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SENSORY CHARACTERIZATION OF HUMAN MILK

by

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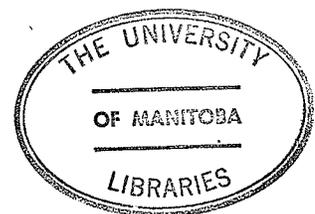
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MASTER OF SCIENCE

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A B S T R A C T

The taste preferences of the human neonate have been studied by various researchers. However, nothing has been reported of the sensory qualities of human milk. Fore and hind milks from a single morning feed collected from twenty-four nursing mothers over three consecutive days were evaluated for the sensory attributes of sweetness, viscosity and mouthcoat by a trained adult panel using magnitude estimation. The presence and intensity of off-flavours were also noted. Milks were perceived as sweet, thin and low in mouthcoat, but significant differences in the three attributes existed between mothers. Hind milks were perceived as more viscous and more mouthcoating than fore milks, yet these differences were very small. Chemical analysis revealed significant differences between mothers in lactose, fat and protein contents. A significantly higher fat content in hind milk ($p = 0.001$) was associated with a significantly higher estimation of physical viscosity ($p = 0.002$). Maternal age was negatively associated with fat content ($r = -0.48, p = 0.02$). Off-flavours noted in fresh human milk were described as metallic, fruity,

sour and spicy. Frozen milk, subject to more frequent and more intense off-flavours, was described as metallic, cardboardy and soapy. Placement of the milks on a sweetness power function permitted a comparison of intensity of the milks to the basic taste stimulant. Further comparisons of sweetness of human milk to that of other infant foods could also be made. Although significant sensory differences were found in human milks, it is unknown whether the human infant could perceive such changes.

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I N T R O D U C T I O N

Human milk is often the first food offered to an infant. With present trends of increased incidence of breastfeeding, longer duration of breastfeeding, and later introduction of solids, the human infant may be exposed to breast milk alone for a few months before other foods are introduced. Therefore, in the study of the effects of early taste experience upon the development of flavour preference in human infants, it is important to consider the sensory qualities of human milk.

There appear to be no studies reported in the literature describing the flavour of human milk. At the same time there exists a folklore of anecdotal literature about the effects of particular foods in the mother's diet upon her ability to lactate, upon the response of the infant at the breast and later gastrointestinal upset of the infant, and upon the flavour of her milk. Many of the reported effects of foods consumed by the lactating woman upon her milk are part of an oral cultural tradition passed from one mother to another. Sometimes this tradition becomes formalized

into proscribed foods for the nursing mother, and often these food avoidances are reinforced by medical and nutrition practitioners in the advice they give to nursing women.

Studies with laboratory animals such as the rat, have shown that rats formed taste preferences and/or aversions through their experiences with mothers' milk. The effect of mothers' milk upon the human infants' later taste preferences is unknown.

It has been hypothesized by Hall (1975a) that compositional changes in the milk during a single feed could act as an appetite control mechanism whereby the infant would somehow perceive flavour and/or textural changes leading to a sensation of satiety and cessation of feeding. As well, the literature on infant feeding abounds with references to the development of "a sweet tooth". This and other problems such as accelerated growth and infantile obesity have, in the past, been blamed on formula feeding, sweetened formulae and early introduction of solids. These accusations have been made without actual quantification of the relative sweetness of human milk as compared to the sweetness of formulae,

commercial or home-prepared, presently used in infant feeding.

Therefore, the purpose of this research was to examine the sensory qualities of human milk. The objectives of this study were:

1. To define the sensory characteristics of human milk by utilizing a trained adult sensory panel.
2. To note and describe off-flavours in fresh human milk and to relate flavours in milk to foods in the maternal diet.
3. To determine if sensory differences exist in milks of different mothers or between samples from the same mother from day to day or within a single feed.
4. To relate the sensory viscosity of human milk to physical viscosity.
5. To determine the effects of lactose, fat and protein contents upon the sensory qualities of human milk.
6. To determine the effects of freezing upon the flavour and texture of human milk.
7. To test for the effects of certain maternal parameters as age, stage of lactation, parity, frequency of nursing and style of nursing upon the sensory, physical and chemical qualities of human milk.

REVIEW OF THE LITERATURE

I. Development of Taste Preference in Infants

A. First Exposure to Taste Stimuli

An infant is likely exposed to its first taste experience in utero, since it has been reported that taste receptors are developed at twelve weeks of age and that a fetus swallows amniotic fluid beginning about this time (Mistretta and Bradley, 1977). In fact, it has been estimated that between 10 to 15 percent of protein requirements of the fetus were satisfied by amniotic amino acids, and that intrauterine growth retardation was associated with fetuses that could not swallow (Brans, 1976).

B. Infants Responses to Taste Stimuli

1. Neonate

Because of the non-verbal nature of the neonate subject, infants' responses to taste stimuli have been measured in various ways: observation of tongue movements (Jacobs et al., 1977; Nowlis, 1977), facial expressions (Steiner, 1977) heart rate (Lipsitt, 1977),

breathing rate and sucking patterns (Johnson and Salisbury, 1975), volume of taste solution ingested (Desor et al., 1973), and film studies (Drewett and Woolridge, 1979).

Similar conclusions have been reached from these various studies regarding the discrimination of taste in the human neonate. It has been demonstrated that the neonate does discriminate among different taste qualities and over a variety of concentrations and tastants. It has been shown that the neonate clearly prefers sweet solutions over non-sweet ones (Aiyar and Agarwal, 1969; Desor et al., 1973; Steiner, 1977). The neonate preferred certain sweet sugars such as sucrose and fructose over less sweet sugars, glucose and lactose (Desor et al., 1973). The infant preferred more concentrated sweet solutions over less concentrated ones (Crook and Lipsitt, 1976; Desor et al., 1973).

Negative responses of infants to sour solutions have been reported (Aiyar and Agarwal, 1969; Steiner, 1977), but these researchers used very high concentrations of tastants in their experiments. By using weaker solutions of tastant, Desor, Maller and Andrews (1975) found that the addition of citric acid to a sucrose solution significantly

decreased the volume ingested by neonates, but a similar addition of bitter tastant, urea, and of salty tastant, sodium chloride, did not significantly alter the amount of sweet solution ingested. Desor, Maller and Andrews (1975) also found that volume of weak aqueous solutions of sour and bitter tastants did not significantly differ from the volume of water ingested. They attributed this lack of difference to a possible aversion to water in the human neonate.

Thus, it seems that the human neonates prefer sweet and are aversive to strong solutions of sour and bitter. It remains unclear whether very young infants find weak bitter solutions aversive.

Salty solutions have not been tested often, due to the fear of introducing a dangerous level of hypertonic solution to the neonate. Johnson and Salisbury (1975) found salt solutions to cause apnea, or to be inhaled, by newborn infants. Negative responses to salty taste were reported by Aiyar and Agarwal (1969) and Jensen (1932). Desor, Maller and Andrews (1975) reported indifference to sodium chloride solution of 3-200 mM (or 0.017 - 1.17 percent) in infants of 1 - 4 days of age. Conner (1979)

reported a case study involving an infant during the ages two weeks to six months, who had rejected one of its own mother's breasts that had previously been infected with mastitis. Analysis of milk from this breast revealed sodium concentration at 108 mEq/l and chlorides at 88 mEq/l; this represented approximately an 88 mM (or 0.5 percent) sodium chloride solution, more typical of serum than of milk. Normal human milk contained 3 and 7 mEq/l of sodium and chloride. The father had tasted the milk from the previously infected breast and had described it as salty in comparison to the sweet milk of the other breast.

Jacobs et al. (1977) tested one neonate with various solutions of tastant administered with a tongue applicator and upon observing responses, set up a category scale of taste preferences based on oral behaviour criteria of licking and spitting. They found consistent rejection of expressed human milk compared to acceptance of 10 percent sucrose and 20 percent lactose solutions. Water and 7.0 percent lactose solutions elicited neutral responses. A bitter solution (quinine sulfate) elicited a rejection and a solution of 0.85 percent sodium chloride led to moderate rejection. Jacobs did not give the details of

handling the expressed milk. It is possible that a flavour change in the milk, such as that due to rancidity, led to the rejection.

Fomon et al. (1969) studied the effect of two formulae of different caloric density upon the rate of growth of male and female infants between 8 and 112 days. Even though significantly less of the more concentrated formula (133 kilocalories per deciliter) was ingested by both male and female infants than of the less concentrated formula (67 kilocalories per deciliter), the rate of gain was greater in those infants consuming the high calorie feed. Those infants consuming high calorie feeds consumed significantly more kilocalories from ages 8 - 84 days but not from 84 to 112 days. Caution is necessary when making the conclusion that infants adjust their calorie intake because of caloric need as was expressed by Anderson and Fomon (1971). Other properties of the formula, namely taste and texture, may have influenced the infants' responses.

2. Older Infants

Feeding studies with older infants have supported the reported literature on the preference of newborn

infants for sweet and rejection of sour and bitter. Fabro (1979) found that the addition of 10 percent sucrose to a smooth-thin texture system (a model purée system) increased the mean acceptance score of the product as determined by mother's observations of her infant's responses. Sour and bitter stimuli decreased the mean acceptance scores of these same model texture systems. Hogue and McDaniel (1980) found support for these results in their studies where varying concentrations of sweet, sour and bitter stimuli were added to the smooth-thin texture system and fed to 48 infants in a similar experimental design as in Fabro's study. Sour and bitter stimuli decreased mean acceptance scores and sweet increased preference for the system. Scores within each tastant of varying concentration used were not significantly different. These researchers found wide variations between infants in acceptance of all samples.

By a variety of methods, other investigators have studied food preferences of infants. These researchers did not relate preference to a specific tastant or texture property (Beal, 1957; Guthrie, 1966; Maslansky et al., 1974). It was assumed that consumption patterns

indicated preferences. Davis (1939), who allowed young children ages 6 - 11 months to select foods, found that taste, smell and other sensory properties apparently influenced selection and, as the study progressed over four and one-half years, the children became more selective, rejecting some foods. Korslund and Eppright (1967) studied taste sensitivities of preschool children and related these to proportions of likes and dislikes reported. Those children with the lowest taste sensitivities tended to accept more foods than those with higher taste sensitivity. Another study by Fomon et al. (1970) compared the responses of 20 four-month old infants to salted or unsalted infant foods. They found that consumption of strained foods did not appear to be influenced by salt content. Another group of seven-month old infants had no preference for either form, salted or unsalted. Thus, it appears that in both young and older infants, taste preferences are present and these preferences will influence consumption of foods offered. Yet, the development of taste preference is a complex process and is influenced by an interplay of many other factors.

C. Effect of Body State Upon Taste Preferences

Nisbett (1972) and Nisbett and Gurwitz (1970) found differences in sweet preferences in neonates according to their sex and birth weight. Females and heavier infants ingested more of a sweeter solution than males and lighter infants, but females were less willing to work harder for these solutions. Overweight infants (with high weight/length³ ratios), but not heavier infants, were also less willing to work harder for food. Researchers made the solutions more difficult to obtain by decreasing the size of the hole in the nipple or by using a highly viscous, hard-to-suck formula. The fact that differences in preference for changes in viscosity were not confounding results was not discussed. These researchers hypothesized that lighter birth weight infants with higher rates of weight gain may have been deprived in utero and may be seeking more food. These infants did, in fact, consume more hard-to-suck and more sweetened formulae.

D. Effect of Age Upon Infants' Response to Taste

Thomas and Murray (1980) conducted a study where they attempted to evaluate if developmental differences existed in taste perception of children ages 5 - 8. Twenty-two

children were offered varying concentrations of 8 different spice distillates in water, and water, in a paired comparison design. The researchers found no significant differences in taste discriminations among age groups or sexes. An adult group used as a control had no significant differences in taste discrimination of spices from that of children, but were able to identify the spice by name more often. Identification of spice by name was low in both groups.

Desor, Greene and Maller (1975) tested groups of 9 - 15 year olds and found age and sex differences in preferences for sucrose and sodium chloride solutions. Taste sensitivity was not tested. When compared to adults, more younger subjects preferred higher concentrations of sweet and salty. Yet, within adult age groups, no further differences according to age and sex for sweet or salty preferences were found.

Korlund and Eppright (1967) found that thresholds in four to six year olds were the same as reported literature values for adults. Although no differences in

taste perception appeared between children and adults in these studies cited, it is not clear how taste sensitivity changes from birth and throughout infancy.

E. Cultural Influences Upon Taste Preferences

Culture has an influence upon the foods selected as suitable for the neonate. The time at which the infant is first put to the breast, the attitude towards colostrum and the use of pre-lactaeal foods have been determined by cultural influence (Jelliffe, 1962; Neihoff and Meister, 1972; Wellin, 1955). In some cultures it has been reported that colostrum was considered harmful or a waste product and was thrown away; therefore, a variety of pre-lactaeal foods was used (Neihoff and Meister, 1972), or the infant was not fed at all, but allowed to suck on cotton dipped in oil (Wellin, 1955). Prelactaeal foods tended to be sweet. In fact, most cultures tended to indulge infants' preferences for sweet (Jerome, 1977). Honey, or sugar and water, and coconut juice, hot honey and mustard oil, and other foods, in a viscous mixture, were fed to ensure the infants' response to eating (Jansen, 1977, abst.; Jelliffe, 1962; and Lindenbaum, 1977, abst.).

The use of 5 percent dextrose solution in today's hospital, as a first feed to determine patency of the gastrointestinal tract, was noted as a culturally-defined practice (Jerome, 1977). According to Brans (1976), sterile water may be more appropriate because it is less irritating to the bronchoalveolar epithelium but it, and saline solution, may be unpalatable.

As in the widespread use of sweet, the recognition of universal responses of infants to certain taste stimuli can be seen again in the use of bitter compounds to initiate weaning. Spreading of bitter tastants, quinine or bitter saps, upon the mother's nipples as a weaning agent, was noted by Neihoff and Meister (1972) and Jelliffe and Jelliffe (1974; 1978).

By studying culturally-defined foodways, the possible relationship between early experience with sweet taste and later preference for sweet, can be examined. Both Jerome (1977) and Muto (1977) reported that cultural groups following a non-sugar tradition did not reject sweets when they were introduced into the culture. Yet, acculturation to sour and bitter taste has been noted (Jerome, 1977; Maller and Desor, 1974).

Each culture appears to define its own beneficial foods for its infants, often based upon palatability as well as nourishment, yet Jerome (1980) found that immigrant women in the United States would adopt foods of their new culture for their infants if they thought they were good for them.

F. Infant Responses to Texture

Whereas infants and children have been reported to exhibit texture preferences, (Fabro, 1979; Harasym, 1977; Szczesniak, 1972), the responses of neonates to texture is unknown. Innervation of the oral cavity of the fetus by twelve weeks gestation accompanied the development of the taste buds (Mistretta and Bradley, 1977). About 540 milliliters of amniotic fluid is swallowed per day near term (Brans, 1976). This fluid is the infants' first exposure to texture.

The texture parameters likely to be experienced by the neonate are viscosity and mouthcoat. Astringency was also reported by Malcolmson and McDaniel (1980b) to be a texture component of infant formulae.

The mechanism of suckling has been described by

Applebaum (1970). When nursing at the breast, the infant takes the nipple and areola into the oral cavity and with its tongue, draws these tissues out until the nipple reaches well into the mouth and presses onto the hard palate. The tongue and cheeks exert negative pressure against the nipple and sucking begins. The gums compress the areola, squeezing milk into the back of the throat. Milk flows against the hard palate from the high pressure system of the breast.

When the adult senses the texture parameter of viscosity, it has been described as a combination of forces applied by the tongue and the rate of tongue movement when a liquid is drawn into the mouth and passed between the tongue and hard palate (Parkinson and Sherman, 1971). In sucking, the viscosity of a liquid might influence its ease of removal from the breast. It is not clearly understood how the neonate might perceive the sensation of viscosity. The neonate has a very limited capacity to manipulate its tongue, cheeks and jaws (Szczesniak, 1972). The infant is reported to be able to only accept liquids which merely require swallowing, a reflex action. This limitation in movement of oral components could limit the infants'

ability to detect textural changes in liquids.

In some of the studies previously cited under discussion of taste stimuli, where comparisons in response of infants to sugar solutions and various milks were made, the investigators did not report on the relative viscosities of the various test solutions (Desor et al., 1977; Jacobs, 1977; Johnson and Salisbury, 1975). It is not without possibility that differences in viscosity may have affected the infants' responses. For instance, in the study of Desor et al. (1977), infants preferred 0.3 M sucrose solution (10 percent) over Similac 13, a low-density proprietary formula. There would have been an obvious difference in sweetness; if the two solutions offered had been equal in sweetness but different in textural parameters, one wonders what the infants' responses might have been.

II. The Human Neonate and Sucking Behaviour

Sucking is a highly complex activity, internally regulated and externally stimulated. It is synchronized with other physiological functions like swallowing and breathing. Sucking behaviour has been studied intensively

by Gunther (1961); Wolff (1968); Lucas et al. (1979); Drewett and Woolridge (1979). Methods of study have involved films (Drewett and Woolridge, 1979), and specially-constructed regulated feeding bottles so that fluids were delivered at constant rate and under controlled pressure (Kron and Litt, 1971).

Wolff (1968) described two modes of sucking in human infants: the non-nutritive mode of a typical burst-pause rhythm, which occurred when infants sucked on blind teats and the nutritive mode, a slower, more continuous rhythm. These rhythms appeared to be controlled by the brain but could have been in response to milk flow. In cinematographic studies, Drewett and Woolridge (1979) demonstrated that sucking rhythms at the beginning and the end of a feed tended to be typical of non-nutritive suckling, whereas the rhythm in minutes 2 to 4 of a 10 minute feed were more typical of nutritive sucking rhythm. In accompanying studies, Lucas et al. (1979) showed that 50 percent of the volume of breast milk was ingested during the first two minutes of suckling, and 80 to 90 percent was ingested by four minutes suckling. This four minute event was the point of switching to non-nutritive suckling.

Johnson and Salisbury (1975) observed breathing and sucking rhythms upon feeding various fluids to neonates. Patterns of sucking and breathing differed with different fluids given. They observed 44 infants fed with expressed breast milk and prepared cow's milk formulae. The pattern of sucking observed in expressed breast milk was the "burst-pause" rhythm typical of human neonate non-nutritive sucking as described by Wolff (1968). (These researchers had not used expressed breast milk from the infant's own mother). Johnson and Salisbury found disturbed breathing and altered sucking patterns with artificial milk.

Nursing has been described as a two-way process between mother and infant (Lozoff et al., 1977). The mother responds to visual, auditory and tactile stimuli of her infant with a let-down reflex where milk is actually ejected from the breast. Milk flow is partly a maternal response; the infant removes the milk by mouthing and collecting milk from lacteals, or ducts in the breast (Applebaum, 1970). An infant can be seen to gulp milk when the let-down reflex is strong; milk often spurts or drips from the other breast at this time (Pryor, 1973).

Continued sucking leads to further milk production (Applebaum, 1970; Hytten, 1954a, e).

It is difficult to determine which factors are involved in the changes of suckling rhythm and in the termination of feeding. Some infants have been reported to nurse with renewed vigor at the second breast (Hall, 1975a; Pryor, 1973). Milk could be expressed from a breast after an infant was finished feeding (Hall, 1975a). It is possible that the infant responds to decreased milk flow, or to the more viscous milk being harder to suckle, or to some other changes in sensory stimuli. Infants have been reported to be highly sensitive, even at neonate stage. MacFarlane (1975) described how infants six to ten days old responded to their own mother's smell by turning their heads more frequently to their own mother's breast pad.

III. Development of Appetite Control in the Infant

Control of appetite and/or food intake depends upon a highly complex interaction between an individual's nervous, endocrine and motor systems and upon environmental, social and psychological factors affecting the individual. How

important these various factors are in determining long range relationships between consummatory behaviour and regulation of body weight is a very difficult problem to solve.

Theories of food intake regulation are numerous, however, in the development of appetite control in the human neonate, the relative importance of the various factors is unknown. In a widely-cited article, Hall (1975a) hypothesized that the changing composition of human milk in a single feed at the breast could somehow, on the basis of the milk's taste and texture composition, help in the development of appetite control. By an inverse hypothesis, Hall, also questioned whether the constancy of artificial formulae would fail to elicit development of such a control mechanism. In her article, Hall (1975a), appeared to base her hypotheses, in part, upon the assumption that bottle-fed babies were found to be fatter than those babies who were breastfed. Also, she questioned whether the cause of termination of a feed by the infant would be due to lack of milk, because she reported that she had been able, in one situation, to express manually, 52 milliliters of milk from a breast after the feed.

From the earlier studies of Taitz (1971), Eid (1970), Ounsted and Sleigh (1975) and Shukla (1972) the theory that bottle feeding led to an increased incidence of infantile obesity became widely accepted. These studies had been based on cross-sectional studies of infants without data being reported correlating energy intake or feeding practices with obesity among the obese infants in the samples (Dubois, 1979). More recent studies of Poskitt and Cole (1977; 1978); deSwiet et al. (1977); Dubois et al. (1979) and Yeung (1979a) have failed to find a correlation between the type of feeding and the incidence of obesity. In fact, with these longitudinal studies, whether an infant was breastfed or bottlefed and the timing of introduction of solids did not play a causal role in determining subsequent body weight. These researchers found that total calorie intake per se was the determining factor in infant weight gain.

It has also not been established that infantile obesity predisposes the individual to childhood obesity and to later adult obesity. Eid (1970) had observed a correlation between weight gain in infancy and obesity at age six but longitudinal studies have been reported to show that the

relationship between infant weight and childhood weight weakens as the time span investigated lengthens (Dine et al., 1979; Poskitt and Cole, 1977; 1978; Yeung, 1979a). Even the definition of infantile obesity was vague and varied from study to study (Dubois et al., 1979).

Since infant feeding is a two-way process, other factors in breastfeeding, such as an efficient let-down reflex in the mother which will enable her to deliver milk to the infant, will influence the outcome of this process. One might expect variation among mothers in this neural-hormonal response and in the response of her infant to a variety of milks and feeding situations. Maternal attitudes were more of a determining factor in the development of an appetite control than the nature of the food offered (Dubois, 1979; Myers, 1979). Sims and Morris (1974) observed that authoritarian attitudes towards children were associated with higher caloric intakes and a greater weight/height relationship in preschool children.

Hall (1975a) reported on the change in milk composition of one infant at one feed. The milk to be analyzed had been collected manually at the other breast while the infant was nursing on one breast. There would be many

problems with this procedure. The let-down reflex could have been altered in such an artificial situation. Milk flow in the expressed breast depends on milk let-down, which may not have occurred. It cannot be assumed that the milk flow and the milk from the expressed breast were the same as the infant was receiving while nursing. Lucas et al. (1979) had shown by cross-sectional studies that the majority of milk was obtained by an average of 4 minutes. Drewett and Woolridge (1979) had reported the change in infants mode of sucking at this time also. In Hall's study, the single infant nursed for 16 minutes during which time collection was made. No note was made of changes in sucking rate or rhythm. Rate of milk flow may change over a feed even though milk can be expressed from the breast at the end of the feed. It is not known if the rate of milk flow influences the infant at the breast and its sucking rhythm.

IV. Infant Feeding Practices

A. Incidence of Breastfeeding

World trends in incidence of breastfeeding indicate that there is a significant decline occurring among certain

population groups. According to preliminary results of the WHO Collaborative Study of 9 countries, incidence and duration of breastfeeding depends on socioeconomic group, urban-elite, urban-poor, traditional-rural, with the greatest decline in developing countries in the urban-elite group (Hofvander and Petros-Barvazian, 1978).

In western, industrialized countries, an increase in breastfeeding has been occurring in the past few years (Hofvander and Petros-Barvazian, 1978; Jelliffe and Jelliffe, 1978b). These findings are supported by some Canadian studies. Incidences of 58 percent of mothers initiating breastfeeding in a Saskatchewan study (Bergerman et al., 1979) and of 58 percent in a Manitoba study (Clark, 1978) and of 70 percent in Toronto studies (Tse et al., 1978) were reported. Yet, an incidence of 17 percent was reported in a Newfoundland study (Alton-Mackey and Orr, 1978).

In the Alton-Mackey and Orr study, only 6 percent of rural mothers were breastfeeding at the time of home interviews within 4 months of the infant's birth, whereas 20 percent of a metropolitan sample were. Similar results were found by this author (Barker, 1979) by surveying

hospital records in two rural Manitoba hospitals. In one community hospital serving a large native population, 25 percent of mothers attempted breastfeeding and only 8.3 percent solely breastfed, of infants - mothers surveyed in a 6 month period. In another community, a farming one, 66 percent of mothers breastfed in hospital. Harasym (1977) found that 72 percent of her sample breastfed in hospital, and Fabro (1979) found that 84 percent did. Both these studies represented biased samples of mothers attending a large city hospital prenatal clinic. These various studies show that even within Canada, breastfeeding incidence varies among communities. Based on large surveys, more recent American statistics (Martinez and Nalezienski, 1979) revealed that a resurgence of breastfeeding has taken place in the United States since 1971, and it has involved all economic groups and educational levels. In 1978, 46 percent of all mothers of infants answering the survey (n = 25926), reported breastfeeding in hospital, a change from 25 percent in 1971.

B. Type of Formulae Used

The decline in breastfeeding before 1970 was accompanied by changes in the types of formulae used by non-breastfeeding mothers. According to Fomon (1971), the use of evaporated milk formula declined from 40 to 5 percent during years 1950 to 1970, and the use of commercial formula increased from 15 to 80 percent, while breastfeeding did not decline greatly.

Myers (1979) in presenting data from Nutrition Canada Survey, showed that the type of milk consumed by infants varied according to the income level of the family. Lowest and low income classes showed greater trend towards whole milk and evaporated milk, whereas two percent and skim milks were used by other income groups.

In the Newfoundland study of Alton-Mackey and Orr (1978), of the 82 percent of infants not breastfed in hospital, 60 percent received commercial milk formulae and 22 percent received evaporated milk formula. At home, the formulae had been changed so that commercial formulae were used by 34 percent of metropolitan mothers, 35 percent of urban and 20 percent of rural, whereas evaporated milk formula was given to 43 percent of metropolitan, 47 percent of urban and 72 percent of rural

babies.

In the rural Manitoba community surveyed by this author (Barker, 1979), with the high proportion of native babies, because of a high hospital readmission rate, infant charts could again be surveyed to determine which method of feeding the mother was using at home within 4 months after birth. Of twelve original infants, seven were on evaporated milk formula, one was on commercial formula, two were on soybased formulae, and two were unknown. Commercial formula had been used in the hospital. These differences in milk choice may have been selected on the basis of economics or accessibility.

Martinez and Nalezienski (1979) reported an overall percentage of formulae use in 1978 in the United States as follows: 59 percent of all babies whose mothers were surveyed were consuming commercial formulae; 0.5 percent, evaporated milk formula; and 0.1 percent, whole cow's milk, at one week of age. The remaining 45 percent were being breastfed.

Both Harasym (1977) and Fabro (1979) found non-breastfed infants were receiving predominantly commercial formulae.

Since it may be possible that the sensory

characteristics of early foods fed to an infant would influence the child's later preferences, these varying and changing statistics are of interest to those researchers studying taste development.

V. Composition of Human Milk

Human milk production is under the control of a complex of hormonal and neural processes. Although many hormones are involved, two are of predominant interest: prolactin from the anterior pituitary, controls milk synthesis in the alveolar cells of the mammary gland and oxytocin from the posterior pituitary gland, controls release of milk from the breast. Knowledge of the control of synthesis of particular nutrients is limited, but biochemists and physiologists have been able recently to measure (many constituents) more precisely with modern analytical techniques.

Normal physiologic variation exists in the constituents of human milk. Physiologists have pointed out that the milk of each mammalian species is particularly suited to the growth and nutritional needs of that species (Jelliffe and Jelliffe, 1978b). Human milk has been reported as

higher in lactose but lower in protein and minerals than milks of many other mammals.

Variability of human milk constituents has been described at length in the literature. These variations have been the result of various factors - of normal physiological variation (Hyttén, 1954a; e; Jelliffe and Jelliffe, 1978a; Jelliffe and Jelliffe, 1978b; Jensen et al., 1978; Lonnerdal et al., 1976; Picciano and Guthrie, 1976); of maternal diet (Guthrie, 1977; Insull et al., 1959; Mellies et al., 1978; Potter and Nestel, 1976; Read et al., 1965); of maternal drug intake (Catz and Ciaccio, 1972; O'Brien, 1974); of environmental pollution (Atkinson, 1979), and of many other factors including length of pregnancy (Atkinson, 1978), disease in the breast (Conner, 1978) and emotional stress (Sevy, 1971). The reader is referred to these particular articles for further elaboration of each topic.

For the purpose of this study, there will be a brief summary of the literature as it relates to gross composition; that is, fat, protein and lactose contents and their variabilities from mother to mother, from day to day and within a single feed.

A. Carbohydrate Content of Human Milk

Lactose, the main carbohydrate of human milk, has been found only in mammalian milks and has been reported to show the least variation of milk constituents (Hytten, 1954a; e; Jelliffe and Jelliffe, 1978b). Table 1 contains a summary of mean reported values in the literature for lactose. Since many of the analytical methods exploited the fact that lactose is a reducing sugar, these lactose values were likely overestimated. Other minute amounts of reducing sugar would be estimated also. Mean lactose content ranged from 5.0 to 7.4 grams per deciliter.

Other carbohydrates were described by Jelliffe and Jelliffe (1978b) and by Ebner and Schanbacher (1974). These were present in very small quantities and included: galactose, fucose, glucosamine, sialic acid and various oligosaccharides found only in lacteal secretions. An important oligosaccharide was "bifidus factor" described by G jorgy (1971) which promoted sole colonization of the infant digestive tract with Lactobacillus bifidus bacteria. Most oligosaccharides seemed to contain a common "gal-glcNAC-gal-glc" unit. Ebner and Schanbacher (1974) noted that human milk oligosaccharides varied with Lewis or

Table 1
Reported Mean Values for Lactose Content for
Human Milk

Reference	Values g/100 ml	n	Sources of information to author/methods of analysis
Morrison (1952)	7.2	-	Review of literature
Macy, Kelly, Sloan (1953)	6.0 - 7.6	1500	Review of literature
Hyttén (1954a; e)	6.29 ± 0.45	150	7 day milk
	6.87 ± 0.26	150	1 month milk
Macy, Kelly (1961)	6.8	216	
Edozian (1976)	7.9 - 9.6	25	Low protein diets, Nigeria
Jelliffe and Jelliffe (1978)	5.0 - 7.6	-	Review of literature
Dept. Health Soc. Security 12, London (1977)	7.4	96	(Fehling's solution for reducing sugars)
Forsum and Lonnerdal (1979)	7.22 ± 0.44	6	Enzymatic method
Lauber and Reinhardt (1979)	6.74 ± 1.04	33	Phenol-sulfuric acid method-reducing
Hall (1979)	8.29 ± 0.10	15	Fore milk
	7.95 ± 0.22	15	Hind milk Iodoimetric method

blood type antigens.

B. Fat Content of Human Milk

The fat content of human milk shows the greatest variation of all milk constituents, as reported in early studies of Gunther and Stanier (1949) and Hytten (1954a; e), and reconfirmed by Jensen et al. (1978). Lipids in milk are the main source of calories and are important precursors in the development of the central nervous system and the lipid-rich brain. Human milk is 98 percent triglycerides according to Jensen et al. (1978).

The reported mean values of fat content from a number of studies on human milk are in Table 2. Variations occur between studies because of differing times of collection, amounts collected, and methods of analysis. Mean values reported ranged from 3.1 to 4.5 grams per deciliter.

The variability of human milk makes representative sampling difficult. Variations in constituents have occurred between mothers, within the same mother from day to day, from morning to evenings, from one breast to another and throughout the months of lactation. It is particularly important to obtain a representative aliquot

Table 2
Reported Mean Values for Fat Content of
Human Milk

Reference	Values	n	Sources of information to author/method of analysis
Macy, Kelly, Sloan (1953)	3.1 - 5.2	(1500)	Review of literature 1500 publications
Morrison (1952)	3.3	-	Review of literature
Hyttten (1954a; e)	3.17 \pm 0.78	150	24 hour sample of 7 day milk
Macy, Kelly (1961)	4.5	216	
Picciano, Guthrie (1976)	3.05 \pm 0.91	50	Early morning milks; (Nakai and Le (1971); daily mean
Dept. Health Soc. Security 12, London (1977)	4.2	96	(Rose-Gottlieb method)
Jensen (1978)	3.2 - 3.5	-	Review of literature

of at least a 24 hour sample to have a fat content typical of a particular woman. In Table 3, the change in fat content over one feed is reported from two studies. Hind milk was found to be higher in fat content than fore milk in both studies (Hall, 1979; Hytten, 1954b). The fat content has been reported to vary throughout the day with the lowest values reported in early morning (6:00 hours) and peaking of values at 10:00 hours (Gunther and Stanier, 1949; Hall, 1979; Hytten, 1954; c;). Hytten (1954b) reported slightly different values for fat content depending upon which breast was suckled first. He proposed that mixing of fore and hind milks took place to some extent in the second breast during let-down reflex while the infant suckled at the first breast. This mixing tended to raise the initial fat content in the second breast, but did not significantly change the overall fat content of milks from the two breasts.

Maternal diet influences fatty acid composition of human milk. According to studies of Insull et al. (1959) and of Read et al. (1965), during energy equilibrium, the fatty acids of human milk resembled those of the diet. When caloric inadequacy existed, fatty acids of the milk resembled the depot fatty acids of the mother. They also

Table 3

Reported Values for Fat Content of Human Milk During a Single Feed

Reference	Values g/100 ml		Comments
	Fore Milk	Hind Milk	
Hytten (1954a; e)	0.45 to 5.55	2.90 to 10.05	Time of day for collection not specified; Range reported for 53 samples; Gerber method of analysis; Hytten took 12 ml aliquots during single feed.
Hall (1979)	Mean: 2.42 ± 0.3 Range: 0.87 to 5.05	7.48 ± 0.57 3.02 to 12.05	Time of day not specified; 15 samples of 6 mothers; Gravimetric method of analysis after solvent extraction. "Foremilk" represented sample before feed and "hindmilk" after feed.

found that the most important dietary factor influencing fatty acid composition was the level of carbohydrate. A high carbohydrate diet favoured mammary gland synthesis of fatty acids, particularly lauric (C12:0), myristic (C14:0), and inhibited removal of long chain fatty acids from the blood. Linoleic acid levels were reported to be influenced by maternal diet also. Jensen et al. (1978) reported a variation in linoleic acid (C18:2) from one to 43 percent depending on the mother's diet. Guthrie (1977) reported an increase in C18:2 levels of milks of American women over values reported 20 years earlier. Human milk contained almost no short chain fatty acids of chain lengths less than ten (Jensen et al., 1978). Fatty acid composition did not vary throughout the day or over a single feed (Emery et al., 1978).

C. Protein Content of Human Milk

The proteins of human milk can be divided into two groups (1) milk - specific proteins, synthesized in the secretory cell, such as the caseins, lactalbumin (part of the lactose synthetase enzyme system) and the anti-infective proteins, lactoferrin, lysozyme, and immunoglobulin A; and

(2) serum-derived proteins such as other immunoglobulins, and serum albumin. Besides having a lower protein content than cow's milk, human milk has a different casein: whey ratio. Early researchers paid much attention to casein: whey ratios and to curd tensions formed upon gastric hydrolysis; these differences were incorporated into "humanized" commercial formulae. Recent information stresses that differences in protein go far beyond those early differences. Hambreus et al. (1978) suggested that protein content of human milk was actually 0.9 g/100 ml instead of 1.2 g/100 ml or higher, as earlier reported for human milk. Hambreus reported the non-protein nitrogen at 23 percent of total nitrogen. Therefore, the use of Kjeldahl estimation of total nitrogen times a conversion factor of 6.25 or 6.38 became invalid as a measure of total protein content. In Table 4, the variety of reported values for protein in human milk are summarized. Again, the variety of analytical methods has influenced the results reported.

VI. Flavour of Milk

A. Off-Flavours in Human Milk

Publications written for nursing mothers and advice

Table 4
Reported Mean Values for Protein Content of
Human Milk

Reference	Values g/100 ml	n	Source of information to author/methods of analysis
Morrison (1952)	1.3	-	Review of literature
Macy, Kelly, Sloan (1953)	0.9 - 1.6	1500	Review of literature
Hyttén (1954 a; e)	1.44 ± 0.22	150	7 day milk
	1.07 ± 0.16	15	3 - 6 weeks
	0.91 ± 0.12	15	76 weeks
Macy, Kelly (1961)	1.1	216	(Kjeldahl N and protein precipitation)
Lonnerdal, Forsum, Hambreus (1976)	0.88 ± 0.14	6	(Amino acid analysis)
Edozian (1976)	1.20 ± 0.21 to 1.61 ± 0.15	12	Mothers on different protein intakes Kjeldahl N x 6.25
Dept. Health SS 12 (1977)	1.07	96	(Amino acid N x 6.38)
Picciano, Guthrie (1976)	0.76 - 2.04	50	(Nakai and Le, 1971)
Hall (1979)	1.00 ± 0.06	15	Foremilk
	1.36 ± 0.16	15	Hindmilk (Lowry, 1951)

given to mothers by the medical profession have often included specific foods to be avoided by lactating women, mainly because of off-flavours expected in milk and because of gastrointestinal upset expected in infant. No studies were found specifically relating foods eaten by the mother and appearance of off-flavours in her milk.

Mothers have been warned to avoid numerous foods. Some of these recommendations and their sources are summarized in Table 5. Foods most commonly implicated were gas-forming vegetables, spices and chocolate. As well, in an article written for physicians (Smith et al., 1978), the authors cited these same foods as causing gastrointestinal distress in the infant. The statement was made that "it takes about four to six hours for food metabolites to get into breast milk", (Smith et al., 1978: 95) but no specific reference or study was cited to document their statement. Taylor and Worthington (1977: 169) stated that "there is no basis for avoiding garlic, curry, sulfur-containing vegetables, or any other nourishing food because a woman is breastfeeding".

Sims (1978) surveyed 61 lactating women on the sources and types of dietary advice they had received. Over 90

Table 5

Summary of Advice Given in Publications Written for Mothers for Food Avoidances
During Lactation

Source (Reference)	Advice
Lambert-Lagace (1976)	Avoid "foods which give the mothers' milk an unpleasant taste, such as strong-tasting vegetables (ie., the cabbage family, onion, garlic)" and "highly seasoned foods". (p. 97).
Manitoba Dept. of Health and Community Services Publication (1978)	"if you eat a strong-flavoured or gas-producing food (such as cabbage, turnips, onions, radishes, dried beans or cauliflower) and it upsets the baby, omit it from your diet". (p. 4).
Ross Laboratories "Breast Feeding Your Baby" (1975)	"foods such as onions, members of the cabbage family, tomatoes, chocolate, spices and seasonings have been troublesome for some nursing mothers by causing their babies to refuse milk so flavoured". (p. 11).
Eiger and Olds (1972)	"some foods eaten in large amounts will give the milk a distinctive taste"... "other women find that their nursing babies sometimes suffer gas after their mothers eat such gas-producing foods as cabbage, broccoli or brussels sprouts". (p. 58).
Pryor (1973)	"Volatile oils which give most spices their characteristic odors and flavours, pass through milk unchanged. This does not mean that a nursing mother has to avoid spicy foods... there is no evidence that the breast fed baby objects in the slightest to garlic -or curry- flavoured mothers' milk. (p. 59).

percent of her sample indicated that they ate differently while nursing, and 75 percent reported avoiding certain foods during lactation. Specific foods cited were the familiar ones: cabbage, beans, garlic, onions, chocolates and alcoholic beverages. The most common advice received by these women from their pediatrician was to avoid chocolate and spicy foods.

B. Cultural Influences Upon Diet During Lactation

Proscribed foods for women during the reproductive years were commonly reported from different ethnographies (Jelliffe, 1962; Niehoff and Meister, 1972; Wellin, 1955). In one culture, lactation was seen as illness and the women fed sparingly (Katona-Apte, 1977, abst.). Snow and Johnson (1978) found many food beliefs in 40 low income, multiethnic women attending a modern health clinic in Michigan. Dietary restrictions in the postpartum period were frequent, sometimes lasting forty days, although only 5 of the 40 mothers specified foods to avoid during lactation. Spicy foods and cabbage were reported likely to "sour" the milk.

It may be surprising to find such beliefs in one's



own culture, yet without controlled study, it is impossible to separate fact from belief or to determine whether the cultural beliefs cited in section VI, A, are rooted in fact. Walter (1975) collected beliefs on food and lactation passed by oral tradition in the United States. She found chocolate to be the food held in greatest trepidation, but found also, the same foods were prescribed and/or proscribed in different parts of the country.

C. Off-Flavours in Animal Milk

1. Cow (Bovine Milk)

Because of its economic value, cow's milk has been intensively studied for the presence and cause of off-flavours. In a comprehensive review, Shipe et al. (1978) attempted to classify the particular off-flavours by name and causal agent. These off-flavours appeared to fall into two groups: those caused by storage and handling of milks and those caused by transmitted flavour from feed, or environment of the cow. Transmitted flavours are of particular interest in this study. Flavour transfer in the cow has been reported to occur by the respiratory route and/or the digestive system. Rumen bacteria also played

a role. Depending on the flavour component, it took 4 to 12 hours to excrete or metabolize the product. Also mentioned by Shipe and coworkers, was salty flavour of milk from mastitic cows. Sulfur compounds were proposed as a common source of off-flavour. Based on these studies, it is possible that the human female could transmit strong volatile flavour components into her milk by a respiratory or gastrointestinal route.

2. Other Animals

Various studies on early ontogenesis of learning have utilized the ability of the mother rat to excrete flavour compounds into her milk. The effect of an animals' early food experience on later acceptance of foods was studied by adding garlic juice to mothers' diet (Capretta and Rawls, 1974). The researchers found that neonate rat experience with flavoured mothers' milk was a potent cue in development of preferences for such flavours later in life. When combined with garlic and water diet for 5 days during postweaning stage, strong and lasting preferences were reported in the rats.

After a number of studies with preweanling rats and

mothers' milk (Galef and Clark, 1972; Galef and Henderson, 1972; Galef and Sherry, 1973), it was concluded that mothers' milk was an important mechanism for transferring of gustatory cues reflecting the mothers' diet which would influence selection of foods in the postweanling diet.

Martin and Alberts (1979) found that preweanling rats could not be made aversive to a flavour in mothers' milk within the nursing situation. It was as if the maternal cues of the nursing situation were immune to aversive conditioning. Suppression of nursing as a measure of learning may be beyond the capabilities of some altricial neonates (those species have long nursing periods). Although the concept of imprinting as an effect in inducing attachments to objects and food sources in developing young of some species has been studied, these same effects were discussed by Rozin (1978) as unlikely and maladaptive processes for mammals, particularly humans. He stated that because milk is the first food for all mammals, imprinting would be especially maladaptive, since milk is generally not available after weaning, except for humans. And, in the case of humans in some cultures preferring a pungent flavour like chili, Rozin (1978)

stated that young children do not like chili even though they may have been exposed to small amounts of its flavour in mothers' milk. Some cultures proscribed chili as a food for young children.

D. Flavour Changes Occurring in Human Milk Upon Storage

Because of renewed interest in human milk banking, the effects of means of storage upon milk have been studied in relation to maintenance of anti-infective properties and nutritional properties (Ford et al., 1977; Hernandez et al., 1979; Roy and Lescop, 1979). No studies have been found which discussed the effects of storage upon the flavour and texture of human milk.

Like raw bovine milk, fresh human milk contains enzymes. Lipases have been described by Hernell et al. (1977) and Hall (1975b). One of these lipases, a bile-stimulated lipase, has been demonstrated to hydrolyze long-chain triglycerides under conditions found in the small intestine (Hall et al., 1979). Because pancreatic lipase activity is reduced in the neonate, milk lipase likely plays an important physiological role in digestion

and absorption of lipids by the neonate (Hall et al., 1979). Bile-stimulated lipase was reported to be secreted in high amounts in human milk (Hall et al., 1979).

Lipases have been reported to be active at -10 to -20° Celsius (Webb and Johnson, 1974). Activity of lipases in fresh human milk might be expected to lead to rancid or lipolyzed off-flavour. Webb and Johnson (1974) described rancid flavour as "bitter soap-like" flavour. Factors reported as enhancing lipolysis are agitation, freezing and thawing of raw milk (Hall et al., 1979). In reports from mothers of rejection of frozen expressed human milk by the baby, the rejection has been attributed to refusal of infant to accept a different nipple (F. Andrusiak, La Leche League, personal communication). It is possible also, that a high degree of milk rancidity led to rejection of the milks. Holder pasturization of raw milk at 55°C destroyed almost all lipases (Webb and Johnson, 1974). Often, though, heat treatment of fresh human milk has been avoided in an attempt to retain anti-microbial factors (Ford et al., 1977); also, mothers at home have frozen breast milk without heat treatment.

E. Drugs or Food Metabolites Excreted Into Human Milk

Numerous studies have analyzed drug excretion into human milk. The quantity excreted depended upon maternal dosage, drug kinetics, pH of drug, ionization of drug, fat solubility of drug (O'Brien, 1974). Because of the lipid content of human milk, the mammary gland has been reported to excrete fat soluble drugs and environmental pollutants such as certain pesticides, and industrial chemicals, polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) (Atkinson, 1979).

Excretion of particular compounds in foods which could exert pharmacologic action have been studied. Theobromine reported present in chocolate was studied by Resman et al. (1977). A chocolate bar containing 240 mg of theobromine was eaten by each of 6 nursing mothers. This represents a single pharmacologic adult dose. Theobromine found in milk was matched closely to that in maternal plasma, 2 to 3 hours after ingestion. Because of slow metabolism of this drug in the neonate, it is possible that the infant would receive a pharmacologic dose, especially with repeated ingestion of chocolate by its mother. Infants in the Resman et al. study showed no ill effects during

the study. Possible results would have been central nervous system stimulation, diuresis, stimulation of cardiac muscle.

Caffeine has been reported to have been excreted into human milk at one percent of amount ingested, but no effects on the infant have been reported (O'Brien, 1974).

Nicotine studied by Ferguson et al. (1976) revealed 91 ppb in milks of an average but a range of 20 to 512 ppb among women who smoked 10 - 30 cigarettes per day. No adverse effects in infants were reported by these mothers. Amount of nicotine did not correlate well with timing or amount of smoking.

Other reports of food compounds entering milk are of rhubarb, senna and certain allergens as egg and wheat, (O'Brien, 1974). One interesting case involved a mother who had eaten 2 to 3 pounds of carrots each week and produced carotinaemia in her infant (Thomson, 1943).

Illingworth (1953) discussed foods which may affect the baby through mothers' milk and stated that evidence for these beliefs was lacking. On examination of colic and its relationship to foods eaten by the mother, Illingworth found that only onions were noted by more than one mother. All other foods noted by nine mothers of 57

who related colic to foods eaten, were cited only once each.

M E T H O D S A N D M A T E R I A L S

I. Hypotheses

The following hypotheses were formulated to carry out the objectives: (See comment at the end of Hypotheses).

- Ho 1: There will be no difference in sweetness of fresh human milk between mothers or within the same mother from day to day or within a single feed.
- Ho 2: There will be no difference in perceived viscosity of fresh human milk between mothers or within the same mother from day to day or within a single feed.
- Ho 3: There will be no difference in perceived mouthcoat of fresh human milk between mothers or within the same mother from day to day or within a single feed.
- Ho 4: There will be no difference in physical viscosity of fresh human milk between mothers or within the same mother from day to day or within a single feed.

- Ho 5: There will be no difference in total lactose content of fresh human milk between mothers or within the same mother from day to day or within a single feed.
- Ho 6: There will be no difference in total protein content of fresh human milk between mothers or within the same mother from day to day or within a single feed.
- Ho 7: There will be no difference in total fat content of fresh human milk between mothers or within the same mother from day to day or within a single feed.
- Ho 8: The perceived sweetness of fresh human milk will not be related to the lactose content of the milk.
- Ho 9: The perceived viscosity and mouthcoat of human milk will not be related to the total fat content of the milk.
- Ho 10: The perceived viscosity and mouthcoat of human milk will not be related to instrumental viscosity.

Ho 11: Maternal parameters of maternal age, lactation stage, frequency of nursing, parity and nursing style will not be associated with sensory, physical or chemical properties of fresh human milk.

It will be noted that Hypotheses 1 - 7, and 10, are actually more than one hypothesis worded into one statement.

II. Preliminary Work

A. Contact With La Leche League

Approval for the involvement of mothers of the La Leche breast feeding organization was obtained from La Leche League International in September, 1978 (Appendix A). La Leche League has certain guidelines to be followed when mothers are involved in studies. These guidelines appear in Appendix B.

The area co-ordinator for La Leche League in Winnipeg, Manitoba, Canada, was contacted. Discussions were held between two La Leche leaders and the researchers to determine the feasibility of conducting the study and, in particular, of obtaining sufficient quantities of fore and hind milks.

This researcher attended an area meeting of fourteen La Leche group leaders in January 1979, to explain the study and to elicit their help in obtaining the names of mothers who may be interested in participating in the study.

B. Approval of Ethics Committee

The experimental design and protocol for selection of subjects was approved by the Ethics Committee, Faculty of Home Economics, University of Manitoba, in December, 1978 (Appendix C).

C. Pre-test on Milk Samples

Samples of human milk from four mothers were obtained and were evaluated by experienced taste panel members. Since variations in flavour and texture were noted, it was felt that a further study could lead to quantitative information of value. Two mothers expressed fore and hind milk samples to demonstrate the feasibility of obtaining sufficient (30 ml) sample for the study.

III. Investigation Phase

A. Selection of Subjects

1. Fresh Human Milk Panels

At the meeting in January 1979, the researcher provided the La Leche leaders with a letter to be given to an interested mother outlining the study and the expectations of the mother if she were to participate (Appendix D). Examples of the questionnaire and the food record form were also included. At the regular monthly meeting of each group, each La Leche leader introduced the nature of the study to approximately ten to twenty nursing or expectant mothers. Names of mothers were sent to the researcher by the leaders during the following two months.

All mothers were then contacted by telephone to determine their interest in the study. Each mother was then visited in her home. Informed consent was obtained at this time using the form in Appendix E. Coded collection bottles were delivered to each mother, as well as the questionnaire, three food record forms and an outline of instructions for handling the milk samples (Appendix E). Mothers were informed that they would be contacted by telephone one week before they were to participate in the study.

2. Frozen Human Milk Panels

Two additional mothers were contacted for inclusion in another panel session held in July, 1979 for evaluation of frozen milk. One mother from the original group agreed to participate in this section of the study also.

B. Criteria for Participation

The population was restricted to nursing mothers of infants two to six months of age. No other limits were placed upon the population such as parity, or age of mother. The eligibility based on the age of the infant was extended to ten months during the recruitment of mothers in order to obtain sufficient mothers for the study. The

feeding of other foods besides breast milk to the infant did not restrict participation; nor did the mother's food habits.

C. Ineligible Contacts

Thirty-one telephone contacts were made and of these, twenty-six mothers were still interested in the study, and therefore, twenty-six home visits were made. All of the visited mothers were willing to participate and were enthusiastic about the study. Of these, twenty-four were included in the study. One mother could not be reached at the time of the study, and one mother had terminated lactation by the time of the study.

D. Collection of Milk Samples

Milk was expressed in the manner most comfortable to the mother, usually by hand. Samples of milk were collected in new, sterile 60 ml glass jars¹ provided by the Department of Foods and Nutrition, University of Manitoba. The jars

¹ Wheaton Snap-Cap Bottle, 60 ml.
Wheaton Scientific, Div. Wheaton Industries, Millville, N.J.

were washed without detergent in a homestyle automatic dishwasher at water temperature of 60°C and allowed to air dry. Plastic lids were lined with aluminum foil to minimize the transfer of plastic odors or flavours to the milks. Mothers were instructed to store milks in the refrigerator immediately after expression. Fore and hind milks were to be separated. Milk samples were collected each morning of the panel within two hours of expression by members of the Department of Foods and Nutrition, and were transported in an ice bath directly to the foods laboratory. Samples were immediately placed in a refrigerator.

E. Experimental Plan for Mothers Supplying Fresh Milk Evaluation

The twenty-four mothers participating in the study were divided into four groups of six mothers each to enable collection of samples and panel evaluation on a manageable number of units. The sensory evaluation panels covered a period of three weeks. Mothers were asked to express milk between 7:00 hours and 9:00 hours for three consecutive days. Fore milk was defined as the first thirty milliliters

expressed from the first breast before the infant was nursed. Hind milk was defined as the final thirty milliliters expressed from the first breast after the infant was removed from the breast. Either the left or right breast was used.

Mothers were contacted a few days before each experimental period and were reminded of the food records. Mothers were reassured that they should save any milk expressed up to thirty milliliters fore milk and hind milk, assuming that some mothers may have problems expressing the full amount at all times.

Figure 1 represents diagrammatically the experimental plan.

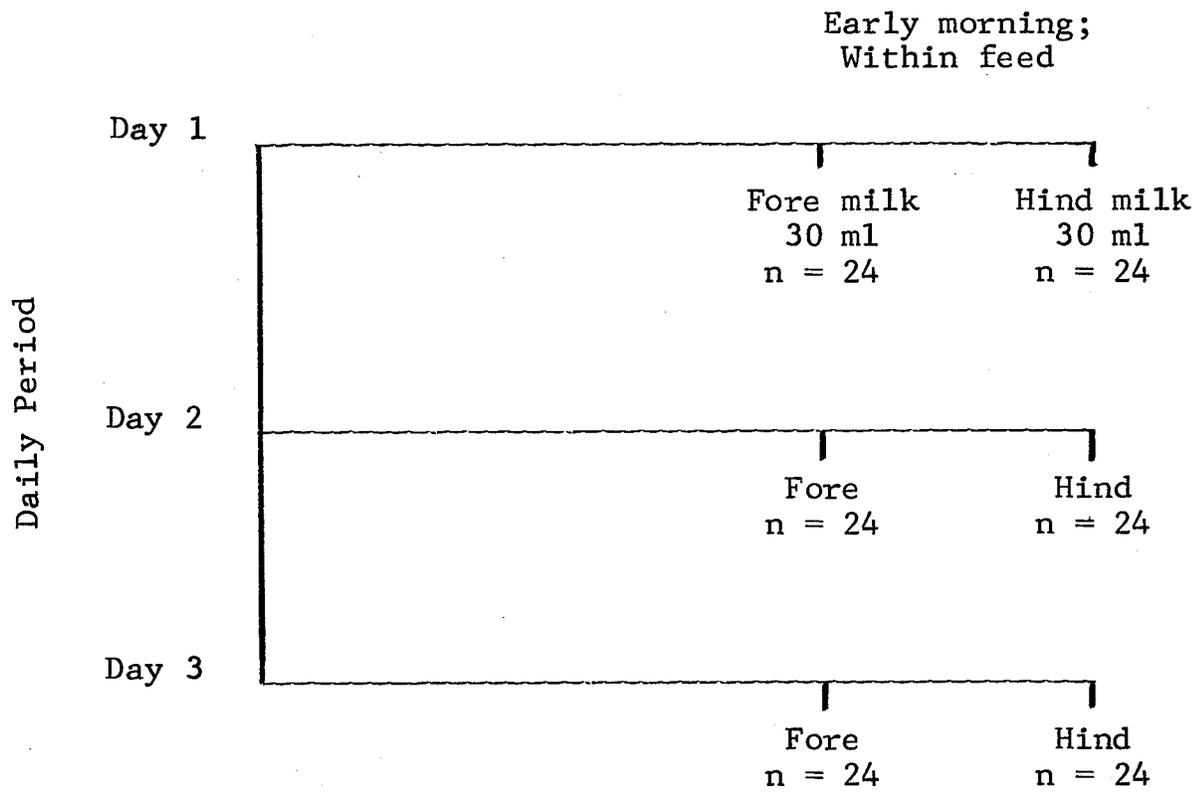
F. Questionnaires

1. Questionnaire Number One

A questionnaire was developed to gain demographic information from the mother. The style and frequency of nursing were also ascertained, as well as a description of the cues that the mother would recognize from her infant as to satiety or termination of nursing (Appendix G). At the time of milk sample collection, the completed

Figure 1

Experimental Plan for Mothers Supplying Human
Milk for Sensory Evaluation



questionnaires were also collected.

2. Questionnaire Number Two

A second questionnaire was developed after the first experimental period and was administered with approval of the La Leche League area coordinator. This second questionnaire sought information about the mothers' practices in freezing breast milk as well as some information on food practices during lactation (Appendix H). The second questionnaire was mailed with a cover letter (Appendix I) to each of the twenty-four mothers, plus two additional La Leche mothers in August, 1979. Completed questionnaires were returned to the researcher also by mail.

G. Food Records

Mothers were instructed to list all foods and beverage consumed within 15 hours of milk expression, beginning at 16:00 hours the previous day, for three consecutive days. Mothers were asked to note, in particular, the intensity of food flavours consumed. Further questions regarding coffee consumption, cigarette usage and medication usage were asked on the food records (Appendix J). Food records were picked up each day of milk collection.

H. Treatment of Milk Samples for Chemical Analysis

Prior to preparation of milk samples for panel evaluations, a two milliliter aliquot of each milk was placed in a coded glass vial², fitted with a plastic stopper. These vials were stored at -18°C until chemical analysis.

IV. Panel Selection and Training

A. Selection of Panelists

A six-member panel consisting of female graduate students and staff of the Department of Foods and Nutrition, Faculty of Home Economics, University of Manitoba, was selected to participate in the study. All six persons had some previous experience in the area of sensory evaluation, and were chosen because of their interest in the project and their ability to perform as judges.

B. Training of Panelists

1. Purpose, Duration and Environment

The six panelists were trained to identify and quantitate

² Vial, Opticlear, 1 dram, 14.5 x 45 mm.
Kimble Products, U.S.A.

the textural characteristics of viscosity and mouthcoat and the taste sensation of sweetness in human milks. A total of six one-hour training sessions were held. Part of each session was held around a large table to facilitate group discussion. During each session, some time was used for group discussion to allow the panelists and panel leader to come to a consensus on the appropriateness of the reference solutions and of the test ballot.

2. Sensory Evaluation Technique

Magnitude estimation, a form of ratio scaling, was used as a measuring instrument throughout the training sessions and for all sensory evaluations conducted in this study. The intensity of a sensory characteristic in a sample was rated against that found in a reference standard solution. For example, when evaluating sweetness, panelists were given a reference lactose solution to which they assigned a score of ten. If sweetness perceived in a sample of milk were half that of the reference, then the sample was given a score of five. If a sample were found to be twice as sweet as the reference, it received a score of twenty. The panelists were familiar with magnitude

estimation and required only one training session to reacquaint themselves with its use.

3. Training in Texture and Taste Evaluations

During the panel training sessions, panelists were presented with reference solutions and samples of varying concentrations of lactose as well as samples of human milk. Solutions of two percent sucrose and seven percent lactose were compared for their sensory qualities. All samples during training were tested at 37°C. Samples were heated in a warm water bath. Panelists practiced the evaluation of the texture parameters of viscosity and mouthcoat and of taste stimulus, sweetness, until all were confident in the use of the techniques of evaluation. The definitions of each sensory characteristic and the criterion of each are listed in Figure 2. Because it was desirable to have a reference whose characteristic fell within the middle of the range of samples, it was necessary to adjust the references for viscosity and mouthcoat until all panel members were comfortable in their uses. For this reason, an infant formula, Similac, was diluted with water to achieve the appropriate consistency. That is, Similac in a 1:3 ratio with distilled water (volume by volume)

Figure 2

Sensory Characteristics: Definitions and Procedures

Sweetness: the pure sweet taste typical of sucrose and generally perceived best at the front of the tongue.

Technique: place sample in mouth, swirl and expectorate. Evaluate sweetness.

Reference: 6 percent lactose (w/v) solution.

Viscosity: the resistance to flow of a liquid when drawn between the tongue and hard palate.

Technique: place a sip of sample in the mouth and rate the force required to draw the sample between the tongue and palate.

Reference: Similac 1:3 water (v/v).

Mouthcoat: the degree of coating or clinging of a sample to the mouth following swallowing or expectoration.

Technique: place sample in mouth, swirl and expectorate. Evaluate degree of coating.

Reference: Similac 1:3 water (v/v).

was used as the reference standard for evaluation of viscosity and mouthcoat of human milk samples. A six percent solution of lactose was chosen as the reference standard for evaluation of sweetness. All references were also used at 37°C.

4. Training in Description of Off-Flavours

Panelists were familiarized with samples of human milk so that they would be able to taste the presence and intensity of off-flavours in the milks. Group discussions took place in order to aid the panelists in their descriptions, but panelists were instructed to use any descriptor they felt appropriate. A five-point intensity scale was used: detectable, slight, moderate, strong, extreme. Panelists were instructed to use one of the five levels of intensity if an off-flavour were present. A sample of the ballot used appears in Appendix K.

Finally, the panelists were familiarized with the entire panel procedure by evaluating six milk samples with the appropriate references and ballot, in the individual booths.

V. Sensory Evaluation of Human Milk

A. Fresh Human Milk Evaluation

1. Experimental Design

The experiments were factorial designs with four factors: mother, day, time (fore and hind milk) and judges. Because of the limited amount of milk available from each mother and because of the variable nature of human milk over time (days and weeks), the experiments could not be replicated. For this reason, judges and their interactions were assigned to be the error term in the analysis of the data.

Because it had been determined in preliminary testing that each mother would be able to supply only 30 milliliters of each of fore milk and of hind milk, only three judges (each needing 10 milliliters of sample) could taste each mother's milk. For this reason, the twenty-four mothers were divided into two groups, each group containing twelve mothers whose milks were evaluated by the same three judges throughout the entire test period. These two groups of mothers are henceforth to be referred to as Group I (Mothers One to Twelve, evaluated by Judges One to Three) and Group II (Mothers Thirteen to Twenty-Four, evaluated by Judges Four to Six). Each mother expressed one fore

milk sample and one hind milk sample for three (3) consecutive days. That is, each mother provided six (6) samples for the study.

On each panel day, judges evaluated fore and hind milks of three mothers. The manner of division of mothers and judges during panel evaluations is illustrated in Table 6. Panel sessions spanned a total of three weeks.

2. Tasting Procedure

a. Environment

Milk samples were evaluated by judges in temperature and humidity-controlled individual sensory booths. Red lights were used to prevent the colour of the samples from influencing judges' perceptions.

b. Sample Preparation

Samples were refrigerated from the time of arrival to laboratory until within one-half hour of panel time. After two milliliter aliquots were removed from the sample for purpose of chemical analysis, samples were placed in coded 100 milliliter glass beakers and placed in a warm water bath heated to 37°C. Due to the limited amount of milk sample, physical viscosity measurements were performed, immediately preceding the sensory panels, on 18 milliliters of each mother's sample. This 18 milliliters of milk was

Table 6

Experimental Plan for Division of Mothers and Samples
Over Judges for Sensory Panel Evaluations

Panels	Group I Mothers* Evaluated by Judges 1, 2, 3	Group II Mothers** Evaluated by Judges 4, 5, 6
<u>Panel Group 1</u> 3 days Fore and Hind each day for each mother	Mothers 1, 2, 3	Mothers 13, 14, 15
<u>Panel Group 2</u> 3 days Fore and Hind each day for each mother	4, 5, 6	16, 17, 18
<u>Panel Group 3</u> 3 days Fore and Hind each day for each mother	7, 8, 9	19, 20, 21
<u>Panel Group 4</u> 3 days Fore and Hind each day for each mother	10, 11, 12	22, 23, 24

* Since Group I mothers were always evaluated by judges 1 - 3, the data from this group of 12 mothers was analyzed as a 12 x 3 x 2 x 3 factorial design (Refer to Section II, Results, p

** Group II mothers were always evaluated by judges 4 - 6; the data from sensory evaluations of this group was also analyzed as a separate 12 x 3 x 2 x 3 factorial design.

returned to the appropriate beaker to be used for sensory analysis. Samples were held at 37°C, therefore, to a total of 30 minutes before panel evaluation.

3. Presentation of Samples to Judges

All samples were served in 40 ml. No. 17 treated Lily creamers, coded with three-digit random numbers. Each judge was presented with six samples, three fore and three hind milks of three mothers for each test day. In addition to samples, trays contained tap-distilled water and unsalted soda crackers for rinsing, two reference solutions, an appropriate ballot and other necessary items.

4. Taste Evaluations

Using magnitude estimation, judges evaluated all milk samples for degree of sweetness perceived in relation to the reference standard of six percent lactose solution.

5. Texture Evaluation

All milk samples were evaluated for perceived viscosity and mouthcoat in relation to a reference standard of diluted Similac.

6. Off-Flavour Evaluations

Judges were asked to describe off-flavours, if present, and to choose one of five intensity descriptors: detectable, slight, moderate, strong and extreme (Appendix K). Each

panelist was instructed to evaluate the sensory parameters in the following order: sweetness and off-flavour; viscosity and finally, mouthcoat.

B. Frozen Human Milk Evaluation

Two mothers participated in this part of the experiment; one mother contacted originally was unavailable at the time of the study. Milks were collected one month prior to the panel sessions and were frozen in the original glass jar in the freezing compartment of a two-door refrigerator at -18°C . Only one sample per mother for each of three consecutive days was collected, again at the first morning feed and from the first breast. Fore and hind milk samples were not separated. Each mother was asked to express sixty milliliters.

On the day of each panel evaluation, the same two mothers expressed fresh milk, as above, for comparison with the frozen sample, for three consecutive days. Milks in all cases were handled and transported in the same manner as in the fresh milk panels.

Frozen milks were warmed to 37°C after thawing, and were presented to each panelist along with fresh milks in the same manner as fresh milk panels.

Each panelist judged four samples per day. The milks were randomized over six judges and over days. Each judge received approximately 10 milliliters of sample.

On the first day (Day 1) of the frozen milk panel session, frozen milks were thawed by placing them in warm water until partially thawed; they were then shaken by hand to accelerate thawing and to homogenize. The milks were then transferred to coded glass beakers and placed in a water bath at 37°C. Since shaking the milk vigorously, appeared to result in milks that caused a severe gag reflex in the judges, care was taken on Day 2 and Day 3 to handle the frozen milks more gently.

Panelists were instructed to note and describe off-flavours and their intensities in the same manner as the previous large panel evaluations.

C. Power Function for Sweetness

In order to derive a power function for sweetness, panelists were instructed to assign ratios to a series of concentrations of sweet stimuli using magnitude estimation.

Varying concentrations of sucrose³ in tap-distilled water were used and were compared to a reference solution of six percent lactose⁴ solution (weight by volume in tap-distilled water). The reference was assigned a score of ten. The order of serving stimuli to the judges was randomized.

Linear regression analysis was used to determine the power function for the sweet stimuli.

VI. Instrumental Measurement of Viscosity of Human Milk

The Brookfield Synchro-Lectric L.V. Viscometer⁵ equipped with an ultra-low viscosity (U.L.) adaptor was used to estimate each sample viscosity. The U.L. adaptor allowed measurements to be made over the range of 0 - 100 centipoises on 18 milliliters of sample.

Samples of milk were taken from the 37°C water bath. The spindle and tube of the viscometer and the graduated

³ Sucrose crystal, lot 514251, Baker Analyzed Reagent, J.T. Baker Chemical Company, Phillipsburg, N.J., 08865

⁴ Lactose, lactose. Lot. 27C-0352, Crystalline, hydrate, Sigma Chemical Company. St. Louis, Mo., 63178. U.S.A.

⁵ Brookfield Synchro-Lective Viscometer L.V. Model with U.L. Adaptor, D.W. Brookfield Ltd. 1938 Mattawa Avenue, Cooksville, Ontario.

cylinder were brought to body temperature in warm water bath between each reading. A water bath was not connected to the viscometer during the readings. The readings were taken over four shear rates of 6, 12, 30, 60 rpm on each sample. It was necessary to gently tap each viscometer tube containing sample before taking the first reading to eliminate air bubbles which prevented the viscometer from attaining zero.

The sole criteria used to determine which milk samples should be read was sufficiency of sample, since 18 milliliters was required for an accurate reading.

Readings were converted to centipoises by multiplying by the appropriate factors (Table 7).

VII. Chemical Analysis of Human Milks

A. Lactose Content

Lactose content of each of 144 milk samples (24 mothers x 6) was analyzed enzymatically by the method of Kurz and Wallenfels (1974) using the reagent kit of Boehringer-Mannheim Chemical Company.⁶ This method employs the use of

⁶ Boehringer-Mannheim Canada Ltd., 11450 Cote de Liesse, Dorval, Que. H9P 1A9

Table 7
Factors Used to Convert Viscometer Readings
to Centipoises
(Brookfield Synchro-Lectric L.V. Viscometer, U.L.)

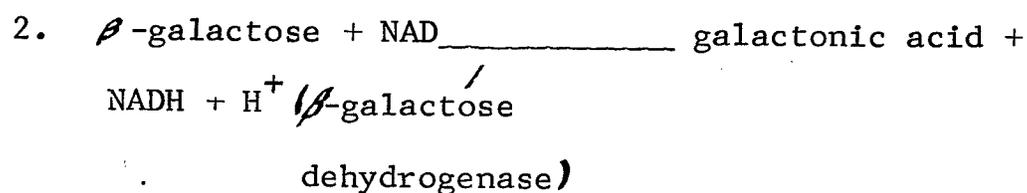
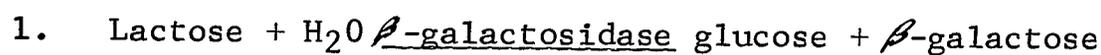
rpm	Range	Multiplying Factor to Yield Viscosity
60	0-10 cps	0.1*
30	0-20 cps	0.2**
12	0-50 cps	0.5
6	0-100 cps	1.0

* 0.4 was deducted from reading before multiplying to correct for windage.

** 0.1 was deducted from reading before multiplying to correct for windage.

(from: D.W. Brookfield Ltd., Cooksville, Ontario).

two enzyme preparations, β -galactosidase and β -galactose dehydrogenase. Lactose is hydrolyzed to glucose and β -galactose in the presence of β -galactosidase and water. β -galactose is oxidized by nicotinamide dinucleotide (NAD) to galactonic acid in the presence of β -galactose dehydrogenase. The amount of NADH formed is stoichiometric with the amount of lactose. The increase in NADH was measured spectrophotometrically at 365 nm on a Unicam SP800 Recording UV Spectrophotometer against water. The following equations represent the experimental reactions:



1. Preparation of Sample

Since the amount of lactose introduced into the cuvette must be between 5 μg and 50 μg , the sample of milk must be diluted sufficiently to obtain a lactose concentration between 0.05 and 1.00 grams per liter. Therefore, one milliliter of thawed, shaken milk sample was measured into a 100 milliliter beaker. Ten milliliters of glass distilled water was added. One half-milliliter of 3.0 Molar

trichloroacetic acid⁶ was added and deproteinization was allowed to proceed for ten minutes. The reaction was stopped by neutralizing with approximately 2.0 milliliters of one Molar sodium hydroxide.⁷ The preparation of these reagents is described in Table 8. Forty milliliters of distilled water was added to the sample and solution in the beaker. The solution was brought to neutrality (pH 7.0) by the use of the Corning pH meter⁸ and with sodium hydroxide and trichloroacetic acid. Solutions were poured into 100 milliliter volumetric flasks and brought to 100 milliliter final volume by addition of distilled water. The solution was filtered into an Erlenmeyer flask using a glass funnel and #2 filter paper.⁹ The slightly opalescent filtrate was used for enzyme assay.

2. Enzyme Assay

The enzyme analysis was performed directly in one

⁶ Trichloroacetic acid A.C.S., Fisher Scientific Co., Fairlawn, N.J.

⁷ Sodium hydroxide, reagent grade, Matheson, Coleman and Bell. Los Angeles, Calif.

⁸ pH meter, Corning Scientific Instruments.

⁹ Filter paper, Whatman Qualitative #2, Medium speed, W and R Bolston Ltd., England.

Table 8

Solutions Used in the Preparation of Human Milk

Sample for Assay of Lactose

Solution	Preparation
	MW = 163.39
3 Molar Trichloroacetic Acid	3 Molar Solution: 245.09 g 500 ml dist. water Trichloroacetic acid
	MW = 40
1 Molar Sodium Hydroxide	1 Molar solution: 20g/500 ml dist. H ₂ O w/v. NaOH

centimeter silica glass square cuvettes as described in Table 9. Kit reagents are described in Table 10. One blank per day was prepared and read. Samples were read against distilled water.

3. Calculations

Absorbance of the blank was determined by subtracting reading one from reading two ($E_2 - E_1$ Blank). Absorbance of the sample was determined in the same manner ($E_2 - E_1$ sample). The difference of these two values represents the change in optical density for the lactose sample. Sample calculations are found in Appendix L.

Samples of milk were analyzed in blocks by day, six samples at a time over three mothers. Random replications were made on 12 samples and experimental error was estimated at eight percent. Results were recorded as grams lactose (plus free galactose) per deciliter. Small amounts of galactose, free or hydrolyzed from oligosaccharides, could increase the analyzed lactose content. The spectrophotometer was not sensitive enough to measure these small amounts of galactose separately.

Table 9
 Procedure for Enzyme Analysis of Lactose Content
 of Human Milk

Reagents	Blank	Lactose Sample
Solution 1	0.20 ml	0.20 ml
Suspension 2	0.05	0.05
Sample Solution	-	0.10
Mix and let stand for 10 minutes at room temperature. Add:		
Solution 3	1.00 ml	1.00 ml
Distilled Water	2.00	1.90
Mix. After 2 minutes read O.D. (E1). Start reaction by addition of:		
Suspension 4	0.05 ml	0.05 ml
Mix. Wait 10 - 15 minutes until reaction is stopped. Read O.D. (E2).		

Table 10

Description of Reagents in Boehringer - Mannheim
Kit for Analysis of Lactose

Reagent	Contents
Solution 1	lyophilizate - citrate buffer (pH 6:6); NAD; magnesium sulfate; stabilizers.
Suspension 2	β -galactoside enzyme preparation
Solution 3	potassium diphosphate buffer, 0.51 mol/liter, pH 8.6; stabilizers.
Suspension 4	galactose dehydrogenase enzyme preparation

B. Fat Content

Total fat content of the 144 human milk samples was measured spectrophotometrically by the method of Nakai and Le (1971). This method was based on the principle of disassociation and solubilization of proteins and fat globules by the addition of strong acetic acid solution, followed with the development of fat turbidity by the addition of a solution of urea-imidazole. Fat turbidity was estimated at 400 nm.

1. Calibration Curve. Preparation of Standard Curve

Dairy coffee cream¹⁰ was purchased and was used to prepare a standard curve. The fat content of the cream was analyzed by the method of Bligh and Dyer (1959). Dilutions of the cream sample were prepared as described in Table 11. Fat concentration per dilution was calculated based upon the analyzed fat content of the initial cream sample (Appendix M). The method of Nakai and Le (1971) was used to estimate fat content of solutions of varying concentrations of cream. A graph was plotted from duplicates and a calibration curve

¹⁰ Coffee Cream. Modern Dairies, Winnipeg, Manitoba.

Table 11
Dilutions of Cream* Used in Preparation of Fat
Calibration Curve

Cream:	Addition of Distilled Water to Final Volume:	Dilution:
1. 0.5 ml	to 10 ml	1:20
2. 1.0	to 10 ml	1:10
3. 2.0	to 10 ml	1:5
4. 4.0	to 10 ml	1:2.5
5. 6.0	to 10 ml	1:1.66
6. 8.0	to 10 ml	1:1.25
7. 10.0	0 water added	1:0

* Cream, coffee, Modern Dairies, Winnipeg, Manitoba.

was drawn (Appendix N). Absorbances of analyzed milk samples were compared to the graph; a corresponding concentration of fat per deciliter was estimated.

2. Preparation of Milk Sample

Milk samples were thawed and homogenized by hand shaking. A sample of 0.05 milliliters was pipetted into a glass culture tube fitted with a teflon-lined plastic screw top. Five milliliters of 97 percent acetic acid¹¹ was added and the solution was mixed to solubilize the protein and fat. Two and one-half milliliters of urea-¹²imidazole solution¹³ (20 percent and 0.2 percent) was added. Reagent preparation is described in Table 12. The solution was mixed on a vortex mixer and allowed to stand for 30 minutes. Absorbance was measured at 400 nm on a SP6-300 Pye Unicam Spectrophotometer against a reagent blank. Milk samples were analyzed in blocks by

¹¹ Acetic acid, glacial, reagent grade, 99.8 percent. Fisher Scientific Company, Fairlawn, N.J.

¹² Urea, crystals. 'Baker Analyzed' reagent grade, Lot No. 39409. J. T. Baker Chemical Co., Phillipsbury, N.J.

¹³ Imidazole, 98 percent by titration, Eastman Kodak Company, Rochester, N.J.

Table 12
Preparation of Reagents for Analysis of Fat Content
of Human Milk

Solution	Preparation
97% acetic acid solution	97 ml of 99.9% acetic acid was brought to 100 ml final volume with distilled water in 100 ml graduated cylinder.
Urea- Imidazole Solution	100 g urea dissolved in distilled water and, 1.0 g imidazole dissolved in distilled water, both brought to final volume of 500 ml.

mother over three days of samples. That is, all the milks for one mother for each of three days (six samples) were analyzed and read at one time. Duplicate analyses were performed on each milk sample. An average of the two absorbance readings were recorded and used to estimate fat content.

C. Protein

Total protein content of each of 144 human milk samples (24 mothers x 6) was estimated by a modification of the method of Lowry et al. (1951). The method is based on a color reaction formed by (1) biuret reaction of protein with copper ion in alkalai and (2) reduction of the phosphomolybdic-phosphotungstic reagent (Folin-Ciocalteu Reagent) by tyrosine and tryptophan present in the treated protein. The final color produced was read spectrophotometrically on a SP-600 Series 2, Unicam Spectrophotometer, which had proved to be a more stable instrument for these readings. Preparation of reagents for this procedure are outlined in Table 13.

Table 13

Preparation of Reagents for Modified Folin - Ciocalteu
Method for Analysis of Protein Content of Human Milk

Reagent	Chemicals and Method
Reagent A	0.4 gm Sodium Potassium Tartrate ¹⁶ 20.0 g Sodium Carbonate ¹⁵ 100.0 ml - 0.5 N Sodium Hydroxide
Reagent B	0.4g Sodium Potassium Tartrate 0.2g Copper Sulfate ¹⁷ 10.0 ml - 0.1 N Sodium Hydroxide Crush copper sulfate crystals with mortar and pestle until a fine powder. Dissolve in NaOH. Add NaK Tartrate gradually while stirring. Stir until clear. Store in dark bottle under refrigeration
Reagent C	1 volume Folin-Ciocalteu Phenol Reagent 5 volumes distilled water Prepare prior to immediate use

15 Sodium Potassium Tartrate, Crystal, Lot. No. 705029,
Fisher Scientific Co. Fairlawn, N.J.

16 Sodium Carbonate, monohydrate crystal, Lot No. 39641,
J. T. Baker Chemical Co., Phillipsburg, N.J.

17 Copper Sulfate, cupric sulfate, Lot No. 31781, J.T.
Baker Chemical Co., Phillipsburg, N.J.

18 Folin - Ciocalteu Phenol Reagent: 2N. Lot. 782217
Fisher Scientific Co., Fairlawn, N.J.

Procedure for Protein Estimation

Bovine Serum Albumin (BSA)¹⁴ was used to prepare standard solutions. These were prepared from a frozen stock solution of 1 mg/ml BSA concentration. Each day, stock solution of BSA was thawed and diluted 10 times to a concentration of 0.1 mg/ml. Blanks and standards were prepared as outlined in Table 14. Frozen human milk samples were thawed, homogenized by hand shaking and diluted 200 times. That is, 9.95 ml of water was added to 0.05 ml of sample, then 1.0 ml of diluted sample was used for analysis. All samples of a single mother (6) were analyzed at one time. Reagents A, B and C were added to each test tube to prepare the blank, the standards and diluted milk samples, according to the plan outlined in Table 14. Duplicates were read and results averaged. Results obtained were recorded as grams of protein per deciliter.

14

Bovine Serum Albumin, crystals, lyophilized, No. A4378, Lot 46C8090, Sigma Chemical Co.

Table 14
 Procedure for Estimation of Protein Content
 of Human Milk

Reagent	Blank	Standards			Human Milk Sample (Diluted)
		1	2	3	
B.S.A. diluted stock (0.1 mg/ml)	-	0.2 ml	0.5 ml	1.0 ml	-
Water	1.0 ml	0.8	0.5	-	-
Milk (diluted)	-	-	-	-	1.0 ml
Reagent A	1.0 ml	1.0	1.0	1.0	1.0
Mix. Start stopwatch; add at 30 second intervals:					
Reagent B	0.1 ml	0.1	0.1	0.1	0.1
Mix on vortex mixer immediately. After 10 minutes (exactly) add:					
Reagent C (fresh)	3.0 ml	3.0	3.0	3.0	3.0
Exactly 20 minutes after adding Reagent B, read absorbance at 700 um. Zero instrument only once since absorbance increases in the blank over time.					

VIII. Analysis of Data

A. Normalization of Magnitude Estimation Data

Magnitude estimation data was normalized by dividing each panelist's estimation by the respective geometric mean of that panelist's estimations. Normalizing data in this manner minimizes the variability in panelists' scores that can occur because panelists are not restricted in their choice of limits of scale. The ratio properties of magnitude estimation generated values are retained in this normalization procedure.

B. Transformation of Normalized Data

Since magnitude estimation data are positively skewed, and since the data fit an exponential mathematical function, logarithmic (base 10) transformation is performed before the data is used in statistical analysis. The data becomes log-normally distributed.

Magnitude estimation data grow exponentially as a function of some physical parameter, according to the following power function equation:

$$S = kC^n$$

where S is sensory estimate, C is a physical parameter, n is the exponent (or the slope after log transformation) and k is a constant.

Transformation to logarithms as in the following equation, renders the relationship a linear one:

$$\log S = n \log C + \log k$$

All further statistical calculations for sensory data were performed on log data.

C. Statistical Treatment of Data

Data from all experiments was analyzed using factorial analysis of variance (ANOVA), from Statistical Analysis Systems (SAS), a computer-based program (Barr et al., 1976). Further testing of the means of significant effects was performed using Tukey's multiple comparisons test (Larmond, 1977). Simple linear regression and multiple regression were performed, again using SAS, relating sensory data to physical data or to maternal parameters.

R E S U L T S A N D D I S C U S S I O N

The results and discussion will be presented beginning with a description of the sample of nursing mothers, followed by presentation of findings related to sensory, instrumental and chemical analysis of milk samples. The significance of these findings and their interrelationships will be discussed. Human milk data from these experiments will be discussed in relationship to other sensory studies on infant foods.

I. Description of SampleA. By Size

Twenty-four nursing mothers in the City of Winnipeg participated in this study. Twenty-one mothers were nursing one infant. One mother was nursing twins and two mothers were nursing two infants each of different ages. All twenty-four mothers who began the study completed milk expression, two questionnaires (Appendix G and H), and three daily food records (Appendix J). The first questionnaire (Appendix G) provided demographic information

on the mother and her infant and on her nursing practices. The sample is described here. Information from the second questionnaire is discussed in sections IV, A and B.

B. By Age of Mother

The ages of the mothers ranged from twenty-five to thirty-five years of age. The mean age was twenty-nine years (Table 15). The narrow age range was probably not representative of all nursing mothers, but may have represented those mothers attending La Leche League meetings.

C. By Age of Infant

The ages of the infants at the time of milk expression, and therefore, the stage of lactation in weeks, ranged from eight to thirty-four weeks. Mean age was twenty-three weeks (Table 16). Human milk produced at eight to thirty-four weeks is considered mature milk (Hyttén, 1954a; e). The sample was restricted in infant age to ensure mature milk and to avoid milk of late lactation. Also, La Leche League does not recommend use of mothers with infants less than six weeks of age for participation in

Table 15
Description of Sample by Age of Mother
n = 24

<u>Years</u>	<u>Number</u>	<u>Percentage</u>
25	4	16.7%
26 - 30	14	58.3
31 - 35	6	<u>25.0</u>
		<u>100.0%</u>

Mean age = 28.9 years

Table 16
 Description of Sample by Age of Infant and Weeks
 of Lactation

n = 24

Age Weeks (Weeks of Lactation)	Number	Percentage	Cumulative
≤ 10	2	8.3%	8.3%
11 - 15	2	8.3	16.6
16 - 20	6	25.0	41.6
21 - 25	2	8.3	49.9
26 - 30	9	37.5	87.4
31 - 35	3	<u>12.5</u>	99.9
		<u>99.9%</u>	

Mean age = 23 weeks

projects requiring milk samples (F. Andrusiak, 1978, personal communication).

D. By Nursing Frequency per Twenty-Four Hour Day

Twenty-three mothers completed this section of the questionnaire. Some mothers reported a range of nursing frequencies. In these cases, the average reported frequency was used in data analysis (Table 17). The mean frequency per day reported was eight (7.96). The range was from five to twelve nursings per day. The frequency of nursings appeared to be related to nursing style discussed in E. In fact, when the correlation matrixes in multiple regression programs were examined (Section VIII, Results), nursing frequency and style were significantly intercorrelated. Many of the mothers were nursing on one breast only per feed and appeared to have increased their frequency per day because of this practice. The mother of twins nursed twelve times per day, one twin per breast. The time interval between nursings and the time the milk is in the breast has been reported to influence fat content of the milk (Gunther and Stanier, 1949; Hytten, 1954b; e). Further discussion of the effects of maternal

Table 17
 Description of Sample by Nursing Frequency per
 Twenty-Four Hour Day
 n = 23

Frequency (Times Per Day)	Number	Percentage	Cumulative
5	2	8.7%	8.7%
6	5	21.7	30.4
7	3	13.0	43.4
8	5	21.7	65.1
9	1	4.4	69.5
10	4	17.4	86.9
11	1	4.4	91.3
12	2	<u>8.7</u>	100.0
		<u>100.0%</u>	

Mean nursing frequency = 7.96

parameters upon the milks takes place in section VIII.

Many mothers also reported nursing more than one time throughout the night. In this study, the first morning feeding was chosen in an attempt to standardize the milk collection between the mothers, but, with the existence of varying time intervals since the previous feeding, some doubt exists as to the reliability of this standardization procedure. It may have been preferable to have chosen a feeding time that occurred within a set interval in hours from the previous feed. The mothers who practiced demand feeding may not have been able to accommodate the researcher. It is the practice of La Leche League not to interfere with the nursing process. The availability of taste panel members and the mechanics of milk collection also helped determine the use of early morning milks.

E. By Nursing Style

Ten mothers reported nursing on one breast only per feed. It was assumed that the other thirteen reporting mothers offered both breasts per feed.

F. By Parity

Ten mothers were primipera (having had their first infant) and fourteen were multipera (with from two to four infants). The number of lactations per mother agreed with parity; that is, no mother had previously given birth and not nursed the infant.

G. By Termination of Feeding

Mothers in this study appeared to be very aware of their infants' responses at the breast and allowed the infant to signal the end of the feed. Only two mothers indicated that they commonly terminated the feed for their own reasons. This section of the survey is of particular interest since Hall (1975a) hypothesized that a change in milk composition, flavour or texture could be leading to sensation of satiety in infant. The question was addressed to whether the infant could and did signal the end of feed, and whether the mother recognized and acted upon the signal. The type of cue used by the infant may have also depended upon developmental age (Table 18).

Table 18
Means by Which Mothers Recognized Infants Were
Ready to Terminate the Feed

Cue Described	Frequency Reported*
Stops sucking	3
Goes to sleep	9
Lifts head away	11
All of above (at certain times)	7
Other: (sometimes in combination with above):	
plays with breast	2
playful	1
crying	2
stiffens back	1
distracted by surroundings	2
lets go of breast	1
pushes mother away	1

* Mothers often reported more than one cue.

H. By Miscellaneous Factors

Three of the twenty-four mothers were cigarette smokers. Yeung (1979b) found that cigarette smoking had a negative effect upon breastfeeding; cigarette smokers were found to be less inclined to breastfeed, or breastfed for shorter time periods. Seventeen mothers drank coffee or tea, in varying frequency. Two mentioned decaffeinated coffee consumption. Medication usage was low. Three mothers reported regular usage during the three days of the experimental period. Only two other mothers mentioned medication usage one of the three experimental days. Medications cited were multivitamin with iron, insulin and an antihistamine.

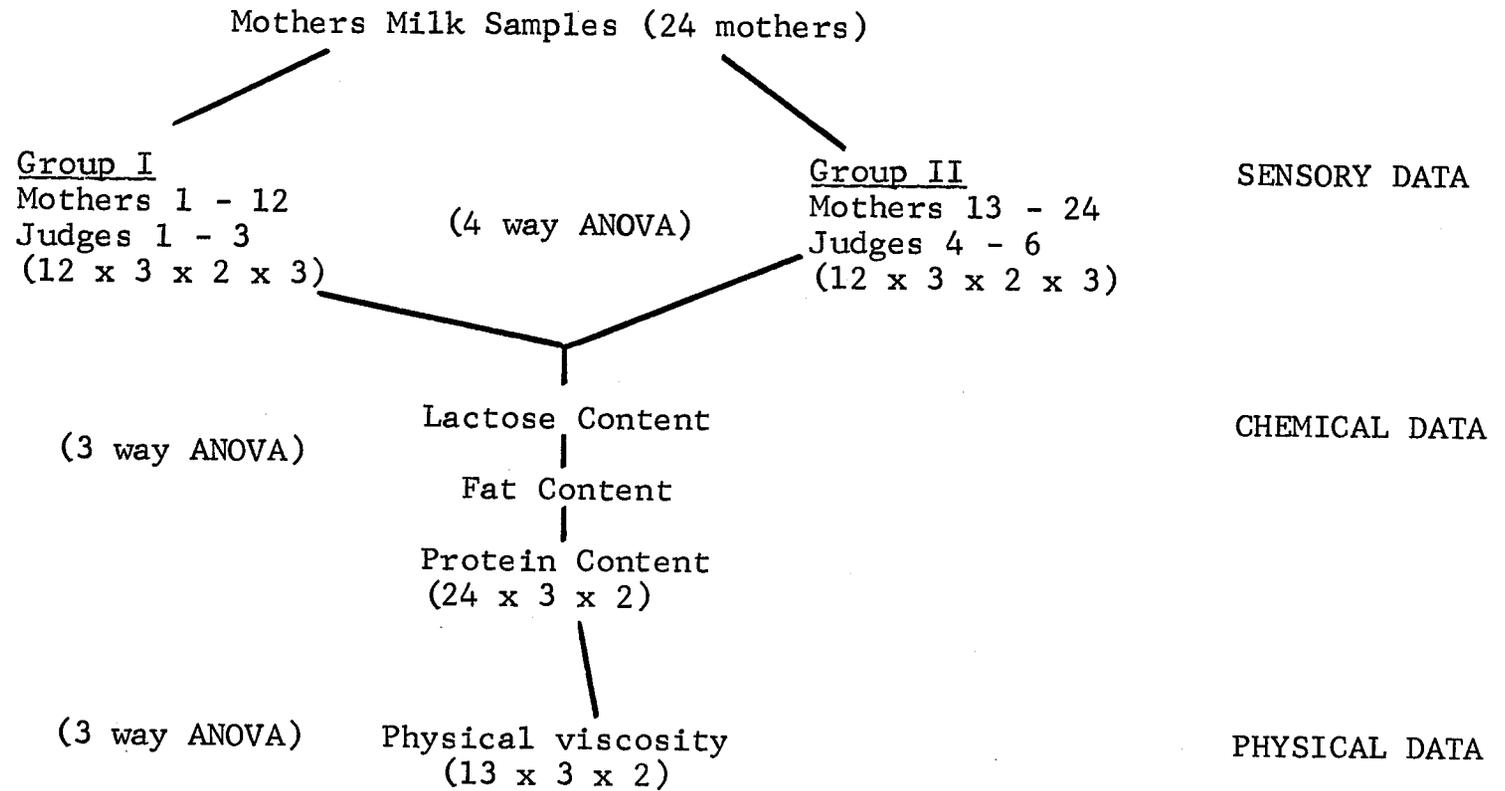
II. Discussion of Analysis of Data

A. Summary of Statistical Analyses Performed

To assist the reader, the manner in which the data from experiments on fresh human milk was handled is summarized in Figure 3. In all cases, a computer-based programme (SAS) of factorial analysis of variance was used. All testing was performed at the five percent level of significance.

Figure 3

An Overview of the Treatment of Data in Statistical Analysis



In all cases of factorial analysis of variance (ANOVA) in this study, it was not possible to estimate true error since the experiment could not be replicated. Mothers could not provide enough milk sample for replication of the experiment. Mothers' milks are subject to the variability that is usual in biological systems; also, the mothers could not act as their own controls in this experiment. Because true error could not be estimated, it was necessary in each procedure to assign a factor or an interaction term as the error term. In the sensory experiments, the judges and all interactions with the judges were delineated as the error term. The ANOVA model for analysis of data from sensory experiments is exhibited in Appendix O. In ANOVA of physical-chemical data - lactose, fat, protein content and physical viscosity, the second-order interaction term of mother x day x time was chosen as the error term. Days could not be considered replications because it was not known that days would not show a significant effect or that days would not interact significantly with another factor. The ANOVA model for physical data is in Appendix P. The numbers of judgements and/or observations included in the mean score of each

variable or interaction term are listed in Appendix Q and R, for ANOVA on sensory and physical data.

The computer-based ANOVA program of SAS was able to perform the analyses on single replications per cell and upon results with missing observations. Missing observations occurred when the mother was unable to supply sufficient sample for tasting by all three judges or for measurement on the viscometer for all three days of the study. Tukey's multiple comparisons test (Larmond, 1977) at five percent level of significance was performed upon means of significant effects to determine where the significant differences lay.

B. Statistical Analysis of Relationships Between Data

Linear regression was used to assess the relationship between sensory and physical data. The SAS computer-based programme called General Linear Model (GLM) was used. A multiple regression programme called "PROC CORR" in SAS was used to determine the effects of maternal age, stage of lactation, frequency of nursing, style of nursing and parity upon both sensory and physical data. A procedure of stepwise variable selection was employed. These

programmes were clearly outlined by Younger (1979).

III. Sensory Characteristics of Fresh Human Milk

Using a six-percent lactose solution as a reference, judges evaluated samples of mother's milk for sweetness. Judges One to Three evaluated three mothers' milks over a three-day span. On subsequent days they also tested nine other mothers' milks for a total of twelve mothers. A Factorial Analysis of variance (ANOVA) was performed on this data. Judges Four to Six judged twelve other mothers' milks in a similar design. A second factorial ANOVA was performed on this data. Table 19 summarizes the factors tested in ANOVA and their significances. Complete ANOVA tables are found in Appendix Si, ii for each group of mothers. The following hypotheses were tested:

There will be no differences between mothers in perceived sweetness estimates on samples of fresh milk.

The null hypothesis was accepted for means of mothers of Group I ($p \leq 0.068$, df 11, 138) and rejected in mothers of Group II ($p \leq 0.041$, df 11, 134). Table 20

Table 19
 Summary of Variable Effects in ANOVA of Sweetness
 Estimates of Fresh Human Milk

Source of Variation	Group of Mothers	
	Group I Mothers 1 - 12 p value	Group II Mothers 13 - 24 p value
Mother	(0.067)	0.041*
Day	0.000***	0.008**
Mother x Day	(0.784)	(0.875)
Time	(0.702)	(0.294)
Mother x Time	(0.560)	(0.814)
Day x Time	(0.328)	(0.437)
Mother x Day x Time	(0.769)	(0.996)

(p value) not significant

* Significant at $p \leq 0.05$.

** Significant at $p \leq 0.01$.

*** Significant at $p \leq 0.001$.

Table 20
Panel Magnitude Estimate Mean Scores for Sweetness
of Fresh Human Milk

Mother No.	Mean by ^x Mother	Mean by Day			Mean by Time	
		Day 1	2	3	Fore	Hind
1	0.938	1.077	1.058	0.724	1.059	0.830
2	0.991	1.302	1.209	0.618	0.926	1.060
3	1.248	1.268	1.277	1.200	1.072	1.566
4	1.211	1.316	1.277	1.056	1.345	1.090
5	1.087	1.728	0.861	0.864	1.220	0.986
6	1.119	1.205	1.217	0.925	1.158	1.041
7	1.014	1.293	1.065	0.753	1.109	1.079
8	0.909	0.835	1.105	0.815	0.851	0.971
9	0.996	1.216	1.065	0.762	0.993	0.998
10	0.866	1.089	0.838	0.712	0.810	0.926
11	0.975	1.083	1.199	0.752	0.919	1.052
12	0.781	0.834	0.899	0.643	0.793	0.769
Reference I	1.0632					
Overall	0.999	1.165 ^a	1.072 ^a	0.800 ^b	1.008	0.987
Means ± SD	+1.509*	+1.637	+1.403	+1.488	+1.519	+1.601
13	0.952	0.947	1.043	0.873	0.983	0.923
14	1.075	1.132	1.051	1.046	1.004	1.152
15	1.152	1.339	1.023	1.117	1.151	1.153
16	0.978	0.902	1.068	0.972	1.027	0.937
17	0.923	0.983	1.030	0.777	0.945	0.902
18	0.862	0.921	0.943	0.737	0.876	0.848
19	1.049	0.978	1.145	1.031	1.091	1.008
20	0.915	0.989	1.023	0.764	0.969	0.868
21	0.982	1.045	1.034	0.785	1.009	0.944
22	1.046	1.016	1.107	1.038	1.029	1.107
23	1.129	1.098	1.148	1.144	1.046	1.219
24	1.000	0.963	1.069	0.973	1.099	0.911
Reference II	0.699					
Overall	1.000	1.020 ^{ab}	1.052 ^a	0.931 ^b	1.016	0.984
Means ± SD	+1.289	+1.273	+1.284	+1.310	+1.274	+1.314

ab Means with the same superscript are not significantly different ($p \leq 0.05$).

x The number of judgements included in each variable mean is found in Appendix Q.

lists the panel magnitude estimation mean scores of sweetness by mother, by day, by fore and hind milk (time), as well as by mother - day and mother - time. As can be seen in this table, the standard deviations of all means were large, indicating a probable large variability between judges' scoring.

Tukey's multiple comparisons test (at five percent level of significance) was applied to the magnitude estimation mean scores of mothers' milks of Group II to see where the differences lay. Only the extreme values were significantly different from one another (Table 21). The mean reference value of 0.6994 was lower than all of the mothers' sweetness scores, significantly lower than nine mean scores. A ratio of $1.152/0.8620$, or 1.34, was necessary between Group II mothers' mean sweetness scores to achieve significance.

Although Tukey's procedure was not applied to mean sweetness scores of mothers in Group I because they were not significant at 5 percent level, it can be seen in Table 20, that four of the mean sweetness scores in Group I were sweeter than the mean reference score of 1.063. The range of scores for mothers' means of Group I was

Table 21

Tukey's Multiple Comparison Test on Mean Sweetness

Scores by Mothers of Fresh Human Milk

Group II Mothers, 13 - 24

Mother Number	Sweetness Estimate (Antilog Score)
15	1.152 a
23	1.129 ab
14	1.075 ab
19	1.049 ab
22	1.046 ab
24	1.000 ab
21	0.982 ab
16	0.978 ab
13	0.952 ab
17	0.923 abc
20	0.915 abc
18	0.862 bc
Reference: (6 percent lactose solution)	0.699 c

abc scores with the same superscripts are not significantly different ($p \neq 0.05$).

(least significant difference = $\sqrt{0.0332/18} \times 4.62 = 0.2430$)

1.211 to 0.781 or 1.55. Possibly, because of a larger error term in this analysis, these mean scores were not significant. Standard deviations in scores of Group I were larger than those of Group II.

There will be no difference in perceived sweetness of fore and hind milks.

As can be seen in Table 20, there was no effect of change over a single feed on the sweetness of mother's milk ($p = 0.702$, $df 1$, 11 for Group I; $p = 0.294$, $df 1$, 11 for Group II). The null hypothesis was accepted.

There will be no difference in perceived sweetness of the milks over three consecutive days.

The null hypothesis was rejected in both analyses. In both groups of mothers and judges, a significant day effect occurred ($p = .00001$, $df 2$, 22 Group I, and $p = .008$, $df 2$, 22 Group II). Tukey's procedure was applied to mean sweetness scores by day in both Groups I and II of mothers. In Group I, Day 3 was significantly less sweet than Days 1 and 2. In Group II, Day 3 was significantly less sweet than Day 2 but not from Day 1. The downward trend in sweetness is obvious in most mothers' mean scores for sweetness by day in Table 20. The reason

for this decline in sweetness by day was unknown, and would not have been expected because there was no significant change in lactose content or fat content over the three days. Perhaps, the judges were becoming accustomed to the sweetness in the milks and were, therefore, judging sweetness lower on the third day of panels, even though judges continued to use the six-percent lactose reference throughout. Also, since temperature constancy of the small samples had been difficult to maintain, a change in temperature of the samples could have influenced judges' perceptions.

There were no significant first-order or second-order interactions in either groups of data.

B. Estimates of Sweetness Intensity of Human Milks Expressed as an Equivalent Concentration of Sucrose

Linear regression analysis was applied to the data from trained panel magnitude estimation of varying concentrations of sucrose in water. The slope of the resulting regression line or the exponent in the power function, $S = kC^n$ was 1.16 ($S = 0.3090 C^{1.16}$).

The mean magnitude estimates of sweetness of human

milks were expressed as concentrations of sucrose in water by use of the sweetness power function.

First, the value of the lactose reference used in the power function taste panel expressed as a sucrose concentration was determined. Judges gave the lactose reference (six percent lactose w/v water) used in the sucrose power function a mean logscore of -0.1951 (0.6381 antilog value).

The following calculations show how the equivalent sucrose concentration for lactose was determined:

The power function formula is $y = ax^b$ or $\log y = \log a + b \log x$, and therefore, $\log x = \frac{\log y - \log a}{b}$

To solve for $\log x = \frac{-0.1951 - (-.5100)}{1.16} = 0.2715$

Antilog of 0.2715 is 1.8684, which represents the percent sucrose concentration equivalent in sweetness to perceived magnitude estimate of six percent lactose reference. The magnitude estimate of lactose reference is

$$y = 0.3090 (1.8684)^{1.16} \text{ or } 0.6381.$$

To determine the equivalent sucrose concentrations of the mean sweetness estimates of each mothers' milks, the following calculations were made:

A ratio (R) of the mean milk sweetness score to the mean reference score of that group of judges (that is, the mean reference score for sweetness of Judges One to Three tasting milks of mothers of Group I) was determined in each case. This ratio (R) was then multiplied by y, (0.6381) the magnitude estimate of six-percent lactose solution reference in the power function test. The product of (R) (y) = y' , the magnitude estimate for each milk.

The percent sucrose concentration (x) was determined by solving for x in the following formula:

$$x = \frac{y' - a}{b} \text{ or } \log x = \frac{\log y' - \log a}{b}$$

Therefore, antilog of x is the percent sucrose concentration that represents the sweetness estimate of the milk. A sample of these calculations for one mothers' milks is shown in Appendix T.

Table 22 lists the trained panel estimates of perceived sweetness in selected mothers' milks expressed as log, antilog, and concentration of sucrose in water. The equivalent sweetness range in Group I milks was 1.43 to 2.15 percent sucrose and the mean of that group was 1.77 percent. The equivalent sucrose concentrations of

Table 22

Trained Panel Estimation of Sweetness in Selected
Human Milk Samples (Highest and Lowest)
Expressed as a Concentration of Sucrose in Water

Mother No.	Mean Sweetness Estimate		Equivalent Sucrose Concentration Percent
	Log Value	Antilog Value	
3 (highest)	0.0961	1.2477	2.15
12 (lowest)	-0.1075	0.7807	1.43
Mean Group 1	-0.0002	0.9995	1.77
15 (highest)	0.0615	1.1521	2.87
18 (lowest)	-0.0645	0.8620	2.24
Mean Group 2	0.0000	1.0000	2.54
Overall Mean (24 Mothers)	-0.0001	0.9997	2.12

perceived sweetness of milks of Group II mothers ranged from 2.24 percent to 2.87 percent with a mean of 2.54 percent. Overall mean magnitude estimate for milks of twenty-four mothers was 0.9997 (antilog value) which was equivalent in sweetness to a 2.12 percent sucrose solution.

These values are illustrated on the sucrose power function in Figure 4.

C. Sensory Evaluation of Viscosity of Human Milk

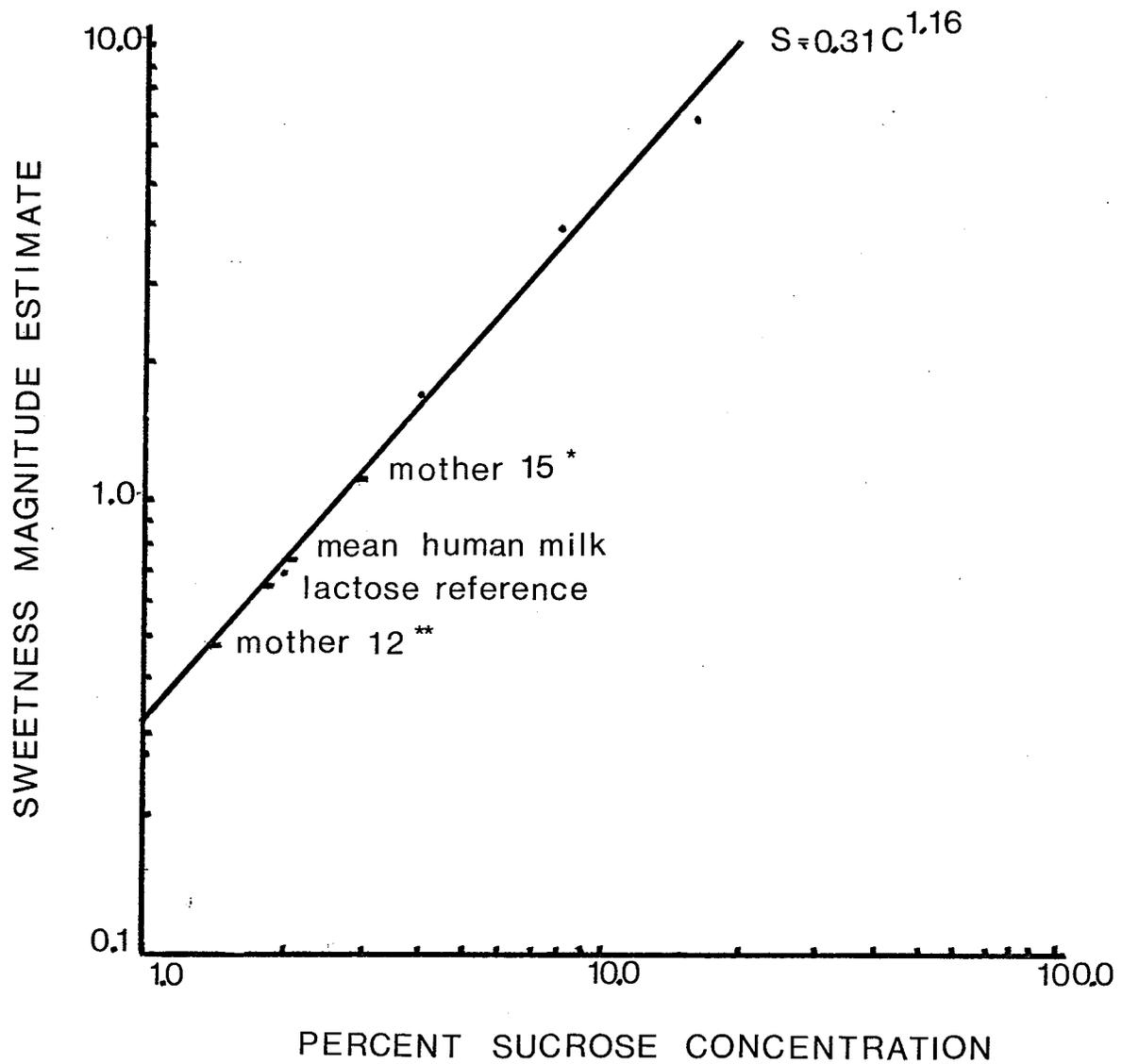
Judges evaluated samples of fore and hind milks of twenty-four mothers over three consecutive days for perceived viscosity according to the experimental plan described in Section V, A, 1 Methods. The diluted Similac reference was assigned a value of 10; samples of milks were compared to the reference by the ratio scaling technique of magnitude estimation. Table 23 summarizes the factors tested by ANOVA and their significances. Appendix Ui, ii contains the complete ANOVA tables for analysis of data from each group of mothers.

The following hypothesis were tested:

There will be no differences between mothers in perceived viscosity estimates of samples of fresh milk.

Figure 4

SWEETNESS POWER FUNCTION AS DETERMINED BY
 TRAINED SENSORY PANEL AND SWEETNESS
 PERCEIVED IN SELECTED HUMAN MILKS
 EXPRESSED AS CONCENTRATIONS OF SUCROSE



* sweetest

** least sweet

Table 23
 Summary of Variable Effects in ANOVA of Viscosity
 Estimates of Fresh Human Milk

Source of Variation	Group of Mothers	
	Group I Mothers 1 - 12 p Value	Group II Mothers 13 - 24 p Value
Mother	0.000 ^{***}	(0.348)
Day	(0.945)	(0.803)
Mother x Day	(0.584)	(0.234)
Time	0.043 [*]	0.009 ^{**}
Mother x Time	(0.741)	(0.693)
Day x Time	(0.734)	(0.770)
Mother x Day x Time	(0.203)	(0.285)

(p value) not significant

* significant at $p \leq 0.05$

** significant at $p \leq 0.01$

*** significant at $p \leq 0.001$

The null hypothesis was rejected for mean viscosities in mothers' milks of Group I ($p = 0.0001$, df 11, 138) but not for those mothers in Group II ($p = 0.348$, df 11, 134). Table 24 contains the panel magnitude estimation mean scores of viscosity by mother, by day, by fore and hind milk (time) and by mother - day, and by mother - time. The range of perceived viscosity mean scores by mother in Group I was 1.394 to 0.719 or a ratio of 1.939. That is, milks of Mother Two with the highest mean viscosity score were judged to be twice as viscous as the milks of Mother Four, with the lowest mean score. By referring to Table 25, containing the results of Tukey's test ($p \leq 0.05$) for Group I mothers, it can be seen that a ratio of 1.394/0.881, or 1.582, was necessary to achieve significant differences between mothers' mean viscosity scores. Eleven of twelve mothers' milks were judged as less viscous than that of the reference, five significantly so. The range of mean scores by mother in Group II was 1.090/0.905, or a ratio of only 1.204. This was not sufficient to lend significance to the viscosities of the milk of these mothers. Most mothers' mean scores were close in value to the reference, with a normalized mean value of

Table 24
Panel Magnitude Estimate Mean Scores for Sensory
Viscosity of Fresh Human Milk

Mother No.	Mean by Mother*	Mean by Day			Mean by Time	
		Day 1	2	3	Fore	Hind
1	1.248	1.202	1.255	1.289	1.242	1.254
2	1.394	1.150	1.489	1.581	1.422	1.361
3	0.856	0.805	0.722	1.055	0.816	0.919
4	0.719	0.775	0.626	0.767	0.702	0.737
5	0.770	0.717	0.725	0.880	0.647	0.916
6	0.881	1.009	0.679	1.022	0.928	0.831
7	1.069	0.976	1.217	1.027	1.013	1.128
8	0.958	0.987	0.985	0.904	0.868	1.058
9	0.861	0.941	0.835	0.813	0.828	0.896
10	1.207	1.424	1.209	1.022	1.094	1.333
11	1.105	1.169	1.159	1.014	1.175	1.021
12	1.066	1.056	1.291	0.887	0.922	1.232
Reference I	1.349					
Overall \bar{X}	0.9964	0.997	0.983	1.002	0.9478	1.045
+ SD	+1.505	+1.437	+1.588	+1.506	+1.521	+1.489
13	1.066	1.026	1.132	1.043	1.037	1.096
14	1.090	1.075	1.091	1.105	1.091	1.089
15	1.090	1.058	1.100	1.112	1.022	1.162
16	0.9649	0.930	0.964	1.002	0.883	1.055
17	0.966	1.005	0.882	1.018	0.882	1.059
18	0.928	0.959	0.818	1.019	0.895	0.963
19	1.015	1.030	0.946	1.075	1.045	0.906
20	0.999	1.042	0.877	1.093	0.977	1.023
21	0.983	0.978	0.953	1.058	0.938	1.056
22	1.029	1.160	1.048	0.932	1.051	0.956
23	1.034	1.005	1.134	0.972	0.990	1.081
24	0.905	1.060	1.052	0.664	0.787	1.040
Reference II	0.9956					
Overall Mean	1.006	1.020	0.991	0.999	0.9607	1.0520
+ SD	+1.255	+1.180	+1.250	+1.378	+1.351	+1.159

* The number of judgements included in each variable mean is found in Appendix Q.

Table 25

Tukey's Multiple Comparisons Test on Mean Viscosity

Scores by Mothers of Fresh Human Milk

Group I Mothers, 1 - 24

Mother Number	Viscosity Estimates (Antilog)
2	1.394 a
1	1.248 ab
10	1.207 abc
11	1.105 abcd
7	1.069 abcde
12	1.066 abcde
8	0.958 abcde
6	0.881 bcde
9	0.861 bcde
3	0.856 bcde
5	0.770 de
4	0.719 e
Reference: (diluted Similac)	1.349 a

abc scores with the same superscripts are not significantly different ($p \leq 0.05$).

(least significant difference = $\sqrt{0.0260/18} \times 4.62 = 0.1756$)

0.996. Seven mothers were perceived as more viscous and five as slightly less viscous, than the reference, but none were significantly so.

There will be no difference in perceived viscosity estimates of fore and hind milks.

In both groups of mothers, the null hypothesis was rejected ($p = 0.043$, $df 1, 11$ for Group I and $p = 0.009$, $df 1, 11$ for Group II). In Group I, overall mean viscosity value for hind milk was 1.045 and for fore milk was 0.948, whereas in Group II, hind milk mean viscosity value was 1.052 and fore milk, 0.961. Therefore, hind milk was judged to be more viscous than fore milk, but only slightly so, 1.10 and 1.09 fold in both groups I and II, respectively. Nine of twelve mothers in both Group I and II had hind milks judged to be more viscous than fore milks, but in most cases, the differences were very small. There were smaller differences found between hind and fore milks, 1.10 fold, than were found among mothers - 1.939 fold in Group I.

There will be no difference in perceived viscosity estimates of the milks over three consecutive days.

The null hypothesis was accepted in both groups of

mothers ($p = 0.945$, $df = 2, 22$, Group I; $p = 0.803$, $df = 2, 22$ in Group II). Mean viscosity scores appeared to be very consistent from day to day except for Mothers Six, Ten and Twenty-four.

In all of the data, large standard deviations occurred reflecting large judge variability in response to oral viscosity. The standard deviations of mean viscosity scores for Judges Four to Six were consistently lower than those for Judges One to Three (see Table 24). These Judges, Four to Six, then appeared to be more in agreement in their scoring. Inconsistencies in data of the two groups of mothers, illustrate the difficulties present in the sensory perception of viscosity. Another possibility was that some cue or cues other than changes in viscosity were stimulating the judges' responses. Malcolmson (1978) found that judges perceived changes in viscosity at differing rates when evaluating plant protein model systems of increasing solids concentration. Some panelists were more sensitive to differences than others. Some of the judges variability in this data could have been due to variability in each judges' perception of differences in viscosity.

A recurring problem throughout statistical analysis of all of the sensory data has been in the original experimental design where, due to limited milk samples, the experiments could not be replicated. Therefore, true error could not be estimated. Also, since time was a fixed variable (fore versus hind), it (E_{ms}) was tested against $E(ms)$ of mother x time interactions. When this interaction term was small, time effect became significant. If time had been considered a random effect and had been tested against error (which included all judge interactions), its effect would not have been significant in the sensory viscosity data. Because judge effect and all interactions with judges had been selected as the error term in this data analysis, these judge-variable interactions could not be tested. It is possible that such interactions would have affected interpretation of the significant main effect of time (fore versus hind) in this data.

D. Mouthcoat

Judges evaluated fore and hind milks of twenty-four mothers over three consecutive days as described in the

experimental plan, Methods V, A, 1. Factorial analysis of variance was applied to the data in the same manner as in viscosity and sweetness evaluations. Table 26 summarizes the factors tested and their significance. The complete ANOVA tables for Group I and II analyses are in Appendix Vi and ii.

The following hypotheses were tested:

There will be no differences between mothers in perceived mouthcoat of fresh milk samples.

The null hypothesis was rejected in Group I mothers ($p = 0.0001$, df 11, 138) and was accepted in Group II mothers ($p = 0.234$, df 11, 134). Table 27 contains the panel magnitude estimation mean scores by mother, by day and by time as well as by mother - day and mother - time. Significant differences were found in mean perceived mouthcoat of mothers' milks in Group I. There was a range of 1.354 to 0.759, or a ratio of 1.784, in panel magnitude estimation mean scores per mother. A ratio of $1.354/0.872$ or 1.553, was necessary to achieve significance between mothers in Group I (Tukey's multiple comparisons test, $p \leq 0.05$) (Table 28). Milks of eleven of twelve mothers in Group I were judged as less mouthcoating than

Table 26

Summary of Variable Effects in ANOVA of Mouthcoat

Estimates of Fresh Human Milk

Source of Variation	Group of Mothers	
	Group I Mothers 1 - 12 p value	Group II Mothers 13 - 24 p value
Mother	0.000 ^{***}	(0.234)
Day	(0.584)	(0.264)
Mother x Day	0.028 [*]	(0.338)
Time	0.015 [*]	(0.504)
Mother x Time	(0.789)	(0.401)
Day x Time	(0.667)	(0.357)
Mother x Day x Time	(0.199)	(0.734)

(p value) not significant

* significant at $p \leq 0.05$

** significant at $p \leq 0.01$

*** significant at $p \leq 0.001$

Panel Magnitude Estimate Mean Scores for Sensory
Mouthcoat of Fresh Human Milk

Mother No.	Mean by Mother*	Mean by Day			Mean by Time	
		Day 1	2	3	Fore	Hind
1	1.180	1.140	1.207	1.194	1.219	1.142
2	1.354	1.132	1.487	1.520	1.317	1.421
3	0.759	0.633	0.596	1.148	0.671	0.915
4	0.783	0.962	0.687	0.726	0.781	0.785
5	0.813	1.074	0.706	0.710	0.693	0.955
6	0.872	0.956	0.968	0.728	0.890	0.852
7	0.903	0.857	1.175	1.349	0.982	1.248
8	0.948	0.905	0.780	1.206	0.880	1.021
9	0.932	0.909	0.804	1.108	0.815	1.066
10	1.233	1.175	1.241	1.283	1.242	1.223
11	1.129	1.448	1.059	0.968	1.087	1.186
12	1.021	1.094	1.047	0.929	1.031	1.011
Reference I	1.288					
Mean	1.0001	1.000	0.956	1.039	0.9451	1.0583
± SD	±1.499	±1.434	±1.578	±1.505	±1.5639	±1.4305
13	1.022	0.801	1.163	1.155	1.025	1.019
14	0.863	1.186	0.944	0.806	0.743	1.002
15	1.118	0.974	1.265	1.135	1.190	1.051
16	1.068	1.003	1.154	1.052	1.096	1.041
17	0.992	0.928	1.056	0.995	0.981	1.002
18	0.943	0.933	0.954	0.943	1.027	0.866
19	1.059	1.076	0.989	1.116	1.072	1.047
20	1.035	1.266	0.873	1.003	1.029	1.041
21	0.984	1.021	0.901	1.090	0.954	1.030
22	0.998	0.832	1.114	1.084	0.968	1.114
23	1.035	0.935	1.159	1.022	0.948	1.130
24	0.931	0.929	1.018	0.854	0.911	0.951
Reference II	1.0088					
Mean	1.0022	0.960	1.038	1.010	0.9897	1.0157
± SD	±1.2989	±1.345	±1.263	±1.280	±1.3038	±1.2935

* The number of judgements included in each variable mean is found in Appendix Q.

the reference; four were significantly less so. Mean mouthcoat scores of mothers' milks of Group II were not judged to be significantly different from one another. The range of panel magnitude estimation scores was from 1.118 to 0.863, or a ratio of only 1.3. Judges evaluated these mothers' milks to be very similar to the reference in mouthcoat with six mothers' milks judged to be slightly more mouthcoating and six less, than the reference.

These results can be compared to those of Malcolmson and McDaniel's (1980b) work on the sensory evaluation of textural characteristics of infant formulae. They found a range of 1.58 to 0.44 in panel magnitude estimation mean scores for evaluation of mouthcoat in thirteen different infant formulae. This represented a 3.6 fold difference. They also found a 2 fold difference between home-prepared cow's milk formula recommended for a 1 - 2 week infant and that recommended for a 5 - 6 month infant. This was a significant difference. Since 1 - 2 week formula was cow's milk diluted 2:1 with water (with sugar added) and 5 - 6 month formula was cow's milk with no changes, the difference in mouthcoat of these two formulae

Table 28

Tukey's Multiple Comparisons Test on Mean Mouthcoat

Scores by Mother of Fresh Human Milk

Group I Mothers, 1 - 12

Mother Number	Mouthcoat Estimates (Antilog)
2	1.354 a
10	1.233 ab
1	1.179 abc
11	1.129 abcd
7	1.107 abcde
12	1.021 abcde
8	0.948 abcde
9	0.932 abcde
6	0.872 bcde
5	0.813 cde
4	0.783 de
3	0.759 e
Reference: (diluted Similac)	1.288 a

abc scores with the same superscripts are not significantly different ($p \leq 0.05$).

(least significant difference = $\sqrt{0.0241/18} \times 4.62 = 0.1692$)

could approximate the differences found between mothers in this study, with a ratio of 1.78 over the entire range of mouthcoat scores. One might have expected, therefore, based on Malcolmson and McDaniels' experience with infant formulae, that the differences in panel magnitude estimation mean scores per mother would be very small. Further discussion of human milk in relation to the study on infant formulae can be found in Section IX, Results, page 215.

There will be no differences between perceived mouthcoat estimates of fore and hind milks.

The null hypothesis was rejected in mothers of Group I ($p = 0.015$, $df 1, 11$) but accepted in mothers of Group II ($p = 0.504$, $df 1, 11$). Hind milks in mothers of Group I were judged to be significantly more mouthcoating than fore milks, however, a ratio of only $1.058/0.945$ or 1.12 , was found between hind and fore milks in Group I mothers. Eight of the twelve mothers had mean mouthcoat scores for hind milk that were greater than those for fore milk, but of these eight mothers, the degree of change from fore to hind milk varied. That is, Mother Three had a 1.36 fold change, whereas Mother Eleven showed only a 1.09 fold change.

In mothers of Group II, no significant difference was found between mouthcoats of hind and fore milks. The panel magnitude estimation mean scores were 1.016/0.990 or 1.03 fold, a negligible difference.

There will be no differences in perceived mouthcoat estimates of the milks over three consecutive days.

The null hypothesis was accepted in both groups of mothers ($p = 0.584$, $df 2, 22$ in Group I and $p = 0.264$, $df 2, 22$ in Group II). In Group I mothers there was a significant mother by day interaction ($p = 0.028$, $df 22, 138$). This means that mean mouthcoat estimates over the three days were not consistent for each mother. This effect can be seen in Table 27 where there is variation in judgements for many mothers over the three days. For example, Mother Three increased over three days in mouthcoat estimates, whereas Mother Five decreased. More consistent scores can be seen in mouthcoat estimates over three days in Group II mothers, where no significant mother by day interaction occurred.

The result of analysis of mouthcoat data can be compared to the results of analysis of viscosity data. In both groups of data, Judges One to Three evaluating

Group I mothers, found highly significant differences between mothers' mean estimates, but Judges Four to Six failed to find any significant differences in milks of Group II mothers. No day effects were found in either set of data, but a difference did exist in the effect of time (fore versus hind milk) in the two sets of data, viscosity and mouthcoat. Although judges found significant differences between fore and hind milk in both groups of mothers for sensory viscosity, only Judges One to Three, evaluating Group I mothers, found significant differences between fore and hind milk for mouthcoat. A comparison of Tukey's tests on mean estimates of mothers' milks for viscosity (Table 25) and for mouthcoat (Table 28) for Group I mothers in both cases, revealed that the same mothers' milks were rated as more viscous and as more mouthcoating (Mother One, Two and Ten) and, also as less so (Mothers Three, Four and Five). Milks of Mother Two were found to be more viscous and more mouthcoating than the reference. Because these two groups of data were in agreement, it appears that perception of viscosity and mouthcoat as sensory parameters were being influenced by similar factors in the milks. Also, judges were performing well

to identify these differences in both sets of data.

IV. Description of Off-Flavours in Human Milk and Discussion of Food Practices of Sample During Lactation

A. Off-Flavours in Fresh Human Milk

During panel evaluation of fresh milk samples, judges were asked to note the presence of any off-flavours, to describe them with words of each panelists' choice and to indicate the intensity of off-flavour by choosing one of five intensity descriptors (Appendix K).

A variety of off-flavours were noted and described. A possible total of four hundred and thirty-two (432) judgements were made upon all fresh milks; that is, eighteen judgements per mother (6 milks x 3 judges) and over twenty-four mothers. There were eighteen cases of missing observations, leaving four hundred and fourteen (414) actual judgements made.

Table 29 contains the descriptors used and the frequency of occurrence of panelists' descriptions. In thirty percent (30.4%) of the judgements on the samples, some off-flavour citation occurred.

Table 29

Descriptors Used by Panelists to Describe Fresh Human Milk Off-Flavours

Descriptor	Number of Times Noted	Range of Intensities	Possible Cause of Off-Flavour
metallic	29	detectable - moderate	
tinny	2	slight, moderate	oxidized
fruity	25	detectable - strong (2)	
candy apples	1	strong	microbial;
musty	2	slight	transmitted
earthy	1	slight	
sour	11	detectable - moderate	
old, rotten	1	moderate	lipolyzed
soapy	1	slight	
off	1	moderate	
cooked milk	3	moderate	
ice cream, vanilla	11	detectable - strong (1)	heat-induced
sulfur	1	strong	
caramel	6	detectable, slight	
milky	2	moderate	
coconut	6	slight - strong (1)	transmitted
salty	5	detectable - moderate	
hot, spicy, peppery	10	slight, moderate	miscellaneous
limey	4	slight	
perfumy	1	slight	
peppermint	1	slight	
beany	1	slight	
astringent	1	slight	
Totals	126/414 (30.4 percent)		

Shipe et al. (1978) classified off-flavours in cow's milk based on causative factors. They used a classification system of seven categories and listed some descriptive terms usually associated with these causative factors (Table 30). The off-flavours used by panelists in this study of human milk were also organized into groups based on possible causes of off-flavour (Table 29). These groupings were chosen by the experimenter. It was very difficult for the judges to describe the off-flavours and was also difficult for the researcher to associate them with causes. Possible sources of off-flavours in expressed human milk are lipolyzed flavours, oxidized flavours, heated flavours, microbial off-flavours and transmitted flavours from the mothers diet or environment. Each possible cause will be discussed briefly and will be related to the descriptors used by panelists in this study.

Lipolysis, as the result of action of lipases present in raw milk, could free fatty acids which of themselves could lead to off-flavours or which could form soaps with the cations, sodium and potassium. In human milk, the fatty acids released would not be the low molecular weight ones, such as butyric acid (C4:0), typical of cow's

Table 30

*Categories of Off-Flavour in Bovine Milk

<u>Causes</u>	<u>Descriptive Terms</u>
Heated	Cooked, caramelized, scorched
Light-induced	Sunlight
Lipolyzed	Rancid, bitter, butyric
Microbial	Acid, bitter, fruity, malty, unclean
Oxidized	Papery, cardboard, metallic
Transmitted	Feed, weed, cowy
Miscellaneous	Astringent, bitter, chalky, salty

*(from Shipe et al, 1978).

milk. These short-chain fatty acids are virtually absent from human milk (Jensen et al., 1978) and the first fatty acid in more than trace amount is capric acid (C10:0) reported at 1.3 percent of total fatty acids (Jensen et al., 1978). A bitter, rancid or soapy off-flavour in fresh expressed breast milk could reflect lipolysis. Only three descriptions used by panelists in this study seemed to reflect rancidity in the milks: "soapy" and "off", "old, rotten". As will be seen in further discussion of frozen breast milk off-flavours, lipolyzed off-flavours are more likely to occur during freezing.

Oxidized flavours are possible in expressed breast milk if the milks are left to stand uncovered. A "metallic" or "tinny" description could represent oxidized flavours. Thirty-one of the 126 off-flavour citations in fresh human milk were "metallic" or "tinny". Milks in this study were evaluated within four hours of expression and were left uncovered only at the last half hour when they were heated for panel evaluation. Metallic flavour has also been described as a response resulting from a combination of low concentrations of sour and salty stimuli (Amerine et al., 1965). Also, one judge in this study noted the lactose

reference solution as metallic. The sweet taste in human milk may not be as "pure" as the sweetness of sucrose.

Heated off-flavours could have resulted in the milks from reheating of cooled milks to 37°C. Such descriptors as "cooked milk", "caramel", "sulfur" and "vanilla" used by the panelists may represent heated off-flavours.

Although these terms occurred in 21 of 126 off-flavour citations, they represented only 21/414 total judgements or 5 percent of all samples. If handling of milks in this study had been an important causal factor in off-flavour development, one would have expected to have seen a higher incidence of all off-flavours lipolyzed, oxidized and heat-induced across all samples.

"Fruity" and "musty" descriptors are associated by Shipe and coworkers (1978) with microbial off-flavours. "Fruity" and associated terms were commonly used in these panels: 40 of 126 citations, yet one might expect if they were related to the release of off-flavours by microbial enzyme systems, that, frozen milk samples would also have had a high incidence of similar terms. This was not the case (Table 35). "Fruity" could also represent the flavour of aldehydes, alcohols and ketones (Shipe et al., 1978) and these may have been transmitted from the mother's diet.

Transmitted food flavours from the mother's diet would be extremely difficult to identify in this study. No restrictions were placed upon mothers' food selection prior to milk collection and food volatiles, although leading to off-flavours, may not be recognizable. Off-flavours in cow's milk due to onion were not found to be of a typical onion-like flavour (Dougherty et al., 1962).

This part of the sensory evaluation of human milk was difficult for the judges to perform. The judges received 10 milliliter samples of milk and were asked to evaluate three sensory attributes as well as note off-flavours and their intensities. During panel training, the off-flavours were discussed, but the choice of descriptors was left open to the judges. Judges performed with variability in their abilities to detect off-flavours, their reporting of them and/or their use of particular descriptive terms. It was difficult to know if the mothers' milks were more intense in off-flavour or whether the judge was more sensitive. To demonstrate judge variability, the number of citations per judge were noted. Table 31 contains a summary of the number of times each judge noted an off-flavour over the 72 judgements he/she made. Judges Two,

Table 31

Frequency of Citations of Off-Flavours in Human

Milk by Judge and by Day

(Total Citations on 414j = 126)

Judge	Day			Total	Frequency %
	1	2	3		
1	3	3	3	9	7
2	15	11	10	36	28.5
3	2	15	16	33	26
4	11	11	12	34	27
5	1	4	5	10	8
6	1	-	3	4	3
Totals				126	99.5

Three and Four reported 81 percent of all citations, whereas Judges One, Five and Six reported only 18 percent. Judge Six, in particular, seldom noted an off-flavour (4 of 126 or 3 percent of total cited). This judge was a cigarette smoker. Judges Three and Five cited more often on Days 2 and 3 of the experimental period. Among the 126 off-flavour citations, there was some agreement between judges and some consistency over the same judge when describing a particular mother but no regular pattern emerged. In fact, agreement was the exception. In Tables 32 and 33, data from six mothers are featured. Samples from these mothers had a higher number of citations of off-flavour than those of other mothers and/or reflected more judge consistency, yet the variability is still evident. The mean number of descriptors per mother over all judgements was 5.2 ± 2.9 . Judges One to Three noted an average of 6.4 ± 1.8 per mother (1 - 12) and Judges Four to Six noted an average of 4.0 ± 3.3 off-flavour citations per mother. The total number possible per mother was 18.

Since one of the objectives of the study was to relate maternal diet to mothers' milk flavour, mothers with a higher frequency of off-flavour citations (seven or

Table 32

Off-Flavour in Fresh Human Milk:
 Four Frequently Cited Mothers (Judges 1 - 3),
 Descriptors Used by Judges Over Three Days

Mother Code (no)	Judge	Day 1	Day 2	Day 3	Totals*
229 fore (1)	1	-	-	-	4
	2	coconut	coconut- cooked milk	-	
	3	-	metallic	-	
229 hind (1)	1	-	-	-	3
	2	coconut	coconut- cooked milk	-	
	3	-	metallic	-	
381 fore (7)	1	-	-	-	3
	2	fruity	-	fruity	
	3	-	-	salty	
381 hind (7)	1	-	ice cream	-	6
	2	nutty- coconut	fruity	-	
	3	musty	peppermint	sour	
278 fore (10)	1	-	sour	-	7
	2	sour	fruity	fruity	
	3	metallic	vanilla ice cream	fruity	
278 hind (10)	1	sour	-	-	3
	2	-	-	-	
	3	-	sour	sharp-sour	
868 fore (2)	1	-	-	coconut	6
	2	salty	cooked, coconut	sulfur	
	3	-	metallic	metallic	
868 hind (2)	1	-	-	-	1
	2	coconut	-	-	
	3	-	-	-	

* Total number of off-flavour citations per mother possible = 18; fore = 9; hind = 9.

Table 33

Off-Flavour in Fresh Human Milk:
 Two Frequently Cited Mothers (Judges 4 - 6),
 Descriptors Used By Judges Over Three Days

Mother Code (No)	Judge	Day 1	Day 2	Day 3	Totals*
933 fore (13)	4	sour, tinny	metallic	metallic, sour	5
	5	-	-	old, rotten	
	6	-	-	astrigent	
933 hind (13)	4	sour, tinny	metallic	metallic, sour	5
	5	-	sour	vanilla	
	6	-	-	-	
185 fore (18)	4	hot, peppery	peppery	hot, peppery	6
	5	-	limey - perfumy	limey - perfumy	
	6	-	-	caramel	
185 hind (18)	4	hot, spicy	peppery	hot, peppery	5
	5	-	perfumy	-	
	6	-	-	-	

* Possible total number of notations of off-flavour per mother = 18; by fore or hind = 9.

more) were selected for further attention. Their three-day food records were reviewed and an attempt made to relate particular foods to off-flavour occurrence. Mothers Two and Eighteen (Table 32) reported eating salty and spicy diets, yet this assessment was not quantified. Mothers had been asked to note the intensity of flavours and spices in their diets on the food records, but evaluation and reporting was entirely subjective. It is possible that off-flavours in mothers' milk due to diet are the result of a combination of flavours acting in an additive mode (Parks, 1965).

Three mothers in this sample were cigarette smokers. Two of these mothers (One and Thirteen) had a greater frequency of off-flavour noted in their milks (7 and 10 citations, respectively), yet were lighter smokers (3 - 7 cigarettes per day) than Mother Six who reported smoking 20 cigarettes per day. Mother Six had only five off-flavour citations on her milks. The descriptors used for Mother One (Table 32) were coconut (four times) and metallic (three times) and for Mother Thirteen (Table 33) were sour, metallic, tinny (7 times), old, rotten, astringent, and vanilla (each once). Mother Six, although not listed

in a table, had descriptors of fruity (four) and sour (once). Many of these citations could be resulting from oxidized off-flavours. Ferguson et al. (1976), in measuring nicotine in breast milks, found that the amount of nicotine in the milk did not correlate strongly with either the timing or the amount of smoking. It is possible that other flavour compounds than nicotine resulting from smoking, were influencing the off-flavours of the milks.

The milks of two mothers in the study deserve particular attention when the relationship between diet and milk flavour is being considered: Mother Two (Table 32) and Mother Eighteen (Table 33). In Mother Two, although off-flavours were noted only seven out of eighteen judgements, the descriptors of salty, metallic and coconut were used. This mother had consumed a noticeably salty diet with ham and salted nuts appearing at supper each of the three days preceding milk expression. In Mother Eighteen, fore and hind milks were noted by Judge Four on all three days of evaluation as "hot, peppery, spicy" and by Judge Five as "limey, perfumey, soapy". This mother had recorded eating three spicy meals including such foods as soya sauce, barbeque sauce, bouillon and garlic, as well as taking a

multivitamin with iron preparation each day. Yet, even though the potential for transfer of spices into the milk did exist in the spiciness of the maternal diet, it was not possible for this researcher to make more than an observation of the descriptors, incidences and intensities of each mothers' milks. Added difficulties in relating maternal diet to mothers' milk flavours may have arisen because the time interval between eating and milk expression was sometimes long - up to 10 hours. Any flavours entering the milks from foods eaten may have had time to be excreted. When volatile compounds become lower in concentration in the blood, because of their excretion through lungs or kidneys, an exchange could take place between the milk and blood at the blood-mammary epithelium barrier. Therefore, compounds would be removed from the milk and excreted.

A further difficulty in relating milk off-flavour to maternal diet may have been that mothers in this study were avoiding flavourful foods; therefore, it was of interest to determine whether these mothers were practicing food avoidances. Of the twenty-four mothers responding to the second questionnaire (Appendix H), thirteen (54 percent) practiced food avoidances during lactation. Table 34 contains a list of particular foods

Table 34

Specific Foods Avoided by Mothers During Lactation

n = 13

Food Cited	Number
Chocolate	3
Milk products	2
Egg	1
Fruit	2
Alcohol	1
"Gassy" vegetables	10
"Spicy" foods	5

mentioned by the mothers as avoided and their frequency. These foods are in agreement with those cited by Sims (1978) and Snow and Johnson (1978). It appears that those foods avoided with the greatest frequency are those that could possibly affect milk flavour - "gassy vegetables" and "spicy foods". Seventy-four percent of the mothers in Sims' study (1978) practiced food avoidances during lactation.

B. Off-Flavours in Frozen Human Milk

Two mothers participated in this part of the study. Six panelists evaluated a fresh and frozen milk sample from two mothers over three consecutive days. There were, therefore, thirty-six possible citations of off-flavours on fresh and frozen milk. Table 35 contains the descriptors and intensities, as well as the incidence of reporting off-flavours in the fresh versus the frozen milks. In seventy-five percent of the judgements made upon frozen milks, an off-flavour was noted, compared to thirty-six percent in fresh milk samples. This incidence in fresh milk is comparable to the thirty percent off-flavours noted in fresh milk samples of the larger part of the study. The intensities noted were stronger in frozen milk.

Table 35

Descriptors Used by Panelists to Describe Frozen and Fresh Milk Off-Flavours

Descriptor	Frozen Milk		Fresh Milk	
	No.	Intensity	No.	Intensity
Waxy	2	2 extreme	2	strong
Sour	1	1 slight	1	detectable
Soapy	5	1 strong, 1 slight 3 moderate	3	slight
Evaporated Milk-like 'Regurgitated'	- 1	- 1 moderate	1 -	moderate -
Cooked milk	1	slight	-	-
Peppery	-	-	1	slight
Metallic	12	5 strong, 2 moderate 4 slight, 1 detectable	1	slight
Cardboardy	4	2 extreme, 1 moderate 1 strong	1	extreme
Limey	-	-	1	slight
Astringent	1	slight	-	-
Creamy	-	-	2	slight
Totals	27		13	
Frequency		27/36 = 75 percent		13/36 = 36 percent

It may be that normal or off-flavours present in fresh milk intensify during frozen storage. Those compounds implicated in the characteristic milk flavour have been reported in higher concentrations in off-flavours in bovine milk (Parks, 1967). Descriptors used by panelists in evaluating frozen and fresh milks (Table 35), received a more intense rating in frozen milks. 'Waxy', 'sour', 'soapy', 'metallic' and 'cardboardy' were either more frequently cited or given a more intense rating in frozen milks. According to Shipe et al. (1978) classification, 'cardboardy' and 'metallic' could be associated with oxidized off-flavour. 'Soapy' and 'Waxy' descriptors could represent lipolyzed off-flavours in frozen milks. 'Regurgitated' was also noted by one judge. 'Sickening' was a type of lipolyzed flavour reported by Shipe et al. (1978). This off-flavour was the result of intense agitation of raw milks. Sodium salts of capric and lauric acids were reported to impart a soapy taste to milk (Shipe et al., 1978). Long-chain fatty acids were reported to contribute little flavour. Human milk has been reported to contain capric (C10:0, 1.3 percent) and lauric (C12:0, 3.1 percent) acids (Jensen et al., 1978).

The manner in which the milks were handled on the first day of panels appeared to create a strong response in the panelists. The milks had been shaken by hand under warm water to thaw and homogenize. This treatment was likely responsible for the response of "regurgitated (moderate)" by one judge. Because this treatment evoked such strongly aversive responses in some judges, the milks were thawed more gently on days 2 and 3 of the frozen milk taste panels.

Mothers were asked in the second questionnaire about their practices of freezing expressed breast milk. Of twenty-four mothers answering the survey, twenty-two (92 percent) froze expressed breast milk. Eighteen (75 percent) fed frozen milk to their babies. Of these eighteen, eleven babies appeared to accept expressed breast milk, yet seven mothers reported that their infant had rejected expressed breast milk. Mothers had usually attributed this rejection to the rubber nipple of the bottle, not to breast milk flavour (F. Andrusiak, La Lache League, personal communication).

Nine of the twenty-two mothers freezing milk had tasted their own milk. They were asked to describe the flavour. Table 36 contains descriptors used by these

Table 36
Descriptors Used by Mothers Who Had Tasted Their Own
Milk to Describe Milk Flavour

n = 9

Descriptor	Number
Soapy	4
Sweet	1
Less sweet	1
Same as fresh	1
Oily - sweet	1
'Tin-foil'	1
'Off' - sour	1

mothers. They were untrained in taste description and had not been informed of the descriptors by the researcher. Four of the nine mothers did not report any off-flavours. Of the nine mothers who had tasted their own milks, five reported that their babies rejected the milk. This represents fifty-five percent (5/9) rejecting breast milk, whereas in the total sample, thirty-nine percent (7/18) of the mothers had reported their babies rejecting expressed breast milk. A mothers' own perception of off-flavour could have been affecting her interpretation of her infant's response.

A variety of methods and holding times have been reported for frozen human milk. Mothers were asked to describe their freezing and thawing practices. Table 37 contains a summary of handling practices. It appears that the majority of mothers in this sample, froze milk soon after expressing (0 - 2 hours), and held milks four weeks or less in frozen storage. Thawing techniques varied widely, sometimes depending on the quantity of milk to be thawed.

Because of human milk's anti-infective properties, there has been renewed interest in many hospitals in banking

Table 37

Description of Sample by Practices of Freezing and
Thawing Human Milk
(n = 22)*

<u>Type of Freezer Used:</u>	
freezing compartment refrigerator (one door)	4
freezing compartment refrigerator (two door)	11
deep freeze	10
<u>Length of Freezing Period:</u>	
1 - 7 days	5
1 - 4 weeks	14
>1 month	5
<u>Length of Storage Time Before Freezing:</u>	
0 hours (direct to freezer)	10
1 - 2 hours	4
3 - 4 hours	4
> 4 hours	3
Varies	2
<u>Thawing Techniques Used:</u>	
in warmed water	11
in microwave: 10 - 20 sec.	1
60 sec.	2
in refrigerator: (6 hours)	4
(1 hour)	2
at room temperature (2 - 3 hours)	2
under running water (cool)	3
shaken (often)	1
(once to mix)	9

* Some mothers reported more than one time.

of human milk for use with high risk infants (Welsh and May, 1979), or with those infants whose mothers are unable to supply human milk. In some milk banks, non-heat treated human milk is frozen in an attempt to preserve as many antimicrobial factors as possible (Hernandez et al., 1979). Lipases would be active in these milks, yet no comment was made on the development of off-flavours or of rejection of the milk by the infant. Based on these brief experiments, it would seem advisable to recommend gentle handling of home frozen, non-heat treated human milk during thawing to prevent the development of intense off-flavours.

V. Physical Viscosity of Fresh Human Milk

Only thirteen mothers provided sufficient sample of both fore and hind milks for measurement on the viscometer. The Brookfield Synchro-Lectric L.V. viscometer, with ultra-low viscosity adapter, requires eighteen milliliters of sample for a correct reading. On at least one day, the other eleven mothers in this experiment provided less than this amount. ANOVA was performed on the data, using those values obtained at 30 rpm. The readings at 30 rpm

Table 38

Summary of Variable Effects in ANOVA of Instrumental
Viscosity of Fresh Human Milk

Source of Variation	p Value
Mother	0.000 ^{***}
Day	(0.736)
Mother x Day	0.046 [*]
Time	0.002 ^{**}
Mother x Time	0.001 ^{***}
Day x Time	0.048 [*]

Second-order interaction term (mother x day x time)
was chosen as error term to be used for testing
(p value) not significant

- * Significant at $p \leq 0.05$.
- ** Significant at $p \leq 0.01$.
- *** Significant at $p \leq 0.001$.

Table 39

Mean Instrumental Viscosity Estimates of Fresh Human
Milk in Centipoises *

Mother No.	Mean by Mother **	Mean by Day			Mean by Time	
		1	2	3	Fore	Hind
1	1.47	1.48	1.41	1.53	1.29	1.65
2	1.44	1.39	1.36	1.53	1.29	1.67
4	1.47	1.44	1.58	1.40	1.32	1.63
5	1.41	1.44	1.41	1.39	1.38	1.45
7	1.63	1.66	1.74	1.50	1.52	1.75
10	1.48	1.58	1.41	1.42	1.42	1.57
12	1.40	1.47	1.42	1.30	1.39	1.41
13	1.32	1.28	1.29	1.38	1.28	1.35
14	1.51	-	1.52	1.51	1.50	1.53
16	1.39	1.39	1.34	1.44	1.33	1.45
19	1.31	1.28	1.34	1.31	1.30	1.32
20	1.32	1.38	1.29	1.28	1.26	1.37
24	1.37	1.38	1.41	1.28	1.34	1.40
Overall Means ± SD	1.42 ±0.13	1.43 ±0.18	1.42 ±0.16	1.41 ±0.13	1.35 ±0.095	1.50 ±0.165

* Readings were performed on a Brookfield LV viscometer, UL adaptor, at 37°C at 30 rpm.

** Number of observations included in each variable mean is found in Appendix R.

were chosen for three reasons: (i) according to Shama and Sherman (1973), this shear rate approximated that present in the human mouth; (ii) higher shear rates of 60 rpm were reported to cause turbulence which artificially increased readings (Parkinson and Sherman, 1971) and (iii) during preliminary testing, the readings at 30 rpm were found to be those most reproducible.

Table 38 lists those factors tested in ANOVA and their significances. A complete ANOVA table is in Appendix W. Again, using results at 30 rpm, means by mother, by day and by fore and hind (time) as well as means by mother - day and mother - time are reported (Table 39). Tukey's multiple comparison test at five percent level of significance was used to determine where significant differences lay. The following hypotheses were tested:

There will be no difference in mean physical viscosity values of milks of different mothers.

The null hypothesis was rejected ($p = 0.0001$, df 12, 19). Mean physical viscosity of milks per mother were found to be significantly different from one another. The range of mean physical viscosities per mother was 1.633 centipoises to 1.308 centipoises, or a ratio of 1.25.

By applying Tukey's test (Table 40), many significant differences were found over the range of mean physical viscosities per mother. Because the error term used in data analysis (mother x day x time interaction) was small, only a small centipoise difference was necessary to achieve significance. A ratio of 1.633/1.480 or 1.10 lent significance to mothers' mean physical viscosities.

The results of analysis of data from physical viscosity and from sensory evaluation of viscosity can be compared. For both sensory viscosity for Group I mothers and for physical viscosity of the thirteen mothers tested, significant differences were found between mean values per mother. By comparing Table 25 and Table 40, each containing Tukey's tests on these two groups of data, a similar number of significant differences can be seen, however, only seven of the thirteen mothers whose milks were measured instrumentally were also mothers of Group I in sensory viscosity data where significant sensory differences were found. The mothers whose milks had higher physical viscosity values were not necessarily those which judges had perceived as more viscous. These significant differences are summarized in Table 41 for

Table 40

Tukey's Multiple Comparisons Test on Mean Physical
Viscosity Estimates by Mother of Fresh Human Milk
(at 37°C and at 30 rpm)

Mother	Mean Value Centipoise
7	1.633 a
14	1.513 ab
10	1.480 bc
4	1.473 bcd
1	1.473 bcd
2	1.440 bcde
5	1.413 bcde
12	1.396 bcde
16	1.390 bcde
24	1.370 bcde
20	1.317 e
13	1.317 e
9	1.308 e
Reference	1.58 ab

abc scores with same superscripts are not significantly different ($p \leq 0.05$).

(least significant difference = $\sqrt{0.0049/6} \cdot 5.32 = 0.1528$)

Table 41

Comparison of Tukey's Multiple Comparisons Tests on Two
 Sets of Data: Physical Viscosity and Sensory
 Viscosity, for Mothers in Common

Mother No.	Significance in Physical Data	Significance in Sensory Data
7	a	abcde
10	bc	abc
4	bcd	e
1	bcd	ab
2	bcde	a
5	bcde	de
9	e	bcde

abc mothers with means with same superscripts are not significantly different ($p \leq 0.05$).

the mothers in common in both sensory and physical viscosity data. Mother Seven, with the highest physical viscosity value, was not perceived as significantly different in sensory viscosity by Judges One to Three from any of the other mothers in Group I. Mother Nine, of the lowest physical viscosity value, was perceived by Judges One to Three as significantly lower in sensory viscosity than only three other mothers, Two, One and Ten. Mother Two, with the highest sensory viscosity mean score was not found to be significantly different from the mother receiving the lowest physical viscosity value, but was, in fact, significantly lower in physical viscosity than Mother Seven, with the highest physical viscosity score. Because of these results, significant correlation between physical viscosity and sensory viscosity would not be expected in this experiment.

The physical viscosity of the diluted Similac reference was also measured at 37^o Celsius. Resulting viscosity at 30 rpm was 1.58 centipoises. In the physical viscosity data, one mothers' mean physical viscosity value was higher than the reference; the other eleven mothers were lower in physical viscosity than the reference,

three significantly so. In sensory viscosity data, similar results were obtained, yet, only Mother Nine was significantly less viscous than the reference in both groups of data.

Based on the results discussed, it seems that the instrumental measurement of viscosity was more sensitive to small differences in viscosity than were panelists. With reported power functions of 0.4 (Moskowitz, 1972) where $S = kC^n$ and $n = 0.4$ for viscosity, a range of 1.633/1.308 instrumentally would not be expected to lead to large sensory viscosity differences. In fact,

$$\frac{(1.633)^{0.4}}{1.308} = 1.09 \text{ fold change expected which}$$

represents a very small predicted response in judges. The two-fold difference found by Judges One to Three evaluating mothers in Group I, therefore seems large. It is possible that significant sensory differences should not have been found over such a narrow range of physical viscosities.

There will be no difference between fore and hind milks in physical viscosity.

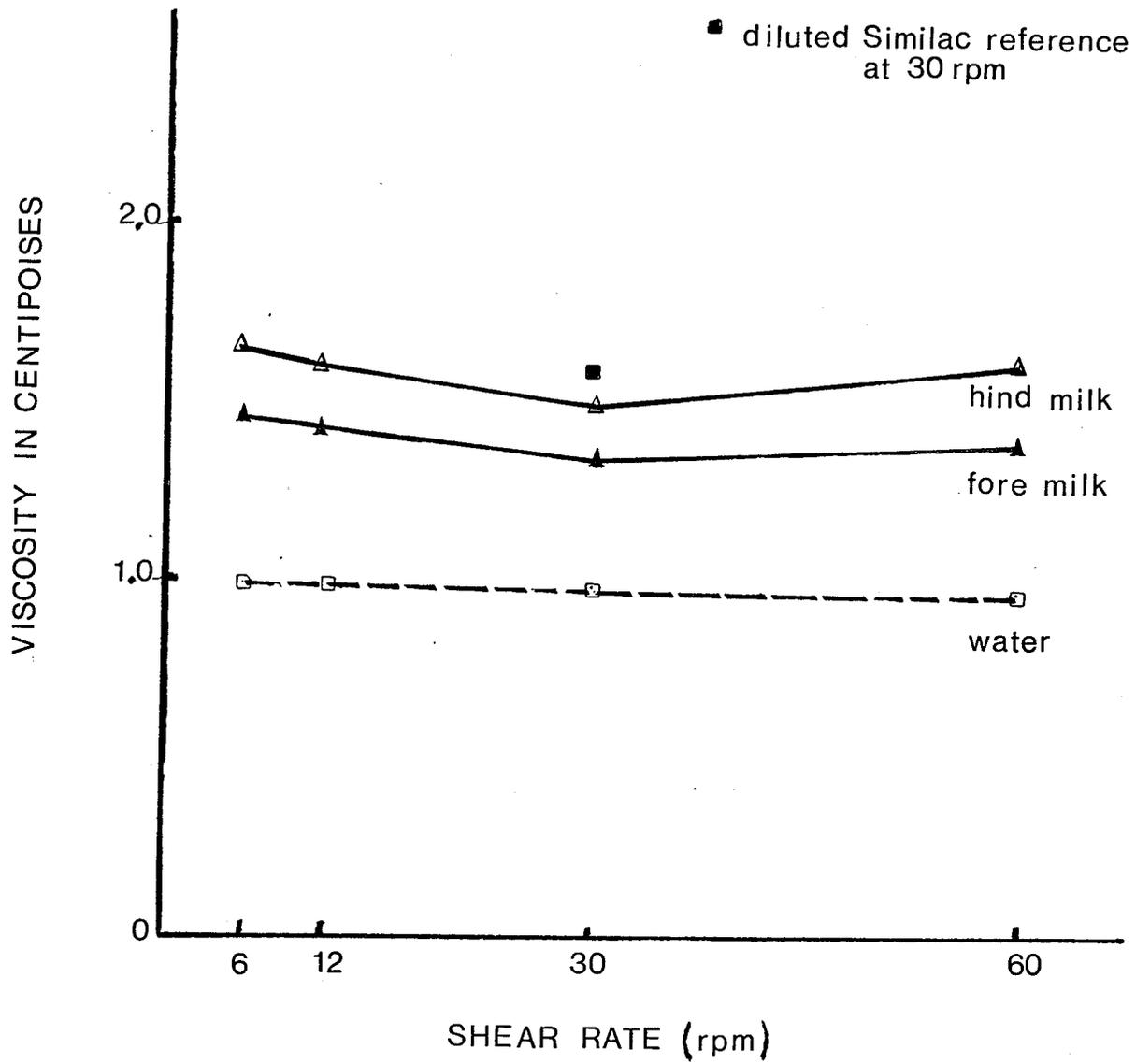
The null hypothesis was rejected ($p = 0.002$, $df = 1, 12$). Hind milk was found to be higher in physical viscosity than

fore milk. Mean viscosity measured for hind milk was 1.499 centipoises and for fore milk was 1.351 centipoises. This was a 1.11 fold change from fore to hind milk on an average of all samples. Hind milk mean values were higher than fore milks in all thirteen mothers, but some differences were small. For example, Mothers One and Two had a 1.3 fold change while Mothers Five and Sixteen had a 1.05 fold change. In Mothers Twelve, Fourteen and Nineteen, change was negligible.

The values of 1.35 centipoises and 1.50 centipoises (cp) for human fore and hind milk respectively, and overall mean of 1.42 ± 0.13 cp, can be compared with values reported for cow's milk (Bateman and Sharp, 1928). Using a Bingham viscometer at 25° Celsius, Bateman and Sharp measured fresh, raw skim and whole cow's milks. They reported values of 1.43 centipoises for skim milk and 1.52 - 1.58 cp for whole raw milk, as well as 1.78 cp for colostrum. They also found that milk was not a true Newtonian fluid but decreased slightly in viscosity with increasing shear stress. The same effect can be seen in human milk data (Figure 5). Over varying shear rates of 6, 12, 30, 60 rpm, the viscosities of human milk decreased

Figure 5

MEAN PHYSICAL VISCOSITIES OF HUMAN FORE
AND HIND MILKS AT 37° C



slightly. The slight rise in viscosity at 60 rpm could have been due to the turbulence described by Parkinson and Sherman (1971). Bateman and Sharp (1928) also demonstrated the difficulty in obtaining measurement on raw milk especially at low shear rates due to separation of cream during the test. This latter problem would be a function of time, more likely to confound readings in a capillary-type viscometer than in a rotational viscometer of the Brookfield Synchro-Lectric model. Nevertheless, it was felt that readings at 30 rpm proved the most reliable.

There will be no difference in mean instrumental viscosities over three consecutive days.

The null hypothesis was accepted ($p = 0.736$, df 2, 23). As in sensory viscosity, days had no significant effect upon the physical viscosity of the milks.

There were many significant first-order interactions between factors tested in ANOVA. Mother - time interaction ($p = 0.002$, df 12, 19); mother - day interaction ($p = 0.046$, df 23, 19), and day - time interaction ($p = 0.048$, df 2, 19) were all significant. These interactions may have been the result of the difficulty of performing

viscosity measurements on this very thin liquid and the difficulty of maintaining accurate temperature conditions during the readings. The decision was made to control the temperature in the manner described in Methods VI, p. 73, instead of using a water bath connected to the viscometer, because of time limitations before panel sessions when prolonged holding of fresh milk would have resulted in deterioration of the sample for tasting purposes. Interaction between factors can also mean that mothers' milks did not change in the same manner for each mother over days or over time (fore and hind). That is, some mothers may have had a greater change from fore to hind or over days than others, and other mothers may have had a negligible change in viscosity. That is, in fact, the variable nature of human milk. The data can still be regarded with confidence when two of the first-order interactions were significant at only $p = 0.048$ and $p = 0.046$. The highly significant mother - time interaction ($p = 0.001$) reflects the varying degree of change in physical viscosity, fore to hind, between different mothers, yet, hind was always more viscous than fore milk. The main effects of mother ($p = 0.000$) and time ($p = 0.002$) can still be

regarded as significant even with the presence of these interactions.

Further discussions on factors affecting physical viscosity of human milks can be found in Section VII, VIII of Results.

VI. Chemical Analysis of Human Milk

A. Lactose Content

Lactose content was analyzed enzymatically and the appearance of NADH measured spectrophotometrically on a UV. Spectrophotometer at 365 nm. By appropriate calculations, results were obtained in grams lactose (plus free galactose) per deciliter. These results are reported in Appendix X.

Means of lactose content by mother, by mother - day, by mother - time, by day and by time are listed in Table 43. Results were analyzed statistically by 3 way factorial analysis of variance using second-order interactions (M x D x T) as the error term. The complete ANOVA table is reported in Appendix Y. Table 42 lists the factors tested and their significances.

The following hypotheses were tested:

Table 42
 Summary of Variable Effects in ANOVA of Lactose
 Content of Human Milk

Source of Variation	p Value
Mother	0.000 ^{***}
Day	(0.228)
Mother x Day	0.000 ^{***}
Time	0.006 ^{**}
Mother x Time	(0.605)
Day x Time	(0)
Mother x Day x Time [*]	

* Second-order interaction (mother x day x time) chosen as error term.

Statistical model for calculating F values in Appendix P
 (p value) not significant

* Significant at $p \leq 0.05$.

** Significant at $p \leq 0.01$.

*** Significant at $p \leq 0.001$.

Table 43
 Mean Lactose (Plus Free Galactose) Contents of
 Human Milks
 (Grams per 100 ml)

Mother No.	Mean by Mother*	Mean by Day			Mean by Time	
		Day 1	2	3	Fore	Hind
1	8.35	7.58	8.39	9.09	8.80	7.89
2	8.76	7.56	8.66	10.05	9.13	8.38
3	8.20	7.48	8.18	8.94	8.44	7.95
4	7.57	7.39	7.43	7.89	7.62	7.52
5	7.63	7.46	7.50	7.94	7.79	7.48
6	7.16	6.93	7.04	7.51	7.42	6.90
7	7.03	7.23	6.73	7.13	7.18	6.89
8	7.49	7.69	7.60	7.19	7.60	7.39
9	7.07	7.50	6.71	7.03	6.99	7.17
10	7.92	7.89	7.98	7.91	8.25	7.60
11	7.71	7.63	7.89	7.61	7.73	7.69
12	7.44	7.74	7.36	7.21	7.39	7.49
13	8.38	8.28	8.34	8.52	8.37	8.38
14	8.10	7.63	8.09	8.59	7.97	8.22
15	8.34	8.41	8.40	8.23	8.32	8.36
16	8.27	7.52	8.17	9.12	8.32	8.22
17	8.37	7.61	8.24	9.27	8.29	8.45
18	8.18	8.66	7.76	8.14	8.33	8.04
19	7.65	8.08	7.31	7.56	7.63	7.66
20	7.11	7.16	7.10	7.08	7.33	6.89
21	7.58	7.86	7.14	7.91	7.56	7.61
22	7.91	7.99	8.03	7.71	7.80	8.02
23	7.64	7.64	7.87	7.40	7.81	7.46
24	7.55	7.81	7.79	7.06	7.76	7.34
Overall Means \pm SD	7.81 ± 0.68	7.70 ± 0.48	7.74 ± 0.59	8.00 ± 0.90	7.91 ± 0.75	7.71 ± 0.61

* Number of observations included in each variable mean is found in Appendix R

There will be no difference in mean lactose content of milks between mothers.

The null hypothesis was rejected. Mothers' milks were significantly different from one another in mean lactose content ($p = 0.000$, df 23, 45). The range of mean lactose values across twenty-four mothers was 7.03 to 8.75 grams per deciliter, a 1.25 fold difference. The overall mean of this sample of mothers was 7.81 ± 0.68 grams per deciliter. This value is somewhat higher than means reported in the literature. Mean values of from 5.0 to 7.6 g/100 ml were reported by Jelliffe and Jelliffe (1978a) in a review of the literature of studies of mothers in developing and industrialized countries. Hytten (1954d) had reported means of 6.87 ± 0.26 g/100 ml over 150 samples of mature milk at one month lactation. A study of 96 women in England was reported with a mean "carbohydrate" content of 7.4 g/100 ml (DHSS, 1977). Hall (1979) had reported a mean of 8.12 g/100 ml over 30 samples of milk. The variety of reported means may reflect the variety of analytic methods. Some studies were measuring reducing sugars and hence, measured all reducing monosaccharides as well as lactose. The analytical method

in this study based upon the enzyme, β -galactosidase, is very specific for lactose, yet could be confused by the presence of galactose or galactose-containing oligosaccharides. An attempt was made to measure separately the presence of free galactose in the milk samples by use of the same reagents. Values of 0.10 to 0.30 g/100 ml were estimated, but it was not known if the SP800 Unicam Spectrophotometer was really sensitive enough to give reliable measures at this low concentration. These values represented approximately two to four percent of the total lactose content estimated. Ebner and Schanbacher (1974) had reported free galactose of bovine milk at 11.7 mg /100 ml (0.0117 g/100 ml) based on gas chromatographic and mass spectrometric analysis techniques. The presence of galactose-containing oligosaccharides in human milk was noted but not quantified.

Tukey's multiple comparisons test was applied to mothers' mean lactose contents to see where significance lay (Table 44). A ratio of 8.75/7.71, or 1.14, was sufficient to achieve significance between means. Although many significant differences were found, they are probably of little sensory significance. A difference of

Table 44

Tukey's Multiple Comparisons Test on Mean Lactose
Content of Milks of Twenty-Four Mothers

Mother	Mean Lactose g/100 ml
2	8.76 a
13	8.38 ab
17	8.37 ab
1	8.35 ab
15	8.34 abc
16	8.27 abc
3	8.20 abc
18	8.18 abc
14	8.10 abc
10	7.92 abcd
22	7.91 abcd
11	7.71 bcd
19	7.65 bcd
23	7.64 bcd
5	7.63 bcd
21	7.58 bcd
4	7.57 bcd
24	7.55 bcd
8	7.49 bcd
12	7.44 cd
6	7.16 d
20	7.11 d
9	7.08 d
7	7.03 d

abc scores with the same superscripts are not significantly different ($p \leq 0.05$).

$$\text{least significant difference} = \sqrt{0.1723/6} \times 5.36 = 0.9084$$

one percent in a solution of lactose of seven to eight percent is very small and probably would not be recognized by sensory panelists. Based on studies of equivalent sweetness of lactose and sucrose, where, at seven percent concentration, lactose is in a ratio of 1:3.5 with sucrose, (Pangborn, 1963), the expected range of equivalent sucrose concentration of this study would be 2.5 to 2.0 percent. The significant range of 7.71 to 8.75 lactose would be equivalent to a range of 2.2 to 2.5 percent sucrose. This difference should be indistinguishable even by a trained sensory judge. It is not surprising, therefore, that judges did not find any significant differences in Group I mothers in sweetness and found only two mothers significantly different in sweetness in Group II mothers. The range in lactose was too narrow, and sweetness perception may have been further confounded by other sensory parameters such as viscosity.

There will be no difference between fore and hind milks in lactose content.

The null hypothesis was rejected ($p = 0.006$, df 1, 23). On the average, hind milk was found to contain significantly less lactose than fore milk. Mean lactose

content for hind milk was 7.71 ± 0.61 and for fore milk was 7.91 ± 0.75 grams per deciliter. The significance achieved lies in the nature of the experimental model where variable time is tested against mother - time interaction. When this interaction is very small, time (fore versus hind) can become significant. The difference is again of no practical significance in sensory evaluation because the difference of 0.02 percent would be too small to be distinguished by panelists. No differences were found between fore and hind milks in sweetness.

Other researchers have reported changes in lactose content in a single feed. Hall (1979) found a decline in lactose from 8.29 ± 0.10 for fore milk to 7.95 ± 0.22 for hind milk in 15 pairs of milks but found these changes non-significant. Hytten (1954) had related changes in lactose to differences in volume of milk secreted. When volume was low, lactose content was low. Lactose is the main osmotic component of milk and has been found to change inversely with other osmolites (Conner, 1979). The changes in lactose content may be partly explained by displacement of the aqueous phase of the milk

containing lactose, by the fat phase as the feed progresses.

There will be no difference in mean lactose content over three consecutive days.

The null hypothesis was accepted ($p = 0.228$, df 2, 46). On the average, means by day were not significantly different from one another, however, the main effect of days is difficult to interpret because of a highly significant mother - day interaction ($p = 0.000$, df 46, 45). This resulting interaction could have been partly an artifact of laboratory analytical error where samples were analyzed in blocks by day. The mean by day trend towards increasing concentration or decreasing concentrations varied from mother to mother.

No other significant interactions were found in the lactose data. The lactose contents of the milks can be compared to the results of sweetness evaluation. Because of the very small ranges of both lactose content and sweetness scores, a strong correlation of these two groups of data would not be expected. Mother Fifteen, with the highest mean sweetness score did have a lactose mean higher than nineteen other mothers, but was only significantly higher in lactose than four mothers. Mother Twenty, although not significantly different in sweetness from

the other mothers, had a lower mean sweetness score than ten mothers of Group II and was significantly lower in lactose than nine of twenty-four mothers. Mothers Thirteen, Seventeen, Eighteen and Sixteen were lower in sweetness, but higher in lactose, than other mothers. In both groups of data, the majority of mothers' milks fell into the non-significant range.

The overall mean of lactose of 7.81 grams percent compares favourably to the overall sweetness mean in sucrose equivalent of 2.12 percent as found by judges. Based on relative intensities of sweetness of sucrose to lactose as found by this panel, the ratio of $7.81/2.12$ or 3.68, is close to the reported approximate ratio of 3.5 (Pangborn, 1963).

B. Fat Content

Fat content was analyzed by the method of Nakai and Le (1971). Results were expressed in grams per deciliter and are reported in Appendix Z. Results were analyzed by 3-way factorial analysis of variance with second-order interaction term (mother x day x time) as the error term. A summary of the factors tested and their

significance appears in Table 45. The complete ANOVA table is in Appendix AA. Means by mother, by time (fore and hind), by day, and by mother - day and by mother - time are reported in Table 46.

The following hypotheses were tested:

There will be no differences in mean fat content between milks of different mothers.

The null hypothesis was rejected ($p = 0.0006$, df 23, 44). Mean fat contents across milks of different mothers were significantly different from one another. The range of means was from 0.57 grams percent to 4.12 grams percent. The overall mean of all milks of twenty-four mothers was 2.17 ± 1.41 grams per deciliter. This value cannot be considered representative of the fat content of milk. These samples were expressed in the early morning. Gunther and Stanier (1949), Hall (1979), Hytten (1954c) and Picciano and Guthrie (1976), all reported lower fat content at early morning feeds. Hall (1979) reported a 2.5 fold change from morning to a mid-day plateau. Hytten (1954c) reported a value of 2.34 grams percent at 6 A.M., representing the lowest value of the day, and peaking at midmorning. Picciano and Guthrie (1976) reported

Table 45
 Summary of Variable Effects in ANOVA of Fat
 Content of Human Milk

Source of Variation	Exact p Value
Mother	0.001 ^{***}
Day	(0.498)
Mother x Day	(0.310)
Time	0.000 ^{***}
Mother x Time	0.005 ^{**}
Day x Time	(0.788)
(Mother x Day x Time) [*]	

Second-order interaction chosen as error term.

(p value) not significant.

- * Significant at $p \leq 0.05$.
- ** Significant at $p \leq 0.01$.
- *** Highly significant $p \leq 0.001$.

Table 46
 Mean Fat Contents of Human Milks
 (g/100 ml)

Mother No.	Mean by Mother*	Mean by Day			Mean by Time	
		Day 1	2	3	Fore	Hind
1	2.35	3.98	1.31	1.76	0.87	3.83
2	3.15	2.02	3.67	3.75	0.82	5.47
3	3.50	2.60	4.44	3.45	0.24	6.75
4	2.14	1.46	2.53	2.43	1.19	3.08
5	1.12	1.15	0.83	1.38	0.62	1.62
6	4.12	5.58	3.00	3.78	2.32	5.92
7	3.11	3.90	3.98	1.47	2.00	4.23
8	2.48	2.95	1.23	3.25	1.98	2.97
9	1.58	0.63	1.48	2.64	1.05	2.11
10	1.58	2.33	1.13	1.28	0.97	2.19
11	3.41	4.28	4.63	1.33	1.10	5.72
12	1.94	2.35	2.75	0.73	1.67	2.22
13	1.26	0.80	1.87	1.43	0.67	1.66
14	2.78	2.98	2.40	2.98	2.52	3.05
15	2.40	1.19	3.08	2.94	1.57	3.23
16	2.43	3.55	1.78	1.95	1.17	3.68
17	1.28	1.18	1.23	1.45	0.67	1.90
18	1.57	1.70	1.23	1.78	0.67	2.47
19	0.57	0.65	0.63	0.43	0.55	0.58
20	1.63	2.95	1.03	0.93	1.08	2.18
21	2.28	2.05	3.08	1.15	1.07	4.10
22	2.17	2.43	2.10	1.99	0.98	3.37
23	1.65	1.65	1.33	1.99	0.57	2.74
24	1.39	1.35	1.35	1.48	0.58	2.20
Group Means ± SD	2.17* ± 1.41	2.32* ± 2.04	2.17 ± 1.89	2.00 ± 1.60	1.13* ± 0.82 a	3.21 ± 2.00 b

* The number of judgements included in each variable mean is found in Appendix R.

an early morning mean of 2.96 grams percent, rising through 3.99 at midday and to 4.51 grams percent at evening. Although these researchers disagreed as to the time of the peak fat values, there was common agreement that early morning fat contents were the lowest of the day. Picciano and Guthrie (1976) had also used the method of Nakai and Le (1971) for chemical analysis of fat content in their study. They reported a range of 0.20 to 10.4 g/100 ml fat over all 450 samples. In this study, the range over all 142 samples analyzed was 0.10 to 9.60 grams percent. Again, a mean value of 2.17 grams percent over 24 mothers seems small in comparison to the literature values just discussed. These milks were expressed from the first breast offered at the feed. It is possible that mixing of the milk in the second breast during let down reflex would raise the fat content of milk from the second breast (Hytten, 1954b). Hall (1979) found an increase in fat content of fore milk in the breast suckled second. It is also possible that asking the mother to express sufficient hind milk interfered with complete emptying of the breast due to her increasing anxiety that she leave some milk in the breast. Terminating the feed early would have reduced the fat content of hind milk.

Tukey's multiple comparisons test (Table 47) was applied to mean fat content of milks by mother. Because of a large error mean square, relatively large differences in fat contents were necessary to achieve significance. A ratio of 4.12/1.39 or a 3 fold difference was necessary for there to be a significant difference between mothers' means. Two mothers at either end of the range appeared to be unusual in that their fat content means were further away from the next nearest value than other mothers' means were from each other, but they were not significantly different from those neighbouring values. The Mothers One to Twelve had milks of mean fat content 2.54 ± 0.92 whereas Mothers Thirteen to Twenty-four had a mean of 1.78 ± 0.64 . This could have affected the sensory evaluation of viscosity over these two groups of mothers, yet the coefficient of variation (SD \div mean) was the same in both groups of mothers (36%). Also, the change over Mothers One to Twelve was 4.12/1.12 or 3.68, whereas the change over Mothers Thirteen to Twenty-four was 2.78/0.57 or 4.88. Yet, it was in this second group (Mothers Thirteen to Twenty-four) with the larger degree of change, that judges failed to find significant differences between mothers in either sensory

Table 47

Tukey's Multiple Comparisons Test on Mean Fat Content
of Milks of Twenty-four Mothers

Mother No.	Mean Fat Content g/100 ml
6	4.12 a
3	3.50 ab
11	3.41 abc
2	3.15 abcd
7	3.11 abcd
14	2.78 abcd
8	2.48 abcd
16	2.43 abcd
15	2.40 abcd
21	2.28 abcd
1	2.35 abcd
22	2.17 abcd
4	2.14 abcd
12	1.94 abcd
23	1.65 abcd
20	1.63 abcd
9	1.58 abcd
10	1.58 abcd
18	1.57 abcd
24	1.39 bcd
17	1.28 bcd
13	1.26 bcd
5	1.12 bcd
19	0.57 d

abc scores with the same superscripts are not significantly different ($p \leq 0.05$).

$$\text{least significant difference} = \sqrt{1.438/6} \times 5.36 = 2.624$$

viscosity or mouthcoat.

There will be no difference between fore and hind milk in fat content.

The null hypothesis was rejected ($p = 0.0000$, $df 1$, 23). There was a highly significant increase in fat content from fore to hind milk. Mean fore milk fat content was 1.13 ± 0.82 grams percent, and mean hind milk fat content was 3.21 ± 2.00 grams percent, a 2.84 fold increase through a feed. Coefficient of variation of fore milk $0.82/1.13$ was 72.5 percent, whereas that of hind milk was $2.00/3.21$ or 62.3 percent. In this study, there appeared to be more variation among fore milk samples than among hind milk, although in both, variation was large. Hall (1979) had reported a similar 3 fold increase from fore to hind milk in fat content of 15 pairs of samples. She reported a mean of 2.42 ± 0.3 (g/100 ml) for fore milk and 7.48 ± 0.57 (g/100 ml) for hind milks. She did not report when these samples were collected. In Table 46, the different responses of each mother over time (fore versus hind) can be seen. In some mothers, the response was greater. Mother Three had a 28 fold change, Mother Eleven had a 5 fold change; yet Mother Nineteen had no change and Mother Twelve

had a 1.3 fold change. Hytten (1954) had reported a maximum increase of 14 fold. Many reasons could account for this individual variability. The time of the feed, that is, the number of minutes the infant suckled, would influence the fat content. Lucas and coworkers (1978) showed that, although on the average the infant would receive the greatest volume in the first four minutes of sucking, richer milk would still be secreted with prolonged nursing. Emery et al. (1978) reported that the relationship between volume of milk expressed and percentage of fat in milk was a curvilinear one; when 60 percent of the volume of milk had been expressed from the breast, only 40 percent of the total fat had been obtained. Fat in breast milk increased as a single nursing progressed. Mothers in this study reported a variety of nursing time periods from two to five to fifteen minutes, but the length of time at the feeding where these samples were expressed was not recorded.

The time interval since the previous feed has been reported to influence the fat content of the milk. A shorter interval between feeds leads to a higher fat content in the milk at that feed (Gunther and Stanier, 1949; Hytten, 1954b; c). Also, milk accumulating in the

breast overnight will be less rich because of the longer time interval. In this group of twenty-four mothers, although an effort was made to standardize the milk collection time, many mothers reported that they were night-nursers, so that varying intervals since the last feed would have existed between mothers. This same principle of time interval would be influencing the variation in milks that might occur because of nursing style; that is, if one breast is offered per feed then possibly the milk would be lower in fat content because that breast might be emptied less frequently. Hytten (1954b; c) took sequential samples of milk in a single feed throughout the entire feed. The rise in fat content was not smooth and in fact, at certain points, dropped when a new lobule in the breast ejected milk, diluting that already in the breast. Some of these factors discussed may explain, in part, why Mother Nineteen had such a low fat content and why the milks did not change over the feed. It would have been interesting to sample this mother at other times during the day. This particular mother was 35 years old with four children, and was nursing two children at the time. As further discussed in Section

IX, Results, maternal age and parity were found to have a negative effect upon fat content of the milk.

There will be no difference in mean fat content of mothers' milks over three consecutive days.

The null hypothesis was accepted ($p = 0.498$, df 2, 46). There was no significant day effect. There was a significant ($p = 0.005$, df 23, 44) mother - time interaction. This has already been discussed in that mothers' milks increased over a single feed in fat content but to different degrees among mothers.

In order to discover whether judges were able to identify those mothers with milks of higher fat content in their sensory evaluation of viscosity or of mouthcoat, comparisons of these three groups of data were made. Mother Six with the highest mean fat content (4.12 g/100 ml) was perceived as less viscous or less mouthcoating than eight of the other eleven mothers in Group I, yet, these other mothers had lower mean fat contents in their milks. Mother Three, also with a high mean fat content (3.50 g/100 ml), was perceived as least in mouthcoat and less viscous than nine other mothers. Mothers Two and Eleven with mean fat contents of 3.15 and 3.41 (g/100 ml) were

perceived as high in mouthcoat and viscosity. Mother Five was perceived as low in both sensory parameters and had a low fat content of 1.12 grams percent. Mother Nineteen with the lowest fat content was not identified by Judges Four - Six as being different from the reference in either viscosity or mouthcoat. Judges were not able to identify those mothers whose milks had a high fat content as being more viscous or more mouthcoating.

C. Protein Content

Protein content was analyzed by a modification of the method of Lowry et al., (1951). Complete results of protein analysis are reported in Appendix BB. Results, as grams per deciliter, were analyzed by 3-way factorial ANOVA with second-order interaction (mother x day x time) as the error term. Table 48 summarizes the factors tested and their significance. A complete ANOVA table is in Appendix CC. Means of protein content by mother, by day, by time, (fore and hind milk) and by mother - day and mother - time are contained in Table 49.

Table 48
 Summary of Variable Effects in ANOVA of Protein
 Content of Human Milk

Source of Variation	p Value
Mother	0.000***
Day	(0.295)
Mother x Day	(0.083)
Time	0.000***
Mother x Time	(0.056)
Day x Time	(0.292)
(Mother x Day x Time) ⁺	

⁺ Second-order interaction (mother x day x time) chosen as error term.

(p value)

* Significant at $p \leq 0.05$.

** Significant at $p \leq 0.01$.

*** Significant at $p \leq 0.001$.

Table 49
 Mean Protein Contents of Human Milks
 (g/100 ml)

Mother No.	Mean by Mother*	Mean by Day			Mean by Time	
		Day 1	2	3	Fore	Hind
1	1.37	1.47	1.21	1.42	1.18	1.55
2	1.22	1.08	1.33	1.26	0.99	1.44
3	1.21	1.11	1.19	1.33	1.00	1.42
4	1.03	0.91	1.04	1.13	0.89	1.16
5	1.16	1.16	1.16	1.15	1.13	1.18
6	1.18	1.00	1.18	1.29	1.50	1.39
7	1.21	1.23	1.27	1.14	1.10	1.33
8	0.84	0.83	0.76	0.92	0.79	0.88
9	0.75	0.71	0.77	0.79	0.74	0.76
10	0.74	0.75	0.72	0.77	0.69	0.80
11	0.84	0.83	0.93	0.77	0.71	0.98
12	0.78	0.84	0.71	0.79	0.76	0.79
13	1.40	1.31	1.47	1.47	1.31	1.46
14	1.86	1.91	1.77	1.92	1.84	1.88
15	2.15	1.95	2.31	2.19	2.10	2.19
16	1.11	1.42	0.89	1.03	1.00	1.22
17	1.00	1.04	0.98	0.98	0.96	1.04
18	1.02	1.02	0.98	1.06	0.99	1.05
19	0.71	0.68	0.71	0.74	0.72	0.70
20	0.61	0.69	0.59	0.55	0.59	0.63
21	0.79	0.77	0.86	0.68	0.70	0.91
22	0.76	0.80	0.71	0.77	0.69	0.82
23	0.83	0.80	0.83	0.87	0.76	0.91
24	0.94	0.93	0.96	0.92	0.89	0.98
Mean	1.06	1.05	1.04	1.09	0.98	1.15*
± SD	±0.39	±0.37	±0.41	±0.40	±0.36	±0.41

* The number of judgements included in each variable mean is found in Appendix R.

The following hypotheses were tested:

There will be no difference in mean protein content between different mothers' milks.

The null hypothesis was rejected ($p = 0.001$, df 23, 43). Mean protein content was significantly different among mothers. A range of 0.61 to 2.15 grams per deciliter was found over twenty-four mothers. The overall mean protein content was 1.06 ± 0.39 grams per deciliter. These values compare well with reported values for protein content in the literature. Hytten (1954d) had reported a mean value of 0.91 grams per deciliter (g/100 ml) in fifteen mothers at more than six weeks lactation. He had used a method of analysis of Kjeldahl nitrogen plus protein precipitation in an attempt to estimate protein versus non-protein nitrogen. His results are in agreement with those of Lonnerdal et al. (1976) who had reported values of 0.8 - 0.9 (g/100 ml) based on amino acid analyses. Picciano and Guthrie (1976) using a chemical method of Nakai and Le (1971) whereby proteins are read spectrophotometrically at 280 nm, reported a range of values from 0.76 to 2.04 (g/100 ml) with a mean of 1.28 ± 0.24 over 50 women. Since this method is subject to interference from nucleic acids, these values could be overestimated. The mean

protein content of 96 British women's milks was reported as 1.07 (g/100 ml) by a method based on amino acid analysis (DHSS #12, 1977). Hall (1979) reported a mean protein content of 1.18 (g/100 ml) over 30 samples from six mothers. She used the same method of analysis as was used in this study (Lowry et al., 1951). She reported a range of 0.65 to 2.61 grams per deciliter.

Tukey's multiple comparisons test (Table 50) revealed many significant differences in the range of means across mothers. A very small error term (the mother x day x time interaction) led to significances with small changes in protein content. Mother Fifteen, with a mean protein content of 2.15 (g/100 ml) had consistently higher values for all six samples analyzed, as did Mother Fourteen. Mothers Nineteen and Twenty had consistently low values over all their milk samples. All the samples for a single mother were analyzed at one time in duplicate.

When the lactose content values and protein content values (means by mother) were examined (Tables 44 and 50), it appeared that a "time" effect, apart from daily or within-feed factors, was influencing the results. Mothers in the first experimental group (1, 2, 3, 13, 14, 15) had higher lactose and protein values than those in the second

Table 50

Tukey's Multiple Comparisons Test on Mean Protein
Content of Milks of Twenty-four Mothers

Mother No.	Mean Protein Content g/100 ml
15	2.15 a
14	1.86 b
13	1.40 c
1	1.37 cd
2	1.22 cde
3	1.21 cdef
7	1.21 cdef
6	1.18 cdefg
5	1.16 cdefgh
16	1.11 efghi
4	1.03 efghij
18	1.02 efghijk
17	1.00 efghijkl
24	0.94 ghijklm
11	0.84 jklm
8	0.84 jklmn
23	0.83 jklmn
21	0.79 jklmn
12	0.78 jklmn
22	0.76 lmn
9	0.75 lmn
10	0.74 mn
19	0.71 mn
20	0.63 n

abc value with same letter are not significantly different.

$$\text{least significant difference} = \sqrt{0.0131/6} \times 5.36 = 0.2505$$

experimental group (4, 5, 6, 15, 16, 17). Those mothers' milks collected and analyzed together seemed to be grouped together in their analyzed values. Since each experimental group was a different group of mothers (refer to Table 6 for further clarity), this "time" effect could not be expected to be influencing an individual mothers' milks. One can speculate that the results were being influenced by the analytical procedure. Yet, lactose contents were analyzed in blocks of milks by day across three mothers, and protein contents were analyzed in blocks of milks by mother across three days. In both cases, though, analysis was performed beginning with the first group of mothers (1, 2, 3, 13, 14, 15) progressing through all samples to the last group (10, 11, 12, 22, 23, 24). In the case of protein analysis, new standards were prepared each day. Fat content values did not exhibit this same "time" effect, even though analyses were performed in the same manner as in protein analysis - blocks by mother across days, beginning at first experimental group.

There will be no difference in mean protein content between fore and hind milks.

The null hypothesis was rejected ($p = 0.001$, df 1, 23).

Hind milk contained on the average, more protein than fore milk, 1.15 ± 0.41 grams per deciliter hind milk and 0.98 ± 0.36 grams per deciliter fore milk. In twenty-one mothers, the hind milk contained greater amounts of protein than fore milk, although the degree of change varied across mothers. Hall (1979) also reported a change in protein content from beginning to end of a single feed. She obtained mean values of 1.00 ± 0.06 and 1.36 ± 0.16 for fore and hind milks of fifteen mothers respectively. She also found variation between mothers in the degree of change such that some mothers' milks showed no change over feed and others showed 1.25 - 3.0 fold change fore to hind. Hytten (1954b) reported a slight increase in casein nitrogen during a feed after fifth day of lactation. Casein tended to be partially absorbed to fat and accompanied its rise towards the end of a feed.

There will be no difference in mean protein content of milks over three consecutive days.

The null hypothesis was accepted ($p = 0.295$, $df 2, 46$). On the average, days did not have an effect upon the protein contents of mothers' milks. This is in agreement with Picciano and Guthrie (1976) who found little between - day

or within - day variation in protein content of milks of 25 mothers. In their study, almost all of the variation in protein content was accounted for by mother variation. In this data, sixteen of the mothers had fairly consistent means by day over the three days, but the other eight mothers (1, 2, 3, 4, 5, 7, 15, 16) had one or two means by day which varied widely from the other means. A significant mother by day interaction might have been expected.

VII. Statistical Treatment of Relationship Between Sensory, Chemical and Physical Evaluation of Human Milk

A. Relationship Between Sensory Evaluations and Physical - Chemical Measurements on Human Milk

Linear regression analysis was used to assess the relationship between sensory and physical - chemical data. All regressions were performed using the computer-based programme of S.A.S. called General Linear Model (GLM). Regressions were performed on log data for both the sensory variable and the physical - chemical variable. This procedure was utilized because of the psychophysical law based on the power function: $S = kC^n$, where the exponential relationship becomes linear with log

transformation: or $\log S = n \log C + \log k$. Because of linearity in log transformation; linear least-squares regression theory can be applied. A summary of the regressions performed and their significances are reported in Table 51. Regression Models appear in Appendices DDi, ii, iii and EE.

No significant linear correlations were found in any of the regressions performed with sensory data. Because no significant correlations were found in those parameters initially tested, further correlations involving mouthcoat data, and protein data were not performed. The reasons for the experiment not proceeding further in testing correlations will become obvious to the reader in the following discussions.

Since no significant linear correlations were found between the sensory data and the physical - chemical data, it is necessary to comment upon the possible reasons for the lack of statistically significant correlations. Firstly, there still exists in the area of sensory perception, a basic argument about whether rheology, the study of deformation or flow of matter, can accurately reflect sensory perception (Kapsalis and Moskowitz, 1978). Even when employing the psychophysical law (Stevens, 1960),

Table 51

Summary of Correlations Performed Between Selected Parameters

Evaluated in Human Milk

Variable y	Variable x	Coefficient of Determination (r^2)	Coefficient of Correlation (r)	Significance of Regression Model ($p \leq$)
Sensory viscosity	Fat content	0.03	0.17	0.432
Sensory viscosity	Physical viscosity	0.02	0.16	0.614
Sensory sweetness	Lactose content	0.00	0.00	0.924

that sensory perception of a stimulus grows exponentially according to the power function $S = kC^n$, it is understood by sensory and psychological researchers that the mechanisms and/or the principles in each area, sensory and instrumental measurements, are not the same. The power function equation as applied by Stevens (1960) performs best when one is studying a simple pure stimulus in a model system, such as sucrose and water for sweetness perception, or silicone oils for tactile viscosity perception (Stevens and Guirao, 1964). This same power function may not predict the response of a taster to a single attribute in a complex food system because of the multiple interactions within the food (Moskowitz, 1977). "Each single texture perception is traceable to the confluence of many physical/mechanical properties of matter acting simultaneously" (Kapsalis and Moskowitz, 1978).

The human mouth processes food as well as measures the taste/texture attributes. That is, time and temperature changes, addition of saliva, breakdown of food components with mixing, chewing, enzymatic processes, all influence the sensory response. These same changes may not occur,

or may be difficult to measure; in the instrumental or chemical estimation.

A second problem can exist in comparing sensory measurements and instrumental measurements. It is possible that the range of forces employed in the instrument may not accurately simulate those forces occurring in the mouth. For example, in viscosity measurement, shear forces applied to a very fluid food have been reported as relatively low (100 dynes/cm^2) and shear rates in the mouth have been found to be high (1000 sec^{-1}) (Shama and Sherman, 1973). The shear rate chosen on the viscometer must be in the same range as that in the mouth to obtain good sensory-instrumental correlations. In these experiments, a 30 rpm shear rate was chosen as representative of that applied to milk in the mouth, while at the same time avoiding a high shear rate (60 rpm) that could lead to turbulence, with artificially increased readings (Parkinson and Sherman, 1971). Further discussion of the choice of shear rate appears in Section V, Results.

The ability to measure the degree of correlation between two variables implies that a range of instrumental and sensory changes have occurred that cover a large enough

span such that association can be made. The range of sensory perception of viscosity in the mouth is very small (Kapsalis and Moskowitz, 1978). A power function exponent of 0.4 has been reported by Moskowitz (1972) in a study of gum solutions and perceived viscosity, and an average exponent of 0.56 by Malcolmson (1978) in a study of plant protein model systems relating sensory perception to physical viscosity. The power function of $S = kC^{0.4}$ implies that a ten-fold change in instrumental viscosity would lead to only a 2.5 fold change in perceived viscosity. Larger changes may have been necessary than occurred in these human milks evaluated for correlation to appear. A mean physical viscosity from 1.50 to 1.35 centipoises (hind milk to fore milk) or from 1.63 to 1.31 centipoises (the range of physical viscosity means across mothers) would be expected to lead to very small perceptual differences. In most of the data in this study, ranges of the means were very small. Mean lactose per mother ranged from 7.03 to 8.75 grams per deciliter. Sensory responses by mother for sweetness was from 0.86 to 1.24. Even with exponents in sugar power functions of 1.3 (Moskowitz, 1970; Stevens, 1960), this small range in sensory response to the

difference in lactose would be expected. Mean fat content per mother, although of a wider range, 0.57 to 4.12 grams per deciliter over twenty-four mothers, did not correlate well with mean sensory viscosity per mother. As mentioned previously, sensory viscosity has been reported to grow slowly in response to physical variables. Also, viscosity is likely an integrated response to a multiplicity of physical properties and not to a single component such as fat content.

This multiplicity of factors associated with fat and viscosity was described by Prentice (1972). He outlined the contribution various parameters related to fat content made to viscosity when discussing viscosity of bovine milk and cream - that is, the fat globule size and frequency, the distance between globules, the forces of attraction between globules, the temperature of the system, all affected physical viscosity measurements, and hence, possibly sensory perception of viscosity, more than just total fat content. In this study, while significances in mean values by mother lay on the outside of the ranges, the majority of means in all sensory evaluations fell into a non-significant range. Strong correlation would have

been difficult to obtain with many values close together.

It is possible that when the medium carrying the tastant is no longer simply water or when the food system being evaluated for a textural attribute is complex, the power function may change. Fabro (1979) found that mouthcoat and viscosity were not perceived as significantly different among samples of model texture systems of pureed infant foods, even though with changes in formulation, she may have expected differences to be perceived. In her study, the power function exponent, n , also changed in basic tastant systems of sweet, sour and bitter, depending on the medium carrying the tastant, whether it was water ($n = 1.41$ for sucrose) or a model texture system ($n = 0.99$). It is possible that sweetness perception in human milk was influenced by other variables present in the milks than solely lactose content, thus preventing correlation between lactose and sweetness perception.

All correlations performed in this study were based upon linear least-squares regression theory. It is possible that other mathematical fractions or equations better describe variables in food systems. It may be necessary

to use non-linear, higher order equations to describe interactions between variables (Kapsalis and Moskowitz, 1978; Moskowitz, 1977). With all the problems inherent in measuring rheological changes in foods and in their subsequent correlations with sensory measurements, it remains that sensory characterization is still the best measure of taste and textural properties of a food.

Even though linear regression did not reveal significant association between sensory and physical - chemical data, some relationships can be ascertained by examining the similarity seen in the results of each parameter estimated. In both sensory and physical viscosities, the majority of samples were evaluated as less viscous than the diluted Similac reference. In both sensory and physical viscosities, the hind milk was found to be significantly more viscous than the fore milk. Fat content also was found to rise significantly in the hind milk over that of the fore milk. Mouthcoat data was in agreement with viscosity data; that is, for Group I mothers, hind milk was significantly more mouthcoating than fore milk. Lactose content mean over twenty-four mothers' milks was 7.81 grams percent. The lactose

reference was 6.00 grams percent (w/v) in water. In Group II mothers, the judges evaluated all twelve mothers' milks to be sweeter than the reference. In Group I mothers, four of the twelve mean scores per mother were sweeter than the reference. Further discussions comparing the data from various evaluations made, can be found under each section (Results) dealing individually with a single parameter evaluated.

B. Relationship Between Fat Content and Physical Viscosity of Human Milk

In an attempt to explain changes in physical viscosity, in relation to changes in composition of the milks, simple linear regression analysis was applied to mean physical viscosity measurements of thirteen mothers and mean fat content estimations of the same thirteen mothers. Results of regression are displayed in Appendix EE. A significant correlation coefficient, r , of 0.65 ($p=0.015$) was found. The coefficient of determination, r^2 , was 0.427, indicating that 42.7 percent of the variation in mean physical viscosity measurement per mother could be explained by changes in mean fat content. Therefore, in this data,

approximately half of the changes in instrumental viscosity values are not explained by changes in fat content. It appears that components of the milks other than fat are affecting physical viscosity. It is possible that total solids and solids not fat, particularly proteins, may be affecting viscosity as well as fat (Prentice, 1972).

A further program of multiple regression of three independent variables - fat, lactose and protein contents, was applied to the dependent variable physical viscosity. There appeared to be collinearity present between fat and protein contents ($r = 0.5096$, $p \leq 0.075$) and between protein and lactose contents ($r = 0.5143$, $p \leq 0.072$) approaching significance at the five percent level. This implies that as protein content rises, the lactose content also increases and as fat content rises, protein content also rises. Because of intercollinearity the variables, protein and lactose content did not add any further information to the regression model than only fat content, in explaining the variation in physical viscosity.

VIII. Relationship Between Certain Maternal Parameters and Properties of Human Milk

A. Explanation of Multiple Regression Program

Programs of multiple regression were run between five independent variables of maternal attributes and each of seven dependent variables evaluated in the milks. These dependent variables are listed in Table 52. The maternal attributes were maternal age, lactation stage in weeks, frequency of nursing per twenty-four hour day, nursing style and parity as described in Section I, Results. The computer-based program of S.A.S. - "GLM; Proc Corr" selection was used with stepwise variable. Indicator variables (1 or 0) were chosen to represent nursing style (1 = both breasts offered at a feed, 0 = 1 breast offered at a feed), and parity (1 = multipera, 0 = primipera). In the other three maternal variables entered, the exact value of the variable was used. For example, the exact value of mother's age in years was entered. Data for these three variables were not grouped. The results of multiple regression are presented in Table 52. An explanation of the table and the procedures involved follows.

In Table 52, under the heading "Independent Variables"

Table 52

Summary of Multiple Regression Analysis Between Maternal Variables and Properties
of Human Milk
(Stepwise Procedure of Variable Selection)

Dependent Variable	Independent Variables: Partial Correlation Coefficients and their significances (r_a)					Multiple Coefficient of Determination of Regression Model		After Variable Selection: Variable Left In		
	Age	Lact.	Freq.	Style	Parity	r^2	p value	r^2	p value	
Fat	-.48	.03	-.03	.12	-.35	0.36	0.12	Age	0.23	0.02
	.02	.90	.88	.56	.09					
Protein	-.35	.21	-.09	-.12	0.11	0.20	0.51	Age	0.12	0.09
	.09	.31	.67	.56	.61					
Lactose	-.22	.34	-.17	.10	.26	0.26	0.32	Lactation	0.18	0.10
	.30	.10	.42	.61	.22					
Physical Visc.	-.38	-.17	-.29	.24	-.30	0.36	0.59	Age	0.15	0.19
	.19	.58	.33	.44	.32					
Sensory:										
Sweetness	.04	-.05	.06	-.13	.02	0.03	0.99	-	-	-
	.84	.81	.76	.55	.94					
Viscosity	-.22	.11	-.12	.11	-.06	0.08	0.89	Age	0.05	0.29
	.30	.62	.58	.59	.76					
Mouthcoat	-.13	-.07	-.08	.11	-.11	0.05	0.96	-	-	-
	.53	.75	.72	.60	.61					

** Significance level of variable entry = .50

Significance level of variable staying in = .10

the individual partial correlation coefficient (r) and their significances (p values) are listed for each maternal attribute tested with each dependent variable. The partial correlation coefficients (measuring the strength of the relationships between that independent variable and the dependent variable) were tested at five percent level of significance (probability $> r$ under $H_0 : \rho = 0$). That means that a correlation coefficient with a p value less than 0.05 was significant. A partial correlation coefficient with a p value more than 0.05 and less than 0.10 was considered a trend in this discussion; that is, that independent variable was affecting the dependent variable at a level approaching significance. Under the heading "Multiple Coefficient of Determination of the Regression Model", are listed coefficients of determination (r^2) and their significances (p values) for each multiple regression model. That is, the effect of all five maternal variables together on the dependent variable and the significance of the model are listed. The coefficient of determination r^2 represents the explained variation of the regression model. For example, in the regression model for fat content, 36 percent of the

variation in fat was explained by all five variables at a level of significance of 0.12. Since many of the individual r were not significant at the five percent level, a procedure of variable selection was performed to reduce the regression model to the number of independent variables best explaining the variation in the dependent variable. The stepwise procedure of variable selection was used. The criteria for determining the best subset of predictors of a set of data were: an increase in r^2 , a reduction in p value and/or a lower standard error of estimate. The variable selection technique began by running a simple regression analysis using the strongest predictor (largest r). Then, the next variable with the largest partial F -value was found, given the strongest predictor, and a two-variable multiple regression was run. Variables were added into the model until partial F -values were too small. That is, a variable with a partial F -value with a level of significance greater than $p = 0.50$ would not enter the model. This was the significance level for variable entry. A variable was dropped from the model when its partial F -value p value was greater than 0.10. The variable had become redundant, and was not adding any further information

to the model. The column in Table 52 headed "After Variable Selection, Variable Left In", lists those maternal variables remaining in the model after stepwise variable selection. Generally, the r^2 was reduced in the new model, but the level of significance (p value) of the model was improved. In most cases, a single maternal variable was left in the model; this occurred because multicollinearity was present between many of the other independent variables in the original model.

As might be expected from the lack of linear correlation between sensory data and physical - chemical data, the correlation coefficients of multiple regressions on maternal parameters and sensory evaluations were very low and not significant. There were some significant linear correlation coefficients in the physical - chemical data. Maternal age, therefore, may have an effect upon the fat content of human milk, but, since fat content did not explain changes in sensory viscosity, one would not expect maternal age to have significantly affected viscosity perceived in mothers' milks in this data. There appears to be a slight trend ($r = -0.22$, $p \leq 0.30$) in the effect of maternal age upon sensory viscosity. This trend

increases in effect of maternal age upon physical viscosity ($r = -0.38$, $p \leq 0.19$) and is stronger and significant in effect of maternal age upon fat content ($r = -0.48$, $p \leq 0.02$). In order for maternal variables to have had an effect upon sensory attributes, one would have to have seen a more direct influence of the physical - chemical properties of human milks upon sensory values.

B. Maternal Variables and Sensory Properties of Human Milk.

Maternal variables will not be associated with the sensory properties of human milk.

The null hypotheses were accepted at five percent level of significance ($r^2 = 0.08$, $p \leq 0.89$, df 5, 18) for sensory viscosity and ($r^2 = 0.03$, $p \leq 0.99$, df 5, 18) for sweetness, ($r^2 = 0.05$, $p \leq 0.96$, df 5, 18) for mouthcoat.

As mentioned previously, maternal variables did not explain any of the variation that occurred in panelists' mean evaluations of the sensory qualities of human milk. For this reason, in these mothers, it cannot be said that age, frequency, lactation stage, nursing style, parity, had any effect upon the sweetness, viscosity or

mouthcoat properties of these milks.

C. Maternal Variables and Fat Content of Human Milk

The maternal variables will not be associated with the fat content of human milk.

The null hypothesis was rejected for variable of maternal age ($r = -0.48$, $p \leq 0.02$, $df 1,22$), but was accepted for all other maternal variables. Maternal age affected the fat content of human milk. With increasing age, one could expect decreasing fat content in these mothers' milks. Also, with increasing parity, there was a trend towards decreasing fat content ($r = -0.35$, $p \leq 0.09$). With the technique of variable selection, only age was determined to be significantly affecting fat content in these mothers. Approximately 23 percent of the variation in fat content was explained by differences in maternal age. This is perhaps surprising because the range of maternal ages in this sample of twenty-four mothers was 25 to 35 years, not encompassing the entire reproductive span of women. There was no interaction between age and parity in this data set, but there was significant interaction between frequency and style ($r = -0.47$, $p \leq 0.02$) and two other interactions between variables were approaching significance

(frequency and lactation stage $r = -0.28$, $p \leq 0.18$) and frequency and parity $r = 0.27$, $p \leq 0.20$). By using a Students' t test on two groups of values, Picciano and Guthrie (1976) also demonstrated a significant decrease in fat content of milks of women over 30 years of age when compared to a group of mothers whose ages were less than 30 years. Parity significantly increased fat content in their sample.

Because frequency and style were significantly interacting in this data, it was not possible to determine the effect of either alone. It has been reported that shorter nursing intervals (higher frequency) resulted in higher fat content of milk (Hyttén 1954b; e; Webb and Johnson, 1974). When a mother offered one breast per feed, she tended to nurse more frequently per day, yet the time interval that milk stayed in the breast may have been the same as for a mother who nursed less frequently but emptied both breasts at a feed.

D. Maternal Variables and Protein Content of Human Milk

Maternal variables will not be associated with the

protein content of human milk.

At the five percent level of significance, no maternal variables were significantly affecting protein content. The null hypothesis was accepted.

After stepwise variable selection procedure, the variable maternal age remained in the model ($r^2 = 0.12$, $p \leq 0.09$, df 1, 22). That is, with increasing maternal age, there was a tendency for protein content of the milk to decrease. At $p \leq 0.09$, 12 percent of the variation in protein content was explained by age. The drop in protein may be that that accompanies a drop in fat content. It may be a decrease in casein which adheres to the fat globules (Hyttén, 1954b; e). In contrast, Picciano and Guthrie (1976) found a highly significant increase in protein ($p \leq 0.001$) with mothers over 30 years in age. There were no interactions between age and other variables in this data.

E. Maternal Variables and Lactose Content of Human Milk

Maternal variables will not be associated with the lactose content of human milk.

In these twenty-four mothers, none of the maternal variables could explain lactose variation between mothers at the five percent level of significance. There was a trend for lactation stage to affect lactose content ($r^2 = 0.18$, $p \leq 0.10$, $df 1, 22$). With increasing length of lactation in weeks, lactose content tended to increase.

F. Maternal Variables and Physical Viscosity of Human Milk

Again, maternal variables were not significantly associated with the mean physical viscosity of mothers' milks. The trend seen in Table 52, of age effect upon physical viscosity ($r^2 = 0.15$, $p \leq 0.19$, $df 1, 11$) might be expected to accompany the age effect on fat content. The effect is probably less strong in physical viscosity because fat content explained only 42.7 percent of the variation in the physical viscosities of the milks.

IX. Comparison of the Sensory Qualities of Human Milk With Those of Other Infant Foods

The results of this study can be compared to the results of other studies performed with infant foods in

the Department of Foods and Nutrition, University of Manitoba. The method of study was either sensory evaluation of infant foods by trained adult panels (Malcolmson and McDaniel, 1980a and 1980b; McDaniel and Harasym, 1979), or infant feeding studies estimating acceptance or rejection of selected taste and texture models of infant foods (Fabro, 1979; Harasym, 1977; and Hogue and McDaniel, 1980).

A. Sweetness of Human Milk in Comparison to That of Infant Foods

By construction of a power function for sucrose and by appropriate placement of human milks on the regression line, one can compare the equivalent sweetness of human milk to that of other infant foods (Figure 4, p. 116). In this study, the equivalent sweetness of human milk was in the range of 1.43 to 2.87 percent sucrose in water solution. The mean sucrose equivalent of human milks was found to be a 2.12 percent sucrose solution. This is sweeter than cow-milk based proprietary formulae, Similac, Enfalac, and SMA, evaluated at 1.2 percent sucrose equivalency by Malcolmson and McDaniel (1980b). The human

milk in this study was less sweet than home-prepared formulae using whole or evaporated milk with added sucrose or corn syrup; that is, formulae recommended for a 1 - 2 week old infant, using whole or evaporated milk (Manitoba Department of Health and Community Services, 1979) and i-Soyalac, were found to be equivalent in sweetness to a six percent sucrose solution (Malcolmson and McDaniel, 1980b).

Human milk sweetness is similar to that of other infant foods, namely cereals (Malcolmson and McDaniel, 1980a); certain infant vegetable - canned carrots, frozen peas (Harasym, 1977); and commercial infant pears and applesauce (Fabro, 1977).

Infants do prefer sweet as documented in "The Review of Literature". The question remains whether they would show varying preferences for sugar-added formulae or for human milk or for commercial cow-milk formulae based on their differences in sweetness, and whether exposure to sweet human milk could influence subsequent sweet preference.

Harasym (1977) found a significant preference of six to sixteen week old infants for sweet among those introduced to fruits before vegetables. Fabro (1979)

found a similar trend, although not significant, in five to eight month infants. Neither study investigated the influence milk source might have played upon sweet preference. Sweetness equivalency in Fabro's study was 10 percent sucrose in a smooth-thin texture system, (or 7.5 percent sucrose in water), as sweet as the sweetest infant formulae and three and one-half times as sweet as human milk. Sweetness influenced acceptance of samples in a study of Hogue and McDaniel (1980), yet significant differences in preference were not found among 10.0, 5.0 and 2.5 percent sucrose treatments. Thus, one would question again whether an infant would be able to recognize and exhibit preference over the small range of differences in sweetness between commonly-used formulae and human milks in this study.

Use of very sweet formulae, home-prepared and soybased, would expose infants to concentrated sweetness. The incidence of use of these sweeter formulae has been reported as low (Clark, 1978; Fabro, 1979; Harasym, 1977; Martinez, 1979), although they are still prevalent in certain areas of Canada. Barker (1979) found a frequency of evaporated milk use of 58.3 percent in a small samples of native

Manitoba mothers and Alton-Mackey and Orr (1978) reported a frequency of 72, 47, 43 percents respectively, in rural, urban and metropolitan Newfoundland communities.

B. Sensory Viscosity of Human Milk in Comparison With That of Other Infant Foods

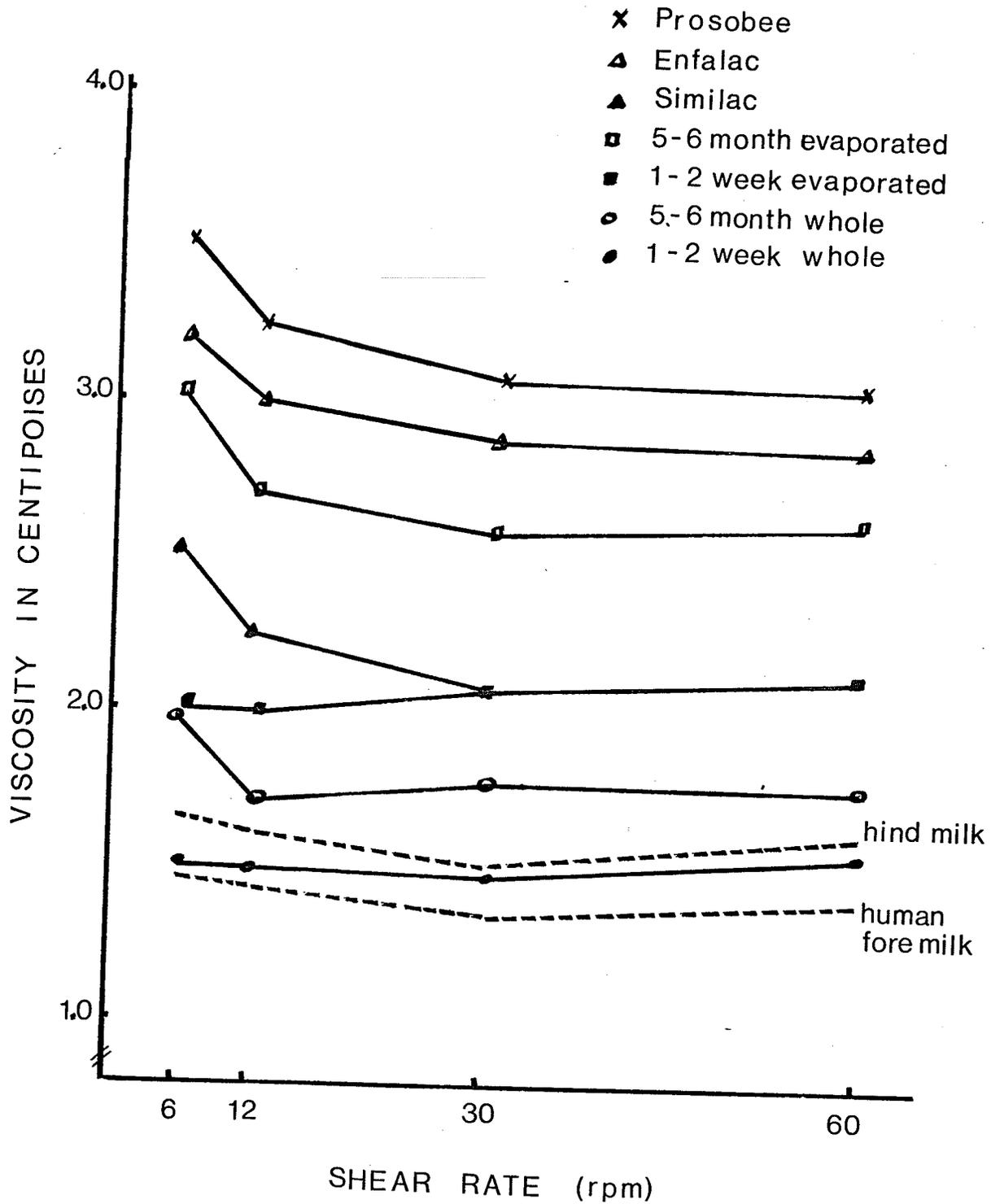
The low viscosity of human milk was reflected in the low sensory viscosity scores, usually less than the reference of diluted-Similac. Human milk can be described as thin and watery. Even hind milk of increasing fat content was found to be relatively thin.

Sensory viscosity was not evaluated in infant formulae by Malcolmson and McDaniel (1980b) because, in preliminary testing, judges thought the samples seemed to be almost equal. It seems unusual that judges could perceive no differences, since in the physical viscosity measurements of the formula (Malcolmson and Kostolansky, 1978) differences from 1.48 to 8.48 centipoises were found. Perhaps the decision was made prematurely not to assess sensory viscosity in panelist training.

The relationship of physical viscosity of human milks to those of formulae can be seen in Figure 6. The

Figure 6

PHYSICAL VISCOSITIES OF SELECTED INFANT FORMULAE AND HUMAN MILK AT 37 °C



components of formulae leading to changes in physical viscosity are likely related to more than total fat content which tends to be similar in all types of formulae. Protein content varies between formulae and human milk. Protein content has been reported as 2.5 percent (w.v) in a soybased formulae (Prosobee, Mead Johnson, 1971) and was found in this study to be relatively low in value in human milk (mean of 1.06 grams percent). Enfalac (Mead Johnson, 1971) was reported as 1.5 percent (w/v) analyzed protein content. Cow's milk has been reported as 3.5 grams percent protein (Fomon, 1974). Also, the physiochemical properties of soybased proteins would be expected to be different from those of milk proteins. Heat treatment and homogenization of cow's milk formulae could be influencing their differing viscosities. Bateman and Sharp (1928) reported that homogenization of whole cow's milk, but not of skim milk, increased viscosity of the milk. Pasteurization was found to decrease viscosities slightly.

C. Sensory Mouthcoat of Human Milk in Comparison to That of Other Infant Foods

Human milk was scored low in mouthcoat in this study. Samples were considered to be lower in mouthcoat than the diluted Similac. It seems likely that human milk would be

found less mouthcoating than most of the infant formulae evaluated by Malcolmson and McDaniel (1980b), possibly equal in mouthcoat to one to two week whole milk formulae, the one found significantly least mouthcoating.

In referring to Figure 6 illustrating the physical viscosities of formulae and human milk, it can be seen that similar differences exist between fore and hind human milk as between one to two week whole milk and five - six month whole milk formulae. These latter two formulae were found significantly different in mouthcoat by panelists in Malcolmson and McDaniel's study (1980b). This fact lends support to the significant differences in mouthcoat found in this present study between fore and hind human milks.

S U M M A R Y A N D C O N C L U S I O N S

Twenty-four nursing mothers from the City of Winnipeg participated in a study on the characterization of sensory properties of human milk. Fore and hind milks from a single morning feed collected over three consecutive days were evaluated by a trained adult panel using magnitude estimation. The sensory attributes evaluated were sweetness, viscosity and mouthcoat.

Judges found the milks to be as sweet as or sweeter than a six percent lactose solution reference. There were differences in sweetness between mothers, a few were significantly different. An effect of decreasing sweetness over the three days of expression and tasting was not explained. Judges, in general, found the milks to be less viscous than the diluted Similac reference. Significant differences in viscosity were found between Mothers One to Twelve evaluated by Judges One to Three. Hind milks were perceived as more viscous than fore milks, although the differences in mean sensory scores were very small. Similarly, in mouthcoat evaluation, small, yet significant differences were noted between hind and fore milks and

between mothers in Mothers One to Twelve evaluated by Judges One to Three. No significant differences were found between Mothers Thirteen to Twenty-four evaluated by Judges Four to Six, in either viscosity or in mouthcoat. Compared to the diluted Similac reference, human milk can be described as thin and watery.

Off-flavours were described in fresh and frozen milk samples and their intensity noted by panelists. Off-flavour notes of varying intensity were found in thirty percent of all judgements made upon fresh milk samples. Descriptors used by judges varied and included such terms as metallic, fruity, vanilla, cooked milk and spicy. Even though some of the descriptors used may have represented off-flavours due to handling of the milks, such as oxidized or lipolyzed off-flavours, it is probable that many of the cited off-flavours were transmitted from the mother's diet or environment. Food records kept by the mother on each of the days previous to milk expression, were examined, however, it was not possible to associate particular foods or reported spiciness of the diet with incidences of off-flavours in the milks. Frozen milks had more frequent and more intense off-flavours described.

Samples of the milks were analyzed in the laboratory for lactose, fat and protein contents. Analysis revealed a range of mean lactose content over twenty-four mothers from 7.03 to 8.75 grams percent with a mean value of 7.81 grams percent. This value agreed well with means reported in the literature on human milk. Days had no effect upon lactose content, but hind milk was significantly lower in lactose than fore milk. Fat content ranged from means of 0.57 to 4.12 grams percent over twenty-four mothers with an overall mean of 2.17 grams percent. This value was low compared to other values reported in the literature, but reflects the tendency of early morning milks and milk from the first breast expressed to be lower in fat. This value is not representative of the fat content of human milk, therefore. Hind milks were significantly higher in fat content (3.21 grams percent) than fore milks (1.13 grams percent). Protein contents ranged across mothers from 0.63 to 2.15 grams percent, with a mean of 1.06 grams percent. Protein content in hind milk was significantly higher than that of fore milk. Significant first-order interactions occurred in lactose and fat analysis data. These interactions reflect the variable nature of human milk

where changes occurred over a feed but at different rates between different mothers. The relationship between chemical composition and sensory evaluation of the samples was examined statistically. Whereas similar trends could be seen in both sets of data, no significant correlations were found between sensory viscosity and fat content. Neither were there significant correlations between sensory sweetness and lactose content.

Instrumental viscosities of a subset of thirteen mothers' milks were determined. Physical viscosities in centipoises ranged from means of 1.308 to 1.633 between mothers and from means of 1.35 to 1.50 between fore and hind milks. These differences were significant. Physical viscosity and fat content were found to be significantly associated ($r = 0.65$, $p \leq 0.015$). Because multicollinearity was present between protein and fat contents and between lactose and protein contents, no further information of the effect of protein and lactose upon physical viscosities could be ascertained.

Mothers were asked by questionnaire about their nursing practices and their infants' responses at the breast. Demographic information on mother and infant was also

collected. Certain maternal parameters as maternal age, lactation stage, frequency of nursing per day, nursing style and parity were related to the sensory and physical properties of the milks using multiple regression. None of the parameters was found to be significantly associated with the sensory properties in the milks. Increasing maternal age was significantly associated with a lower fat content ($r = -0.48$, $p \leq 0.02$).

Mothers were also questioned regarding their freezing practices of expressed breast milk and their food practices during lactation. Eighteen mothers had fed frozen breast milk to their infants. Nine mothers had tasted their own breast milk; a "soapy" off-flavour had been noted by five of these mothers. Food avoidances during lactation were practiced by half of the mothers. "Gassy" vegetables and 'spicy' foods were cited most frequently in avoidances. The avoidance of these foods by half of the sample may have influenced the frequency of off-flavours noted in the milks.

Results of the sensory analysis of human milk were compared to results of other sensory studies on infant foods. By placing human milks on a sweetness power function, comparisons in intensity with a basic taste

stimulus were made. Human milk in this sample of mothers was found to be equivalent in sweetness to a 2.12 percent sucrose solution (0.06 Molar) with a range of 1.43 to 2.87 percent sucrose equivalency across twenty-four mothers. Therefore, human milks in this study were perceived as sweeter than those proprietary cow-milk based formulae evaluated by Malcolmson and McDaniel (1980b) as equivalent in sweetness to a 1.2 percent sucrose solution. Human milk was less sweet however, than home-prepared or soybased formulae with added sugars, evaluated as equivalent in sweetness to a 6.0 percent sucrose solution (Malcolmson and McDaniel, 1980b). Direct comparisons in viscosity and mouthcoat to other infant foods could not be made in this manner.

Based on the results of this study, it would be imprudent to say that formula feeding would lead to the development of a "sweet tooth". The entire problem of the development of sweet preference and its relationship to heredity and/or experience has not yet been resolved. This study should add new information to the search for understanding of this problem. An infant solely breastfed may be exposed to a stronger sweet stimulus than one fed

proprietary formulae.

The results of this study suggest that one must be cautious in making conclusions regarding the flavour and textural qualities of human milk and their subsequent effect upon the nursing infant. Hall (1975a) hypothesized that flavour and texture changes within a single feed could lead to the development of an appetite control. Her hypothesis, although untested, has been widely cited in the literature on human milk.

The sensory differences found in this study over a single feed were small and varied from mother to mother in degree of change.

Another area of study appearing more frequently in the psychological literature is the effect of maternal diet and mother's milk flavour upon learning in laboratory animals such as rats, in their selection of an adult, post-weaning diet. One must be very careful before extending these theories to the human infant and to the effect of human milk flavour on infant's later taste preferences. While off-flavour notes were common (30 percent of total possible samples tested) in fresh human milks in this study, sensory changes fore to hind were small; it is unknown whether the human infant could

recognize these changes. Differences between mothers in sweetness, viscosity and mouthcoat were also small, although significant differences were found. It is hard to imagine the infant recognizing the sensory qualities of its own mother's milk when such small differences exist, yet, it is not impossible. In a study by MacFarlane (1975), neonates turned their heads to their own mother's smell more frequently than not. If neonates could "recognize" their own mother's smell, it is also possible that they could "recognize" the taste of their own mother's milk. Apart from the possible role of the sensory factors in human milk on appetite control, these same sensory factors are likely important in the formation of bonding between mother and infant.

This study on the sensory characterization of human milk was intended to be a preliminary one where the major sensory qualities would be described and quantified. The problem of off-flavours in human milks was introduced. This study could be a starting point for further investigations into the causes and consequences of human milk off-flavours. Hopefully, through scientific research based on well-designed studies, mothers can be provided

with sound information regarding the effect of foods upon the flavour of her milk and upon her infant's response at the breast.

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Good Mothering Through Breastfeeding*The World Over**La Leche League International*

9616 Minneapolis Avenue Franklin Park, Illinois 60131, U.S.A.
Telephone: 312-455-7730

September 22, 1978

Mina R. McDaniel, Ph.D.
Department of Foods and Nutrition
The University of Manitoba
Winnipeg, Manitoba R3T 2N2
CANADA

Dear Dr. McDaniel,

Elizabeth Barker's request for LLL participation in her study of the flavor and texture of human milk has been cleared by our Research Review Committee. This clearance is only for their participation as described in the protocol you sent us. Any significant changes would have to receive a separate clearance. A copy of our policy regarding participation in research projects is enclosed.

Our Committee members did wonder whether what the mother eats might not affect the flavor of her milk so that perhaps a dietary history for the day or so preceding the expression of her milk sample might not be pertinent. Has this been considered?

They suggest that Ms. Baker clearly specify to each mother whether she wishes the first milk from the breast (fore) or hind milk, or a combination; or that this information at least be noted on the sample's container. Along these lines they also suggested it might be interesting to, at some time, compare the tastes of the fore milk (low fat), mid-milk, and hind milk (high fat content) to determine whether the taste may have some influence on the baby's sense of satiety. Barbara Hall did some work which suggests that change in taste and texture of human milk might serve as an appetite-control mechanism in breastfed babies (Lancet 1:779-81, April 5, 1975).

We are notifying Mrs. Judy Burlock, Area Coordinator of LLL of Manitoba, and Mrs. Francis Andrusiak, your local LLL contact, of this clearance. Please make arrangements with Mrs. Andrusiak for contacting women who are interested in volunteering for this study.

We would very much appreciate being informed of any findings or reports and/or copies of any published papers resulting from this research project.

Sincerely yours,

Carolyn Hayes
Carolyn Hayes, Coordinator of LLL
Participation in Research

Enc.
Not for profit organization — Donations tax deductible

Mina R. McDaniel, Ph.D.
page 2

September 22, 1978

cc: Mrs. Judy Burlock
8 Forestview Bay
Regina, Sask. S4R 0A9
CANADA
306-543-0644

Mrs. Francis Andrusiak
101 Elm Park Rd.
Winnipeg, Manitoba R2M 0W3
CANADA
204-233-9249

P.S. Please send us for our file a copy of the instructions for the mothers
when they are settled upon.

Appendix B

*Good Mothering
Through Breastfeeding*



The World Over

La Leche League International

9616 Minneapolis Avenue Franklin Park, Illinois 60131

LLLI Policy with Regard to Participation in Research Projects

All projects must be cleared by LLLI's Research Review Committee. The following requirements govern LLL participation in such projects:

1. Participation is Voluntary. The mothers will be approached first by the La Leche League liaison person who will explain the project and exactly how they can participate. The names of the volunteers may then be given to the researcher to contact personally, or the LLL liaison person may handle this.
2. Babies are not to be deprived, even for a very short period, of either their mother's milk or of mother herself.
3. Babies are under no circumstances to be given food, formula or anything other than mother's milk for the purpose of the study. If during the course of the research project a baby shows he/she is ready for solids, the mother should put her baby's need ahead of the project. If babies are older and already on solids, they should not be given foods other than those to which they've already been introduced.
4. LLL groups and/or individuals who volunteer should not be put to any expense for the study. The LLL liaison person's only responsibility is to inform the women about the project and give them the opportunity to volunteer if they would like to do so. On her own, the liaison person may agree to take on additional responsibilities.

A League group may decide to undertake to act as a collection point for milk samples if they wish. However, collections and transportation are the responsibility of the researcher and there is no reason for League mothers to feel obligated to take this on, or to be burdened in any way.

Appendix C

Submission to Ethics Committee Faculty of Home Economics,
University of Manitoba
December, 1978.

THE SENSORY EVALUATION OF HUMAN MILK

Investigators: Elizabeth Barker and Mina McDaniel

Relationship to subjects: None

Purpose of the research:

Contrary to previous prevailing medical thought, human milk as a biological fluid does not exhibit constancy; in fact, human milk shows great variation of volume and constituents between mothers and within the same mother at different times of sampling. In an often-cited article, Hall (1975) hypothesized that the demonstrated change in fat composition of human milk from beginning to end of feed could act as an appetite-control mechanism in breast-fed infants, which would be less evident in pooled breast milk and commercial infant formulae. It is possible that the variation of constituents in human milk could have physiological significance.

The purpose of this research is to characterize the flavour and other organoleptic properties of human milk, particularly noting those changes which occur during one feed and those differences that exist between mothers.

Methodology:

It is proposed that milk samples will be collected from lactating mothers for sensory analysis by a trained adult panel. Mothers will be volunteers attending La Leche League meetings in the City of Winnipeg and

will be selected with the help of a La Leche League coordinator (Mrs. Ronna Filkow) and La Leche leaders. Approval from La Leche League International has been already obtained. The leaders will act as liaison with the mothers and will obtain names of interested mothers after explaining the study. The investigators will meet with the coordinator and the leaders to explain the study and will maintain contact with these persons throughout the study.

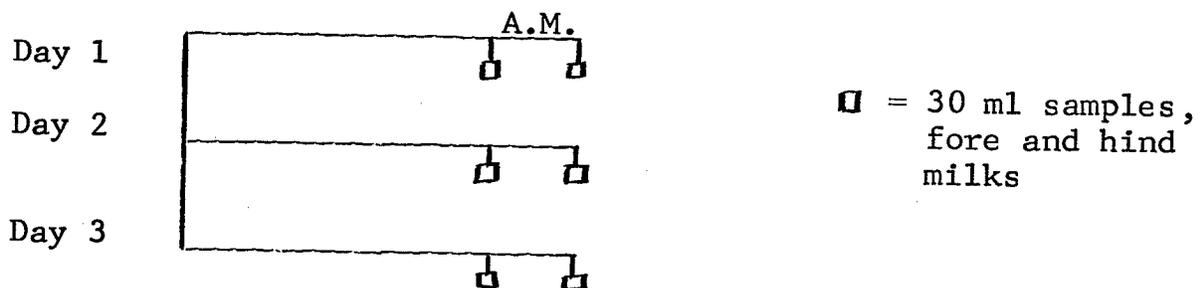
The population has been restricted to mothers of infants 2 - 6 months of age. No other limits have been placed upon the population (age of mother, parity).

A few days before the study, one researcher will deliver containers to each participating mother, at the same time welcoming her to the study, explaining the food record and collection procedures again and answering questions. On the days of the study, milks will be picked up and brought to the foods laboratory for sensory evaluation by a trained panel within four hours of milk expression.

Viscosity measurements will be made on the milks before tasting.

Analytical procedures will be performed on small aliquots of each original sample.

Plan of Experiments:



The study will run approximately six weeks. One group of six mothers will participate each week.

Risk and Benefits to the subjects: None.

Compensation to the mothers: None.

Confidentially ensured: The milk samples will be coded.

Information sought from the mothers:

The mothers will be asked to answer a brief questionnaire and to keep a food record for three days. These forms are included. As well, instructions for handling of samples and a covering letter will be given to each mother.

Proposed time of the study:

Approximately February to April, 1979.

Reference cited:

Hall, B., (1975) Changing composition of human milk and early development of appetite control. Lancet 1:779.

January 10, 1979.

Dr. Vivian M. Bruce,
Chairman, Ethics Committee,
Faculty of Home Economics,
University of Manitoba.

Dear Dr. Bruce:

Re: Reply to the Ethics Committee

Thank you for your letter advising me of the approval of the Ethics Committee of the proposal entitled, "The Sensory Evaluation of Human Milk". The Committee raised some points which I propose to answer as the following:

(1) The researcher will present her study to a group of La Leche leaders. The researcher will obtain names of interested mothers from these leaders after each of the leaders in turn has explained the study to a group of mothers. The researcher will then contact each mother, ascertain her interest in the study, visit her and explain the study further. The questionnaire and food record form will be explained at this time. The researcher will obtain informed consent from each mother at this time, also.

Subsequent contact will be made one week prior to the beginning of the study.

The researcher will collect the milk samples.

(2) On meeting with the La Leche League coordinator, the researchers decided that 30 ml x 2 was a "reasonable" sample of milk to collect from the mothers. Two mothers demonstrated that this would be possible. It may be necessary to limit the study to those mothers that can "donate" this amount of milk, since the researchers feel that this amount of milk represents the minimal useful sample for tasting by two judges.

(3) Confidentiality will be fully explained to the mothers. Mrs. Barker will set up and have access to the code. Initially, the researcher will have the names and addresses of participating mothers, and because she is actually collecting the samples, the mothers will not be anonymous to her. The names will not be used again once the samples are collected.

page 2

January 10, 1979.

I hope these points have answered the concerns raised by the Committee to their satisfaction.

Thank you.

Yours truly,

Elizabeth Barker

Appendix D



THE UNIVERSITY OF MANITOBA
FACULTY OF HOME ECONOMICS
WINNIPEG, CANADA R3T 2N2
TELEPHONE 204 474-9901

DEPARTMENT OF FOODS AND NUTRITION

January 16, 1979

Dear Mother,

I am a graduate student at the University of Manitoba who is presently conducting a study on the flavour of human milk. I am assuming that you have heard about this study from a La Leche leader. Your interest would be appreciated.

Because early food and taste experiences may have some influences on the development of taste and food preferences in later childhood, we are interested in examining the flavours in breast milk. Also of interest is whether unusual flavours of foods consumed by the mother can be detected in breast milk by a trained taste panel.

In order to carry out the study, I would appreciate your help in the following ways: (1) An expression of two samples of milk for three consecutive days at the first morning feeding. This will involve expression of approximately one ounce of milk before the baby nurses (foremilk) and another ounce of milk after the baby finishes (hindmilk). These samples will be taken from the first breast offered to the baby only; (2) Answering some questions prior to the study about your baby and his/her nursing patterns; (3) Keeping a record of foods consumed for one complete day before milk expression. Forms for record keeping will be provided. The study will take place during February and March. Containers for milk collection would be provided. The milk samples will be picked up at your home.

If you are interested in taking part in this study, your name will be given to me by your La Leche leader. I shall then contact you to explain the study further and to answer any questions you may have.

All information given will be available only to myself and to the Department of Foods and Nutrition, University of Manitoba, and will be used only for the purpose of this study.

Thank you for your anticipated interest. If you think you would like to participate, please give your name to a La Leche leader.

Yours truly,

Elizabeth Barker

EB/mk

Appendix E

CONSENT FORM

I, _____, do hereby freely consent to participate in the study conducted by the Department of Foods and Nutrition at the University of Manitoba entitled "The Sensory Evaluation of Human Milk". The conditions of the study have been fully explained to me by the experimenters and I understand them completely. I also understand that I am free to withdraw from the study at any time without penalty and that all information will remain strictly confidential.

Signature _____

Date _____

Appendix F
HUMAN MILK STUDY

PROCEDURES FOR HANDLING SAMPLES

1. Express milk in the way most comfortable for you, into a clean, dry container.
2. Express milk between approximately 7:00 and 8:30 A.M. as follows:
Foremilk - about one ounce or more before the baby nurses on the first breast,
Hindmilk - about one ounce or more after the baby finishes nursing on the first breast.

DO NOT MIX THE MILKS.

3. Transfer the milks into the clean, labelled containers provided by the University of Manitoba. Cover tightly. Refrigerate immediately. DO NOT FREEZE.
4. The milks will be picked up each day between 9:00 and 10:00 A.M. If you will not be home, please call:
Mrs. Liz Barker
474-9901
5. Express milk for 3 days in a row. The milks will be picked up each day.

Appendix G

QUESTIONNAIRE One
HUMAN MILK STUDY

Dates of Collections;

Name of mother _____

Address _____

Telephone No. _____

BABY:

Date of birth _____

Birth weight _____

Present Age of Infant _____

Age of mother _____

Length of pregnancy _____ weeks.

Number of children _____ (including this infant).

Number of children breastfed _____ (including this infant).

1. I nurse my baby _____ times a day.

2. My baby has a night feed. yes _____ no _____

If yes, at _____ A.M.

or, my baby nurses often at night. yes _____ no _____3. My baby nurses about _____ minutes at the first breast,
and about _____ minutes at the second breast.

4. I think that my baby has had enough milk at the first breast because,

_____ he/she stops sucking.

_____ he/she goes to sleep.

_____ he/she lifts his head away.

_____ other reasons, such as,

Appendix H

HUMAN MILK STUDY

Code no _____

Questionnaire Two

1. Frozen Milk Questionnaire

Do(Did) you ever express your milk and freeze it? _____
 If not, please proceed to section 2.

If so, which of the following types of freezing compartments do you use?

_____ freezing compartment in one-door refrigerator
 _____ freezing compartment in 2 door refrigerator
 _____ deep freeze

How long do you usually store the milk? _____ days
 _____ weeks
 _____ months

How long is the milk initially kept in the refrigerator before placing in the freezer? _____ hours

Describe your thawing techniques. . .

in refrigerator _____ time _____
 warmed in any way _____ time _____
 shaken in any way, etc.

Do(Did) you feed the milk to your own baby? _____

Or donate the milk to _____.

How does your baby respond to frozen breast milk?

Appears to like it _____
 Appears to reject it _____

Have you ever tasted thawed frozen breast milk yourself?

Describe the flavour _____.

2. Flavour in Milk

Has your baby ever appeared to reject the breast or appeared "fussy" because of something in your milk? _____

If so, are there any particular foods you avoided?

List them: _____

Please describe the observed response in your baby.

continued

Are there any particular foods that you eat more of because you are nursing?

Where have you obtained information about foods to eat or avoid while nursing?

Books _____ specify, if possible _____

La Leche league _____

Other Mothers _____

Doctors _____

Others _____ specify _____

Decided yourself _____

* * * * *

Appendix I



THE UNIVERSITY OF MANITOBA
FACULTY OF HOME ECONOMICS
WINNIPEG, CANADA R3T 2N2
TELEPHONE 204 474-9901

DEPARTMENT OF FOODS AND NUTRITION

August 3, 1979

Dear

The taste panel evaluations of taste and texture of human milk have been completed. I am now in the process of analyzing the milk samples in the laboratory and will be analyzing the data in the near future.

This study could not have been undertaken without your willing help and cooperation. Most of all, I appreciated the interest that you showed in the study when I talked with you at various times. Working with the members of La Leche league has been one of the most enjoyable parts of my study.

At the end of the taste evaluations, I decided to look at frozen breast milk flavour. Because this part of the study did not involve many mothers, the information gained has led me to many further questions. I also have some further questions about your own dietary habits and your infant's response at the breast.

I would appreciate your taking the time to answer the enclosed questionnaire. Please return it to me in the enclosed envelope at your earliest convenience.

Thank you again for your help.

Yours truly,

Elizabeth Barker

EB/mk

Appendix J

RECORD OF FOODS CONSUMED ON THE DAY PREVIOUS TO MILK COLLECTION

Date: _____

Name: _____

Please list all foods and drink consumed. Pay particular attention to spicy foods, salty foods and "gassy foods".

Breakfast

Snacks

Lunch

Supper

PLEASE ANSWER THE FOLLOWING: (These question are asked because the flavour of the milk could be affected)

How many cups of coffee do you drink each day? _____

Are you at present taking any medications? If so, which ones _____

Do you smoke? _____ If so, how many cigarettes per day _____

APPENDIX K

Ballot Used in Sensory Evaluation of Human Milk Samples

Name _____

Date _____

Sensory Magnitude Estimates on Milk

You have before you six (6) samples of milk and two (2) references - one for sweetness and one for viscosity and mouthcoat.

Please estimate each of the attributes on each sample: one attribute at a time. Do not swallow the samples.

- (1) SWEETNESS and "OFF FLAVOUR"
Taste reference. Place 1/3 sample in mouth, swirl, expectorate. Rate sweetness.

Describe off flavour, if any, and its intensity using terms at the bottom of ballot.

- (2) VISCOSITY Taste reference.
Place second sip of sample in the mouth and measure force required to draw the sample between tongue and palate. Rate viscosity.

- (3) MOUTHCOAT Taste reference
Place third sip of sample in the mouth, swirl and expectorate. Rate mouthcoat.

SAMPLE CODE NO.	MAGNITUDE ESTIMATE VALUE			"OFF" FLAVOUR	
	SWEETNESS	VISCOSITY	MOUTHCOAT	DESCRIPTOR	INTENSITY
REFERENCE	10	10	10	X	X

detectable slight moderate strong extreme

Appendix L

The following formula was used to determine lactose concentrations for each sample:

$$c = \frac{V \times MW}{d \times v \times 1000} \times E \text{ (g/l)}$$

Where V = final volume (ml)
 MW = molecular weight of substance to be assayed
 = extinction coefficient of NADH at Hg365 = 3.4
 d = light path (cm)
 v = sample volume (ml)

$$\begin{aligned} \text{or } c &= \frac{3.30 \times 342.3}{1 \times 0.1 \times 1000} \times E \text{ lac} = \\ &= 11.296 \times \frac{E \text{ lac}}{3.4} \text{ (g lactose) l sample solution} \end{aligned}$$

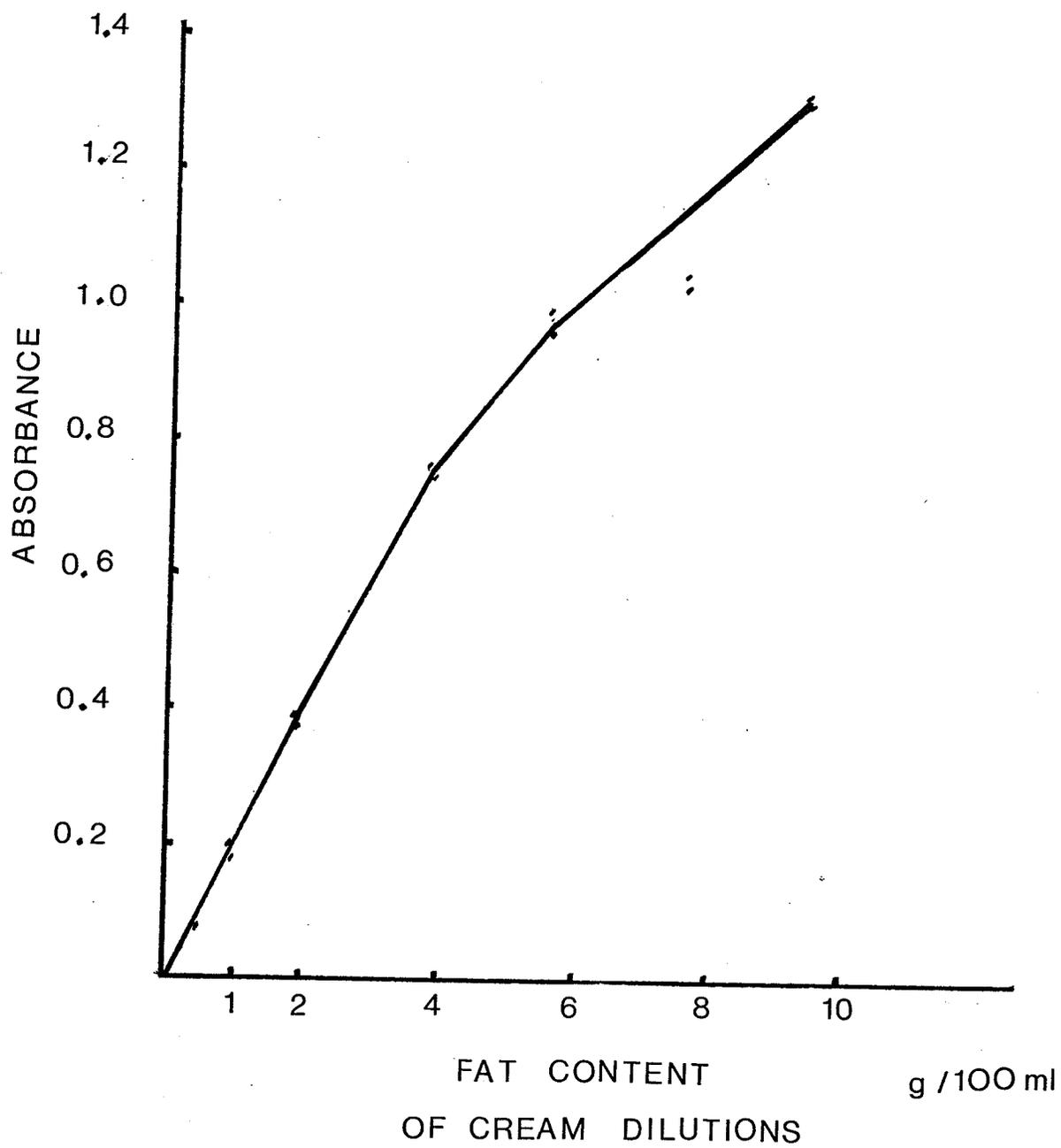
where 3.4 is c or extinction coefficient of NADH at 365 nm.

Since human milk samples had been diluted 1:100, the results were multiplied by dilution factor of 100 to yield lactose content in g/l. Results were presented as lactose g/100 ml obtained by dividing g/l by 10.

Appendix M

Calculated Fat Contents of Cream Sample Dilutions
Used in Preparation of Standard Curves for Fat Analysis
Cream Sample (Bligh and Dyer, 1959) = 9.35 g/100 ml

<u>Dilution</u>	<u>Fat Content g/100 ml</u>
1:20	0.47
1:10	0.94
1:5	1.87
1:2.5	3.74
1:1.66	5.61
1:1.25	7.48
1:0	9.35

STANDARD CURVE FOR ESTIMATION OF
FAT CONTENT OF HUMAN MILK

Appendix O

Sensory Evaluation of Human Milk

Model for ANOVA Testing

Mixed Effects Model 12 x 3 x 2 x 3 Factorial Design

Variable	E (ms)	F Value
Mother	$\sigma^2 + 18 \sigma^2_{\mu}$	$\sigma^2 + 18 \sigma^2_{\mu} / \sigma^2$
Day	$\sigma^2 + 6 \sigma^2_{\mu\delta} + 72 \sigma^2_{\delta}$	$\sigma^2 + 6 \sigma^2_{\mu\delta} + 72 \sigma^2_{\delta} / \sigma^2 + 6 \sigma^2_{\mu\delta}$
Mother x Day	$\sigma^2 + 6 \sigma^2_{\mu\delta}$	$\sigma^2 + 6 \sigma^2_{\mu\delta} / \sigma^2$
Time	$\sigma^2 + 9 \sigma^2_{\mu T} + 108 \sigma^2_T$	$\sigma^2 + 9 \sigma^2_{\mu T} + 108 \sigma^2_T / \sigma^2 + 9 \sigma^2_{\mu T}$
Mother x Time	$\sigma^2 + 9 \sigma^2_{\mu T}$	$\sigma^2 + 9 \sigma^2_{\mu T} / \sigma^2$
Day x Time	$\sigma^2 + 3 \sigma^2_{T\delta\mu} + 36 \sigma^2_{T\delta}$	$\sigma^2 + 3 \sigma^2_{T\delta\mu} + 36 \sigma^2_{T\delta} / \sigma^2 + 3 \sigma^2_{T\delta\mu}$
M x D x T	$\sigma^2 + 3 \sigma^2_{T\delta\mu}$	$\sigma^2 + 3 \sigma^2_{T\delta\mu} / \sigma^2$
Judges & Interactions	σ^2	

Where μ = mother
 T = time
 δ = day

Appendix P

Model for ANOVA for Chemical and Physical Data of Human Milk
Mixed Effects Model 24 x 3 x 2 Factorial Design

Variable	E (ms)	F Value
Mother	$\sigma^2 + 6 \sigma^2 \mu$	$\sigma^2 + 6 \sigma^2 \mu / \sigma^2$
Day	$\sigma^2 + 2 \sigma^2 \mu \delta + 48 \sigma^2 \delta$	$\sigma^2 + 2 \sigma^2 \mu \delta + 48 \sigma^2 \delta / \sigma^2 + 2 \sigma^2 \mu \delta$
Mother x Day	$\sigma^2 + 2 \sigma^2 \mu \delta$	$\sigma^2 + 2 \sigma^2 \mu \delta / \sigma^2$
Time	$\sigma^2 + 3 \sigma^2 \mu \tau + 72 \sigma^2 \tau$	$\sigma^2 + 3 \sigma^2 \mu \tau + 72 \sigma^2 \tau / \sigma^2 + 3 \sigma^2 \mu \tau$
Mother x Time	$\sigma^2 + 3 \sigma^2 \mu \tau$	$\sigma^2 + 3 \sigma^2 \mu \tau / \sigma^2$
Day x Time	$\sigma^2 + \sigma^2 \mu \delta \tau + 24 \sigma^2 \tau \delta$	$\sigma^2 + \sigma^2 \mu \delta \tau + 24 \sigma^2 \tau \delta / \sigma^2$
Error Term	σ^2	

Where μ = mother
 δ = day
 τ = time

Appendix Q

Sensory Evaluation of Human Milk

Data Analysis by ANOVA

Number of observations (judgements) included in the mean sensory score for each variable and interation tested by ANOVA.

eg., Group I: for sweetness*
12 x 3 x 2 x 3 factorial design = 216 observations in total

Main Effects (Means)

Mother	n = 18	(216/12)
Day	n = 72	(216/3)
Time	n = 108	(216/2)

Interactions

Mother x Time	n = 9	(216/24)
Mother x Day	n = 6	(216/36)
Day x Time	n = 36	(216/6)
Mother x Day x Time	n = 3	(216/72)

Error Term was judges and all judge factor interactions.

The analysis was repeated for Group II and for each sensory attribute.

Appendix R

Instrumental and Chemical Analysis of Human Milk

Data Analysis by ANOVA

Number of observations included in mean value for each variable and interaction tested.

24 x 3 x 2 factorial design = 144 volume in total

Main Effects (Means)

Mother	n = 6	(144/24)
Day	n = 48	(144/3)
Time	n = 72	(144/2)

Interactions

Mother x Time	n = 3	(144/48)
Mother x Day	n = 2	(144/72)
Day x Time	n = 24	(144/6)

(Mother x Day x Time) Error Term.

Appendix S_i

Analysis of Variance for Sensory Sweetness

Group I Mothers 1 - 12

Source of Variation	df	SS	MS	F	Prob.
Mother	11	0.6420	0.0584	1.756	0.068
Day	2	1.0408	0.5204	20.027	0.0000
M x D	22	0.5717	0.0259	0.782	(0.783)
Time	1	0.0045	0.0045	0.154	(0.702)
M x T	11	0.3223	0.0293	0.881	(0.560)
D x T	2	0.0419	0.0209	0.840	(0.327)
M x D x T	22	0.5484	0.0249	0.750	(0.768)
Error Term	138	4.5878	0.0332		
Total	209	7.7592			

Appendix S_{ij}
 Analysis of Variance for Sensory Sweetness
 Group II Mothers 13 - 24.

Source of Variation	df	SS	MS	F	Prob.
Mother	11	0.2799	0.0254	1.926	0.041
Day	2	0.1027	0.0513	5.931	0.009
M x D	22	0.1905	0.0087	0.655	(0.875)
Time	1	0.0098	0.0098	1.215	(0.293)
M x T	11	0.0892	0.0081	0.614	(0.814.)
D x T	2	0.0081	0.0040	0.881	(0.437.)
M x D x T	20	0.0922	0.0046	0.349	(0.996)
Error	134	1.7701	0.0132		
Total	203	2.5426			

Appendix T

Detailed Calculations of the Method Used to
Place Sweetness Scores of Human Milks on the
Power Function for Sucrose

Mother 3 Mean Sweetness estimate = 0.0961

$$\text{antilog} = 1.2477$$

$$\begin{aligned} \text{Ratio (R)} &= \frac{1.2477}{1.0632 \text{ mean reference score}} \\ &= 1.1735 \end{aligned}$$

Magnitude Estimate of 6 percent lactose solution = 0.6381

$$\text{ie., } y = 0.3090 (1.8684)^{1.16} = 0.6381$$

$$y^1 = (R) (y) \text{ or } (1.1735) (0.6381)$$

$$= 0.7522$$

$$\log y^1 = -0.1237$$

$$\text{Therefore } \log x = \frac{\log y^1 - \log a}{b}$$

$$\text{or } = \frac{-0.1237 - (-0.5100)}{1.16}$$

$$\log x = 0.3330$$

Antilog $x = 2.1529$ which represents the percent of sucrose solution (w/v) equivalent in sweetness to mean magnitude estimate of milk of mother 3.

Appendix U_j
 Analysis of Variance for Sensory Viscosity
 Group I Mothers 1 - 12

Source of Variation	df	SS	MS	F	Prob.
Mother	11	1.5202	0.1382	5.135	0.000
Day	2	0.0027	0.0013	0.056	(0.945)
M x D	22	0.5196	0.0236	0.908	(0.584)
Time	1	0.0945	0.0945	5.226	0.043
M x T	11	0.1990	0.0181	0.696	(0.740)
D x T	2	0.0207	0.0137	0.416	(0.734)
M x D x T	22	0.7263	0.0330	1.269	(0.203)
Error	138	3.5880	0.0260		
Total	209	6.6710			

Appendix U ii

Analysis of Variance for Sensory Viscosity

Group II Mothers 13 - 24

Source of Variation	df	SS	MS	F	Prob.
Mother	11	0.1318	0.1198	1.132	(0.348)
Day	2	0.0058	0.0288	0.221	(0.803)
M x D	22	0.2863	0.0130	1.229	(0.234)
Time	1	0.0789	0.0789	10.006	0.009
M x T	11	0.0868	0.0079	0.745	(0.693)
D x T	2	0.0066	0.0033	0.264	(0.770)
M x D x T	20	0.2491	0.0125	1.176	(0.285)
Error	134	1.4187	0.0106		
Total	203	2.2650			

Appendix Vj

Analysis of Variance for Sensory Mouthcoat

Group I Mothers 1 - 12

Source of Variation	df	SS	MS	F	Prob.
Mother	11	1.3052	0.1187	4.918	0.000
Day	2	0.0463	0.0231	0.550	(0.584)
M x D	22	0.9253	0.0421	1.743	0.029
Time	1	0.1264	0.1264	8.141	0.016
M x T	11	0.1708	0.0155	0.644	(0.789)
D x T	2	0.0254	0.0127	0.414	(0.667)
M x D x T	22	0.6768	0.0308	1.275	(0.199)
Error Term	138	3.3297	0.0241		
Total	209	6.0628			

Appendix V_{ij}

Analysis of Variance for Sensory Mouthcoat

Group II Mothers 13 - 24

Source of Variation	df	SS	MS	F	Prob.
Mother	11	0.1819	0.0165	1.295	(0.234)
Day	2	0.0403	0.0201	1.414	(0.264)
M x D	22	0.3134	0.0142	1.116	(0.338)
Time	1	0.0064	0.0064	0.477	(0.504)
M x T	11	0.1484	0.0135	1.056	(0.401)
D x T	2	0.0208	0.0104	1.086	(0.356)
M x D x T	20	0.1913	0.0096	0.780	(0.734)
Error Term	134	1.7109	0.0128		
Total	203	2.6156			

Appendix W

Fresh Human Milk

Analysis of Variance for Physical Viscosity

13 Mothers (at 30 rpm)

Source of Variation	df	SS	MS	F	Prob.
Mother	12	0.5826	0.0486	9.812	.0000
Day	2	0.0067	0.0033	0.3106	(0.736)
M x D	23	0.2461	0.0107	2.163	0.046
Time	1	0.3884	0.3884	16.275	0.002
M x T	12	0.2864	0.0239	4.823	0.001
D x T	2	0.0353	0.0176	3.567	0.048
Error Term	19	0.0940	0.0049		
Total	71	1.6395			

Appendix X

Results of Analysis of Lactose Content of Human Milk

(Lactose and Free Galactose Content g/100 ml.)

Mother Code	No.	Day 1		Day 2		Day 3	
		Fore	Hind	Fore	Hind	Fore	Hind
229	1	8.07	7.08	9.07	7.71	9.67	8.90
868	2	7.28	7.84	8.77	8.54	11.33	8.77
966	3	7.44	7.51	8.41	7.94	9.47	8.41
057	4	7.44	7.34	7.28	7.58	8.14	7.64
144	5	7.64	7.28	7.48	7.51	8.24	7.94
504	6	7.48	6.38	7.04	7.04	7.74	7.28
381	7	7.48	7.01	6.84	6.61	7.21	7.04
184	8	7.84	7.54	7.81	7.38	7.14	7.24
152	9	7.51	7.48	6.81	6.61	6.65	7.41
278	10	8.24	7.54	8.24	7.71	8.27	7.54
495	11	7.81	7.44	7.87	7.91	7.51	7.71
899	12	7.87	7.61	7.31	7.41	6.98	7.44
933	13	8.31	8.24	8.44	8.24	8.37	8.67
516	14	6.88	8.37	8.14	8.04	8.90	8.27
258	15	8.47	8.34	8.28	8.51	8.21	8.24
465	16	7.60	7.44	8.27	8.07	9.10	9.14
106	17	7.34	7.87	8.74	7.74	8.80	9.73
185	18	8.87	8.44	7.90	7.61	8.21	8.07
997	19	8.11	8.04	7.31	7.31	7.48	7.64
066	20	7.54	6.78	7.21	6.98	7.24	6.91
834	21	7.74	7.97	7.04	7.24	7.91	-
420	22	8.27	7.71	7.48	8.57	7.64	7.77
731	23	7.84	7.44	7.87	7.87	7.71	7.08
555	24	8.11	7.51	8.04	7.54	7.14	6.98

Appendix Y
 Analysis of Variance for Lactose Content
 of Fresh Human Milk
 24 Mothers

Source of Variation	df	SS	MS	F	Prob.
Mother	23	31.6181	1.3747	7.977	0.000
Day	2	2.6615	1.3328	1.526	(0.228)
M x D	46	20.1555	0.8732	5.067	0.000
Time	1	1.4300	1.4300	9.289	0.005
M x T	23	3.5408	0.1540	0.893	(0.605)
D x T	2	-	-	-	-
Error (M x D x T)	45	7.7549	0.1723		
Total	142	67.1508			

Appendix Z

Results of Analysis of Fat Content in Human Milk

(g/100 ml)

Mother		Day 1		Day 2		Day 3	
Code	No.	Fore	Hind	Fore	Hind	Fore	Hind
229	1	0.55	7.40	0.85	1.76	1.20	2.35
868	2	0.26	3.75	1.65	5.72	0.55	6.95
966	3	0.20	5.00	0.22	8.65	0.30	6.60
057	4	0.87	2.05	2.15	2.90	0.55	4.30
144	5	0.60	1.70	0.45	1.20	0.80	1.95
504	6	1.55	9.60	3.10	2.90	2.30	5.25
381	7	2.25	5.55	2.75	5.20	1.00	1.93
184	8	2.35	3.55	0.75	1.70	2.85	3.65
152	9	0.35	0.90	1.20	1.75	1.60	3.68
278	10	1.50	3.15	0.75	1.51	0.65	1.90
495	11	1.45	7.10	1.45	7.80	0.40	2.25
899	12	1.55	3.15	2.75	2.75	0.70	0.75
933	13	0.63	0.96	-	1.87	0.70	2.16
576	14	2.60	3.35	2.10	2.70	2.85	3.10
258	15	1.00	1.38	0.55	5.60	3.15	2.72
465	16	2.10	5.00	0.65	2.90	0.75	3.15
106	17	1.30	1.05	0.40	2.05	0.30	2.60
185	18	0.90	2.50	0.20	2.25	0.90	2.65
997	19	1.15	0.15	0.40	0.85	0.10	0.75
066	20	2.05	3.85	0.45	1.60	0.75	1.10
834	21	1.30	2.80	0.75	5.40	1.15	-
420	22	0.40	4.45	2.05	2.15	0.50	3.47
731	23	0.55	2.75	0.65	2.00	0.87	4.63
555	24	0.45	2.25	0.65	2.05	0.65	2.30

Appendix A A
 Analysis of Variance for Fat Content
 24 Mothers

Source of Variation	df	SS	MS	F	Prob.
Mother	23	101.8549	4.4287	3.079	0.000
Day	2	2.3580	1.1790	0.707	(0.498)
M x D	46	76.7255	1.6679	1.159	(0.310)
Time	1	153.5040	153.5040	43.030	0.000
M x T	23	82.0498	3.5674	2.480	0.005
D x T	2	0.6821	0.3411	0.239	(0.788)
Error (M x D x T)	44	63.2870	1.4383		
Total	141	480.4614			

Appendix BB

Results of Analysis of Protein Content in Human Milk

(g/100 ml)

Mother Code	No.	Day 1		Day 2		Day 3	
		Fore	Hind	Fore	Hind	Fore	Hind
229	1	1.10	1.84	1.14	1.28	1.29	1.54
868	2	0.93	1.22	1.04	1.61	1.02	1.50
966	3	0.92	1.29	0.94	1.44	1.13	1.53
057	4	0.82	1.00	0.90	1.17	0.94	1.32
144	5	1.12	1.20	1.15	1.17	1.13	1.17
504	6	1.00	-	1.02	1.33	1.13	1.44
381	7	1.04	1.41	1.18	1.36	1.07	1.21
184	8	0.78	0.88	0.70	0.82	0.88	0.95
152	9	0.70	0.71	0.75	0.78	0.78	0.79
278	10	0.70	0.79	0.67	0.76	0.70	0.84
495	11	0.70	0.96	0.70	1.16	0.73	0.81
899	12	0.79	0.88	0.70	0.71	0.79	0.78
933	13	1.26	1.35	-	1.47	1.36	1.57
516	14	1.88	1.93	1.73	1.80	1.92	1.92
258	15	2.01	1.89	1.97	2.65	2.33	2.04
465	16	1.18	1.66	0.85	0.92	0.96	1.09
106	17	1.02	1.06	0.98	0.98	0.87	1.08
185	18	0.94	1.09	0.96	0.99	1.06	1.06
997	19	0.71	0.65	0.72	0.70	0.72	0.75
066	20	0.61	0.77	0.58	0.60	0.57	0.52
834	21	0.73	0.81	0.70	1.01	0.68	-
420	22	0.72	0.87	0.66	0.75	0.69	0.85
731	23	0.74	0.86	0.80	0.86	0.74	1.00
555	24	0.82	1.04	0.82	1.10	1.04	0.80

Appendix CC
 Analysis of Variance for Protein Content of
 Fresh Milk Samples

Source of Variation	df	SS	MS	F	Prob.
Mother	23	18.2727	0.7945	60.649	0.000
Day	2	0.0500	0.0250	1.250	(0.295)
Mother x Day	46	0.9183	0.0200	1.527	(0.083)
Time	1	0.9860	0.9860	43.057	0.000
Mother x Time	23	0.5269	0.0229	1.748	(0.056)
Day x Time	2	0.0332	0.0166	1.266	(0.292)
Error (M x D x T)	43	0.5636	0.0131		
Total	140	21.3506			

Appendix DD i
Analysis of Variance Table for Regression Coefficient for
Sensory Viscosity and Fat Content
of Human Milk

Source of Variation	df	ms	F	p > F	r ²
Model (Fat)	1	0.0026	0.64	0.432	0.028
Error	22	0.0041			
Total	23				

Appendix DD ii

Analysis of Variance Table for Regression Coefficient for
Sensory Viscosity and Physical Viscosity
of Human Milk

Source of Variation	df	ms	F	p > F	r ²
Model (Physical Viscosity)	1	0.0018	0.27	0.614	0.224
Error	11	0.0066			
Total	12				

Appendix DD iii

Analysis of Variance Table for Regression Coefficient for
Sensory Sweetness and Lactose Content
of Human Milk

<u>Source of Variation</u>	<u>df</u>	<u>ms</u>	<u>F</u>	<u>p > F</u>	<u>r²</u>
Model (Lactose)	1	0.00002	0.01	0.924	0.000(4)
Error	22	0.0241			
Total	23				

Appendix EE

Analysis of Variance Table for Regression Coefficient for
Correlation of Physical Viscosity
and Fat Content of Human Milk

Source of Variation	df	ms	F	p > F	r ²
Model (Fat)	1	0.0381	8.21	0.015	0.427
Error	11	0.0046			
Total	12				

Parameter:	Estimate:	T for H ₀ : parameter=0	p > (T):
Intercept	1.29 (a)	24.76	0.0001
Fat	0.07 (b)	2.87	0.015