULTS

Serial No	Age (years)	Sex	Race**	Blood glucose mmol/L Time (mins)						$\hat{g}$(lactose) mmol/L
				0	20	40	60	80	100	
ABNORMAL ($\hat{g}$ < 1.1 mol/L)										
1	17	F	S	3.5	4.2	4.1	4.2	4.5	-	+1.0
2	34	F	S	4.3	3.8	3.6	4.2	4.1	-	-0.7
3	32	F	S	4.4	4.8	4.2	3.9	4.1	-	+0.4
9	46	F	S	5.6	4.7	5.3	5.3	5.2	-	-0.9
10	18	F	S	4.0	3.9	4.7	4.3	3.9	-	+0.7
11	16	F	S	4.7	4.9	4.9	4.8	4.8	-	+0.2
12	31	F	S	5.0	4.7	5.2	5.0	5.0	-	+0.2
15	13	F	S	4.8	4.9	5.4	5.7	5.7	-	+0.9
16	39	F	S	5.3	5.0	5.4	5.2	5.5	-	+0.2
19	--	F	S	5.0	4.6	4.7	4.7	4.9	-	-0.4
21	53	F	S	4.2	3.7	3.7	3.2	4.4	-	+0.2
22	45	F	S	3.3	3.7	3.2	3.4	4.2	-	+0.9
23	48	F	S	4.3	4.4	4.3	4.6	4.3	-	+0.3
24	65	F	S	5.6	5.2	4.8	5.4	-	-	-0.8
25	35	F	T	4.8	4.1	3.8	5.2	3.4	-	+0.3
26	68	F	S	5.3	5.9	5.5	5.1	5.2	-	+0.6
27	70	F	S	4.4	4.4	5.0	5.1	5.2	-	+0.8
28	23	F	S	4.1	4.4	3.5	3.9	4.1	-	+0.3
29	35	F	M	3.8	3.7	3.7	3.7	3.6	-	-0.2
30	39	F	S	4.0	3.8	3.9	3.9	3.8	-	-0.2
31	57	F	S	3.3	3.7	3.8	3.9	4.0	-	+0.7
32	24	F	S	3.5	3.9	4.2	4.1	4.1	-	+0.7
33	18	F	S	4.4	4.3	5.4	5.3	4.8	-	+1.0
34	78	F	S	5.7	4.7	5.0	5.7	4.4	-	-1.3
35	27	F	S	3.1	4.0	4.1	3.6	3.5	-	+1.0
36	18	F	S	3.2	3.4	3.5	3.1	-	-	+0.3
37	23	F	S	1.8	1.7	2.8	2.8	2.5	-	+1.0
38	18	F	S	3.7	4.3	4.3	3.7	4.2	-	+0.6
40	23	F	S	3.4	2.4	3.7	4.3	3.9	-	+0.9
42	22	F	S	4.1	4.2	4.3	4.6	4.7	-	+0.6
43	31	M	S	4.3	4.1	4.4	4.7	5.0	-	+0.7
44	30	M	S	5.5	4.6	4.8	5.3	5.2	-	-0.9
45	24	M	S	5.0	5.3	4.8	5.2	5.1	-	-0.2
46	24	M	S	4.6	3.8	4.1	4.6	4.6	-	-0.8
48	58	M	S	4.4	4.8	5.0	4.2	4.4	-	+0.6
49	16	M	S	5.3	4.5	3.8	4.5	4.6	-	-1.5
50	59	M	S	4.9	5.2	5.5	5.4	5.4	-	+0.6
51	28	M	S	4.9	4.9	5.5	5.0	5.3	-	+0.6
52	30	M	S	4.6	4.7	4.7	4.8	4.7	-	+0.2
53	--	M	S	5.9	6.3	6.2	4.8	5.1	-	+0.4
56	22	M	T	3.7	4.7	4.6	3.8	4.1	-	1.0
57	21	M	T	4.5	4.7	4.8	4.2	4.1	-	+0.3
58	46	M	S	5.1	4.8	5.4	5.2	5.0	-	+0.3
60	38	M	S	4.3	4.3	4.7	4.3	4.3	-	+0.4

Serial No	Age (years)	Sex	Race**	Blood glucose mmol/L Time (mins)						$\hat{g}$(lactose) mmol/L
				0	20	40	60	80	100	
61	22	M	S	4.5	4.4	4.5	4.7	4.8	-	+0.3
62	56	F	T	4.9	5.2	5.1	4.8	5.2	-	+0.3
63	42	-	T	3.9	4.3	4.4	4.3	4.3	-	+0.5
65	30	M	S	4.8	5.3	5.7	5.6	5.7	-	+0.9
66	14	F	S	4.8	3.9	2.9	2.9	4.4	-	-1.9
67	22	F	S	3.4	2.6	3.4	3.5	3.3	-	+0.1
68	32	F	S	4.4	4.8	4.2	3.9	4.1	-	+0.4
69	28	F	S	4.8	4.9	4.8	4.8	4.6	-	+0.1
70	36	F	T	3.7	3.3	3.4	3.3	3.7	-	+0.4
71	38	F	S	4.0	3.8	4.2	3.9	4.1	-	+0.2
72	50	F	S	3.9	4.0	4.7	4.4	4.5	-	+0.8
73	60	F	S	6.9	5.1	5.6	5.4	4.8	-	-2.1
94	18	F	S	3.5	3.9	3.8	3.6	3.6	-	+0.4
95	42	F	S	3.4	3.7	3.7	3.6	3.2	-	+0.3
96	12	F	S	3.4	3.7	3.9	3.5	3.6	3.6	+0.5
97	40	F	S	3.4	3.7	3.8	3.7	3.7	3.4	+0.4
98	24	F	T	3.7	3.4	3.4	3.7	3.4	3.5	-0.3
99	20	M	S							
100	21	F	S	3.6	4.0	4.3	4.3	4.2	3.5	+0.7
101	35	F	S	3.7	3.9	4.3	4.3	3.3	-	+0.6
102	45	F	T	2.9	3.6	3.7	3.7	3.6	3.9	+0.8
103	27	F	S	4.0	4.4	4.7	4.6	4.6	4.3	+0.7
104	24	F	S	3.6	3.6	3.8	3.7	3.7	3.7	+0.2
105	16	F	S	4.3	3.9	4.3	3.7	3.6	-	+0.4
106	55	F	T	3.8	4.2	3.9	3.9	4.1	4.1	+0.3
107	29	F	T	2.4	2.8	2.8	3.4	3.4	-	+1.0
108	22	F	S	3.8	4.8	4.6	3.9	3.7	-	+1.0
109	29	F	S	3.7	4.3	4.6	4.7	4.6	4.4	+1.0
110	25	F	S	3.8	4.3	4.6	4.8	4.1	4.0	+1.0
111	25	F	S	3.8	4.2	4.2	4.1	3.8	-	+0.4
112	20	F	S	3.5	4.0	3.8	3.4	3.5	-	+0.5
113	55	F	S	2.9	3.3	2.8	3.0	2.9	-	+0.4
114	--	F	S	3.7	4.6	3.7	3.9	4.1	-	+0.9
115	23	F	S	4.8	5.3	5.6	5.8	5.3	4.9	+1.0
116	20	F	T	3.3	3.6	3.7	3.8	3.7	3.8	+0.5
117	22	F	S	2.9	3.3	3.8	3.9	3.8	3.7	+1.0
118	33	F	S	2.9	3.9	3.8	3.2	3.6	3.9	+1.0
119	36	F	S	3.4	3.9	3.9	3.7	3.6	3.5	+0.5
120	65	F	S	5.2	5.3	5.4	5.9	6.0	5.6	+0.8
121	17	F	M	4.9	5.4	5.6	5.9	5.4	5.3	+1.0
123	--	F	S	4.3	5.0	4.7	4.8	4.4	4.5	+0.7
124	15	F	T	4.1	4.0	4.2	4.1	3.7	3.8	+0.1
139	48	F	M	5.3	5.3	5.4	5.3	5.3	5.4	+0.1
140	23	F	T	4.2	4.7	4.7	4.1	4.0	3.7	+0.5
141	21	F	S	4.4	4.6	4.7	4.3	4.7	3.9	+0.3

Appendix IV (Continued)

Serial No	Age (years)	Sex	Race**	Blood glucose mmol/L Time (mins)						$\hat{g}$(lactose) mmol/L
				0	20	40	60	80	100	
143	37	F	S	4.7	4.8	5.7	5.7	5.6	4.8	+1.0
144	40	F	M	4.6	4.1	4.1	4.1	4.0	4.2	-0.6
145	35	F	S	4.2	4.1	3.9	3.9	3.8	3.8	-0.3
146	23	F	S	4.3	4.4	4.3	4.4	4.1	4.2	+0.1
147	42	F	T	3.9	4.1	3.9	4.2	4.3	4.2	+0.4
148	21	F	S	4.1	4.3	4.2	4.2	4.4	4.1	+0.3
149	34	F	S	3.4	3.5	3.6	3.7	3.7	3.7	+0.3
150	16	F	S	3.4	3.7	3.7	3.8	4.2	3.8	+0.8
151	26	F	S	3.0	3.2	3.2	3.4	3.2	-	+0.4
152	45	F	S	2.1	2.3	2.4	2.9	2.4	2.2	+0.8
153	28	F	S	3.3	3.3	3.3	3.7	4.2	2.8	+0.9
154	23	F	S	3.1	2.8	3.6	3.7	3.8	4.0	+0.9
155	40	F	M	3.6	3.8	3.8	4.0	3.6	3.7	+0.4
156	20	F	S	3.1	3.6	3.4	3.4	3.9	3.3	+0.8
157	26	F	S	3.6	4.1	4.1	4.6	4.2	4.0	+1.0
158	19	F	S	3.4	3.8	4.1	3.7	3.4	-	+0.7
159	24	F	S	3.6	3.8	4.0	3.6	3.6	-	+0.4
160	39	F	S	2.3	2.9	2.3	2.7	3.1	2.5	+0.8
161	41	F	T	3.2	3.4	3.3	3.7	3.8	3.7	+0.6
162	22	F	S	3.1	3.2	2.8	3.4	3.1	3.2	+0.3
163	15	F	S	4.3	3.6	3.9	4.2	3.3	3.1	-1.2
164	22	F	S	2.9	3.2	3.3	3.7	3.1	2.9	+0.8
165	19	F	T	3.4	3.7	3.7	3.3	3.2	2.6	+0.3
166	30	F	S	5.6	5.9	4.3	3.1	3.3	3.2	+0.3
167	35	F	T	2.4	3.2	2.8	2.6	2.7	2.8	+0.8
168	30	F	T	2.5	3.2	2.9	3.1	2.8	3.3	+0.8
169	31	F	T	4.8	5.8	5.4	4.6	4.7	4.2	+1.0
170	22	F	S	4.1	4.1	4.2	3.8	4.0	3.5	+0.1
171	27	F	S	3.8	4.1	3.8	4.2	4.2	3.9	+0.4
172	16	F	S	4.6	4.3	4.2	4.4	4.4	4.0	-0.6
173	18	F	T	5.1	4.7	4.9	5.0	4.4	4.4	-0.7
174	17	F	S	5.1	4.9	4.9	4.9	5.0	5.1	-0.2
175	19	F	S	5.8	5.8	5.8	6.0	5.6	5.6	+0.2
176	13	F	S	5.3	5.1	5.6	5.6	5.6	5.7	+0.2
177	20	F	S	4.6	5.0	4.8	4.7	4.6	4.4	+0.4
178	28	F	S	4.4	4.9	5.1	4.9	4.8	4.3	+0.7
181	19	F	S	2.6	2.8	2.8	2.5	2.8	-	+0.3
182	35	F	T	3.1	3.4	3.2	2.7	2.9	-	+0.3
183	30	F	M	3.6	3.7	3.5	3.2	3.3	-	+0.1
184	19	F	S	3.3	3.5	4.0	3.2	3.3	-	+0.7
185	20	F	S	3.4	3.7	3.7	3.3	3.2	-	+0.3
186	--	F	M	3.8	3.9	4.2	4.5	4.6	-	+0.8
187	26	F	S	3.3	3.8	4.1	3.7	3.4	-	+0.8
188	18	F	S	4.0	4.7	4.3	4.4	4.1	-	+0.7
189	48	F	S	4.0	4.6	4.7	4.0	4.1	-	+0.7

Appendix IV (Continued)

Serial No	Age (years)	Sex	Race**	Blood glucose mmol/L Time (mins) 0	20	40	60	80	100	$\hat{g}$(lactose) mmol/L
190	60	F	S	3.9	4.9	4.2	3.8	3.9	-	+1.0
191	32	F	S	3.2	3.1	3.9	4.1	3.3	-	+0.9
192	24	F	S	3.4	3.5	3.6	3.4	3.2	-	+0.2
193	32	F	S	3.9	4.3	3.6	3.8	3.8	-	+0.4
194	38	F	S	4.3	5.1	4.7	4.1	4.4	-	+0.8
195	26	F	S	3.6	4.3	3.9	4.0	3.6	-	+0.7
198	--	F	S	4.3	4.7	4.4	4.5	4.8	4.1	+0.6
203	29	F	S	5.8	5.2	5.3	5.6	5.7	-	-0.6
204	30	F	S	4.1	4.3	4.1	4.1	4.2	-	+0.2
NORMAL ($\hat{g}$ > 1.1 mmol/L)										
4	35	F	S	3.7	4.7	6.1	4.0	4.1	-	2.4
5	50	F	S	4.6	8.4	7.0	6.7	6.0	-	3.8
6	22	F	T	3.6	5.5	3.9	3.7	3.7	-	1.9
7	23	F	T	4.3	4.7	5.2	5.7	6.2	-	1.9
8	16	F	S	3.9	5.1	5.0	4.3	4.2	-	1.2
13	36	F	S	4.9	6.9	6.6	6.8	6.8	-	2.0
14	23	F	S	4.6	5.0	5.3	5.8	5.2	-	1.2
17	20	F	S	3.0	4.3	4.0	4.6	3.3	-	1.6
20	24	F	S	3.8	4.3	4.6	5.0	4.9	-	1.2
39	14	F	M	3.4	2.4	3.6	4.8	4.4	-	1.4
47	61	M	S	4.7	5.2	5.4	5.7	6.1	-	1.4
55	23	M	T	3.6	1.1	4.2	4.3	4.2	-	1.3
59	14	M	S	4.1	4.3	4.6	5.3	4.9	-	1.2
64	30	M	T	2.9	4.2	3.6	4.8	5.0	-	1.2
75	37	F	S	5.2	5.9	7.7	6.4	6.5	5.1	2.5
76	28	F	S	3.1	4.2	4.4	4.3	4.3	4.2	1.3
77	22	F	S	4.7	6.6	7.5	6.0	5.2	4.6	2.8
78	21	F	T	3.8	4.8	5.2	4.1	4.4	4.6	1.4
79	60	M	S	6.2	7.4	8.7	9.8	8.3	6.8	3.6
80	28	F	S	3.8	4.9	5.3	5.1	4.5	4.4	1.5
81	41	F	S	3.4	3.9	4.5	4.7	3.7	3.5	1.3
82	45	F	S	4.8	5.6	5.7	6.1	6.3	6.2	1.5
83	20	F	S	4.4	4.6	5.6	5.5	5.8	5.7	1.4
84	53	F	S	3.9	5.0	7.0	6.1	6.3	5.1	3.1
85	57	F	T	3.7	3.6	4.3	4.8	4.1	3.8	1.1
86	26	F	S	2.6	3.8	4.0	4.3	4.6	4.0	2.0
87	13	F	S	3.2	3.8	4.3	4.4	4.2	4.7	1.5
88	26	F	S	4.2	7.1	5.2	4.5	4.6	-	2.9
89	22	M	S	3.4	4.1	5.3	4.6	3.9	3.6	1.9
90	75	F	S	3.9	3.7	4.3	4.8	4.7	5.2	1.3
91	45	F	S	3.2	4.5	4.4	3.8	4.1	-	1.3
92	18	F	S	3.7	4.6	4.7	5.0	5.3	5.4	1.7
93	51	F	S	4.1	5.2	5.1	5.1	4.9	4.4	1.1

Appendix IV (Continued)

Serial No	Age (years)	Sex	Race**	Blood glucose mmol/L Time (mins)						$\hat{g}$(lactose) mmol/L
				0	20	40	60	80	100	
125	30	F	S	3.3	3.7	4.3	4.5	4.1	3.2	1.2
126	28	F	S	3.7	3.9	4.0	5.6	4.9	3.5	1.9
127	18	F	S	3.6	5.5	5.4	6.9	7.4	-	3.8
128	12	F	S	3.0	3.3	4.2	2.8	2.9	-	1.2
129	27	F	T	3.1	3.2	3.9	4.4	4.8	-	1.8
130	25	F	S	3.3	5.7	4.9	5.7	3.7	-	2.4
131	24	F	S	4.6	6.3	7.4	4.2	3.7	-	2.8
132	17	F	S	4.1	2.9	5.0	5.3	4.1	-	1.2
133	18	F	S	4.0	4.7	5.4	6.0	4.9	4.3	2.0
134	19	F	M	2.4	2.6	3.6	4.5	4.1	3.7	2.1
135	17	F	S	1.6	2.4	2.3	2.5	2.8	2.3	1.2
136	25	F	S	3.2	4.1	4.4	3.2	3.1	2.7	1.2
137	23	F	M	4.2	4.6	4.9	5.3	5.4	5.1	1.2
138	18	F	S	2.4	3.8	3.2	3.1	2.9	2.8	1.4
179	23	F	S	4.8	6.8	6.6	4.8	4.5	-	2.0
180	56	F	T	5.8	6.6	6.9	5.8	4.8	-	1.1
196	27	F	T	3.1	3.3	4.0	4.4	4.9	-	1.8
206	21	F	S	4.0	4.9	5.7	4.4	4.8	-	1.7
208	21	F	M	4.2	5.2	5.6	5.6	5.8	-	1.6
210	23	F	S	3.8	4.6	5.3	6.2	5.3	-	2.4
211	25	F	S	5.3	5.8	7.2	6.9	8.0	-	2.7

** S = Sinhalese, T = Tamil

APPENDIX V

LACTASE ACTIVITY, g (lactose) AND SYMPTOMS AFTER ORAL LACTOSE IN SRI LANKAN ADULTS

Serial No	Age (years)	Sex	Weight of mucosa (g)	Lactase activity U/gww	$\hat{g}$(lactose) (mmol/L)	Symptoms of abdominal pain or diarrhoea
Jejunal biopsy						
10	22	F	3.2	0.74	-0.3	+
11	18	F	23.0	0.23	1.4	+
12	30	F	14.5	0.15	0.8	0
13	40	F	60.0	1.03	-0.5	0
14	19	F	9.5	0.59	1.2	0
15	28	F	11.0	2.30	2.8	0
16	18	F	30.0	0.26	1.2	0
17	27	F	5.3	0.69	1.2	0
18	32	F	39.5	0.13	1.0	0
19	22	F	6.8	0.04	2.0	0
20	--	M	21.5	0.34	1.0	0
21	--	F	8.4	0.68	0.6	0
22	--	F	14.5	1.40	--	-
23	19	F	15.0	0.22	--	-
24	30	F	11.0	0.37	0.7	0
25	35	F	4.2	0.44	1.5	+
26	18	F	12.0	0.67	-1.3	0
27	--	F	59.0	0.60	--	-
28	--	F	7.3	0.97	1.1	0
29	22	F	20.5	1.07	1.9	0
30	--	F	36.7	0.53	3.1	0
31	--	F	5.0	0.15	0.8	+
32	--	F	31.3	0.48	2.5	0
33	--	F	21.0	0.07	0.3	0
34	--	F	8.6	0.30	0.4	0
35	35	F	5.4	0.26	0.3	+
36	21	F	3.7	0.44	0.4	+
37	--	F	35.4	0.40	1.1	0
38	37	F	8.3	0.50	1.1	0
39	15	F	25.0	0.22	0.2	0
40	23	F	6.9	0.19	0.5	0
41	21	F	4.5	0.15	0.2	0
Surgical specimens						
1	31	M	35.3	0.31	--	-
2	60	M	31.2	0.37	--	-
3	64	M	47.0	0.31	--	-
4	28	F	51.6	0.00	1.0	0
5	30	F	85.4	0.21	--	-
6	45	M	15.3	2.49	3.7	0
7	35	M	33.5	0.39	--	-
8	--	F	52.2	0.82	0.3	0
9	--	F	95.6	0.57	0.8	0

APPENDIX VI

LACTASE ACTIVITY RELATED TO $\hat{g}$(lactose) AND SYMPTOMS AFTER ORAL LACTOSE LOAD

Outcome of oral lactose load	Serial number	Lactase activity (U/gww)	$\hat{g}$(lactose) (mmol/L)
$\hat{g}$ <1.1 mmol/L, with symptoms	10	0.74	-0.3
n = 4	31	0.15	0.8
	35	0.26	0.3
	36	0.44	0.4
mean ± SD		0.40 ± 0.22	
$\hat{g}$ <1.1 mmol/L, no symptoms	4	0.00	1.0
	8	0.82	0.3
n = 16	9	0.57	0.8
	12	0.15	0.8
	13	1.03	-0.5
	18	0.13	1.0
	20	0.34	0.9
	21	0.68	0.6
	24	0.37	0.7
	26	0.67	-1.3
	33	0.07	0.3
	34	0.30	0.4
	37	0.40	1.0
	39	0.22	0.2
	40	0.19	0.5
	41	0.15	0.2
mean ± SD		0.38 ± 0.29	
$\hat{g}$ >1.1 mmol/L, with symptoms	11	0.23	1.4
n = 2	25	0.44	1.5
mean ± SD		0.34 ± 0.11	
$\hat{g}$ >1.1 mmol/L, no symptoms	6	2.49	3.7
	14	0.59	1.2
n = 11	15	2.30	2.8
	16	0.26	1.2
	17	0.69	1.2
	19	0.04	2.0
	28	0.97	1.1
	29	1.07	1.9
	30	0.53	3.1
	32	0.48	2.5
	38	0.50	1.1
Mean ± SD		0.90 ± 0.76	

APPENDIX VII

ORAL LACTOSE LOAD IN SRI LANKAN CHILDREN

Subject	Age	Sex	Blood Sugar (mmol/L) Time (mins)					$\hat{g}$(lactose) mmol/L
			0	20	40	60	80	
New born babies								
L	1 day	M	4.2	7.2	5.3	39.1	3.2	4.6
SL	1 day	M	3.8	10.9	7.8	9.9	3.9	7.1
0.5 year								
M	0.30	M	4.2	5.3	5.7	7.7	5.8	3.4
FR	0.50	M	6.1	8.7	8.3	8.2	6.9	2.2
SN	0.25	M	2.2	4.4	4.7	4.8	4.6	2.6
PD	0.20	F	4.0	6.9	5.6	4.6	3.8	3.1
NK	0.20	M	3.7	7.4	5.6	4.6	3.7	3.7
D	0.25	F	5.4	9.8	8.6	7.9	7.0	4.4
SB	0.50	M	6.3	12.1	9.4	7.5	5.9	6.2
0.6 - 1 year								
PK	0.80	F	5.3	7.1	6.3	5.7	5.2	1.8
KGD	0.80	M	5.3	5.7	6.1	7.1	5.3	1.8
S	1.0	M	4.0	7.1	5.8	5.2	4.9	3.1
P	1.00	M	5.4	8.5	6.5	5.2	4.7	3.0
KS	0.80	M	5.7	6.1	7.1	7.6	4.7	1.9
MA	1.00	M	5.2	7.6	10.4	8.7	7.6	5.2
NTB	0.90	M	6.2	8.7	7.1	7.6	8.4	2.4
SB	0.80	M	4.9	6.3	6.0	6.0	5.3	1.4
JMSS	0.90	M	3.6	4.6	4.9	6.8	5.5	3.3
I	1.00	F	4.6	7.3	6.2	5.8	8.0	2.7
PMP	0.80	F	4.6	6.4	5.1	4.5	5.8	1.8
USB	1.00	M	4.4	6.5	8.1	8.5	8.1	4.0
KK	0.88	M	4.2	5.2	5.0	5.4	5.0	1.3
IK	1.00	F	5.9	6.3	7.9	6.7	5.5	2.0
SP	1.00	M	5.3	8.4	8.7	7.5	5.8	3.4
1 year to 2 years								
PK	1.25	M	5.2	6.7	7.4	7.9	6.7	2.7
AB	1.20	M	4.7	7.2	7.1	6.7	6.0	2.5
CK	1.40	F	6.7	8.7	8.2	6.7	5.9	2.0
RK	2.00	M	4.5	6.1	6.9	6.1	5.2	2.3
NHB	1.08	M	4.9	7.0	10.7	8.5	6.1	5.8
HMA	1.30	M	4.8	7.2	6.8	6.7	8.1	2.4
G	1.80	M	4.1	5.6	8.6	7.7	6.7	4.5
NPW	1.20	M	3.4	5.6	6.1	6.3	7.4	3.9
S	1.25	M	3.7	5.6	4.6	4.1	3.9	1.8
M	1.30	M	4.1	4.6	6.0	4.6	4.1	1.8
NJ	1.80	M	4.7	6.0	8.4	6.4	5.6	3.7
DM	1.90	M	6.3	9.0	6.9	7.5	7.2	2.7
AD	1.25	M	5.1	7.1	7.6	8.0	6.2	2.9
SK	1.20	M	4.6	8.4	10.6	7.8	5.7	5.9
KC	1.25	F	7.4	13.5	10.2	7.9	7.4	6.1
CJ	1.40	F	4.7	5.6	8.4	9.3	5.1	4.7
TD	1.50	F	4.4	5.6	6.7	7.0	7.8	3.3
NB	1.20	F	2.5	5.6	6.2	7.0	7.5	5.0

APPENDIX VII (Continued)

Subject	Age	Sex	Blood Sugar (mmol/L) Time (mins)					$\hat{g}$(lactose) mmol/L
			0	20	40	60	80	
2 years to 3 years								
GWS	2.20	M	4.3	6.6	6.6	4.7	3.3	2.2
J	2.00	M	3.4	5.0	9.9	5.8	5.3	5.8
AGJ	3.00	M	4.6	5.8	5.5	5.3	5.7	1.2
WL	3.00	F	5.8	6.7	7.1	6.5	5.3	1.3
KS	3.00	M	4.2	6.9	7.4	7.9	6.4	3.7
NK	2.08	M	3.9	4.9	5.4	5.1	4.6	1.4
KMK	2.08	F	4.2	7.9	6.8	6.4	4.9	3.7
S	2.50	M	4.3	6.2	6.7	6.8	5.0	3.1
ST	2.50	M	4.5	5.4	6.8	6.1	5.9	2.3
DK	2.08	M	4.4	1.1	7.4	7.4	6.4	6.7
3 years to 5 years								
PMA	4.70	M	5.7	9.1	8.6	7.7	7.3	3.3
HA	3.25	M	4.0	5.7	5.2	6.0	5.2	1.7
S	4.00	M	3.8	4.1	4.6	5.1	3.9	1.2
PK	3.20	F	2.8	3.6	4.2	3.7	3.1	1.4
UMR	4.00	M	5.4	6.3	7.4	5.9	5.5	2.0
AW	3.08	M	5.1	6.2	6.7	6.7	6.0	1.6
AGJ	4.00	M	3.4	4.9	7.3	5.9	4.6	3.9
6 years to 10 years								
J	8	M	5.1	5.4	7.7	5.7	4.6	2.6
SKR	10	F	5.4	7.8	6.0	8.6	6.3	3.2
J	9	F	3.3	4.3	5.4	5.8	5.6	2.5
TS	8	M	6.3	--	5.6	6.3	6.8	0.5
RB	5	M	5.4	6.4	5.9	6.7	6.3	1.3
BMNB	8	M	3.9	4.4	4.9	5.8	3.3	1.8
SB	8	M	3.8	5.6	5.3	4.4	5.3	1.7
KJ	10	M	3.4	4.1	3.4	3.6	5.4	1.9
SP	5	F	5.4	7.4	8.9	6.9	4.4	3.4
EWM	5	F	4.3	4.8	5.3	4.8	6.3	1.9
MK	6	F	4.8	5.5	6.3	6.5	5.6	1.7
LDP	6	M	4.8	5.3	6.0	6.3	5.4	1.4
J	5	F	5.1	6.4	5.2	4.2	4.2	1.4
NU	7	F	4.8	6.0	6.4	7.4	5.6	2.6
SVD	8	M	4.3	6.0	7.4	7.8	7.4	3.5
PK	5	M	4.4	7.1	7.4	5.6	5.1	3.5
M	10	M	5.7	5.7	5.6	6.1	6.6	0.8
WJK	8	F	3.9	5.8	6.2	6.5	5.8	2.6
A	7	M	2.9	3.9	3.9	4.2	3.8	1.3
NK	5	M	3.8	4.4	6.3	5.8	4.9	2.4

APPENDIX VII (Continued)

Subject	Age	Sex	Blood Sugar (mmol/L) Time (mins)					$\hat{g}$(lactose) mmol/L
			0	20	40	60	80	
11 years - 15 years								
YM	13-	F	4.8	4.9	5.4	5.7	5.7	0.8
KV	13	F	3.4	2.4	3.6	4.8	4.4	1.3
NV	14	M	4.0	4.3	4.6	5.2	4.9	1.3
KJ	14	F	4.7	3.9	2.9	4.2	4.4	1.8
RMS	12	F	3.0	3.3	4.2	2.8	2.9	1.2
PR	13	F	3.2	3.8	4.3	4.4	4.2	3.0
KR	15	F	4.1	4.0	4.2	--	3.7	0.1
PR	15	F	4.3	3.6	3.9	4.2	3.3	0.8
MM	12	F	3.4	3.7	3.9	3.5	3.6	0.6
K	12	F	3.7	4.6	4.3	3.9	4.1	0.9
KM	14	F	4.0	4.2	4.2	4.4	4.2	0.3
VJ	15	F	4.3	5.0	4.7	4.8	4.4	0.7
IM	13	F	5.3	5.1	5.6	5.6	5.6	0.2
S	11	F	4.5	4.9	4.8	4.8	4.8	0.4
KMP	12	F	4.1	4.5	4.6	4.2	4.5	0.5

APPENDIX VIII

Prevalence:	The number of existing cases of a disease in a given population at a designated time.
Prevalence rate:	The total number of all individuals who have a disease at a particular time devided by the population at risk of having the disease.
Incidence:	The number of new cases of a disease in a population within a specified period of time.

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