Gastric pH and microflora of normal and diarrhoeic infants

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SUMMARY The microflora and pH of gastric contents were determined in breast-fed and in bottle-fed normal infants, in well nourished infants with acute diarrhoea and in infants with chronic diarrhoea and protein-calorie malnutrition. The last group of infants was reevaluated after recovery from diarrhoea and protein-calorie malnutrition.

A bactericidal pH effect below 2.5 was observed. Bottle-fed controls had low pH values and low bacterial concentrations, whereas infants with chronic diarrhoea and protein-calorie malnutrition had high pH values and bacterial overgrowth, essentially of Gram-negative bacilli. After recovery, the only remaining alteration was the frequent isolation of yeast-like fungi in low concentrations.

Infants with acute diarrhoea, except for the isolation more frequently of yeast-like fungi, presented no alterations; this seems to indicate that pH alterations and Gram-negative bacilli overgrowth occurred during the evolution of the disease to a chronic state.

Breast-fed normal infants had hydrogen-ion concentrations similar to those of the chronic diarrhoea group, but without Gram-negative bacilli overgrowth, suggesting that other factors, besides pH, regulate bacterial growth in the gastric contents of these groups of infants.

One important function of the gastric acid secretion is to prevent the passage of ingested microorganisms in a viable form to lower segments of the gastrointestinal tract (Drasar, Shiner, and McLeod, 1969). Earlier observations, reviewed by Wolman in 1943, suggest that children with a variety of nutritional and/or gastrointestinal disturbances have a decreased acidity of their gastric contents; this may be one contributory factor to the small bowel overgrowth syndrome described by Coello-Ramirez, Lifshitz, and Zuniga (1972), Gracey and Stone (1972), Mata, Jiménez, Córdón, Rosales, Prera, Schneider, and Viteri (1972), and Challacombe, Richardson, Rowe, and Anderson (1974) in diarrhoeic infants. As those former studies were incomplete and conducted in a heterogeneous group of children (Chievitz, 1921; Klementsson, 1923; Muhl, 1925), our aim was to reassess the deficiency of the gastric acid secretion in infants with diarrhoea and, if proved, to study its possible relationship to alterations in the microflora, not to our knowledge reported before.

Clinical Material and Methods

PATIENTS AND CONTROLS

We studied four groups of 14 infants, from 1 to 12 months of age: (a) breast-fed and (b) bottle-fed normal infants; (c) well nourished infants with acute diarrhoea; and (d) infants with chronic diarrhoea and protein-calorie malnutrition. Twelve infants of the fourth group were reevaluated after recovery from diarrhoea and protein-calorie malnutrition. Both sexes were almost equally represented in the four groups.

We selected breast-fed and bottle-fed controls (some of them also received other foods, depending on age) because of the importance of weaning diarrhoea. They were outpatients from the children's welfare service, criteria for acceptance being the parents' permission, normal physical examination, including weight and height development (Marcondes, Berquó, Yunes, Luongo, Martins, Zacchi, Levy, and Hegg, 1971), satisfactory feeding history, unaltered intestinal habits, no symptoms of disease in the last 15 days; no important infection, no diarrhoea of more than three days' duration, and no treatment with broad-spectrum antibiotics in the

past and for infants over 6 months, a negative parasitic stool examination.

The acute diarrhoea group consisted of bottle-fed infants of normal weight and height with a single bout of diarrhoea up to seven days' duration at the time of gastric aspiration. They were not dehydrated or febrile, not vomiting or receiving medication for the previous eight hours, had no signs of other infections or clinical anaemia and had stools negative for enteropathogenic bacteria as well as for parasites. Some of them had received broad-spectrum antibiotics in the past. Infants in whom the bout of diarrhoea lasted more than 10 days were excluded.

Infants of the chronic diarrhoea group were in the paediatric ward with diarrhoea of more than 15 days' duration (in some of them it was three to five months). All had variable degrees of protein-calorie malnutrition but no signs of kwashiorkor, haemoglobin being above 9 g%. Five of them were receiving cow's milk, and nine a milk substitute (Maffei, 1973). At the moment of gastric aspiration they were afebrile, not dehydrated, not vomiting (24 hours), without symptomatic medication (four hours for calcium carbonate and eight hours for homatropine methylbromide) and with no signs of other infections. They had one to three negative stool cultures and examinations for the presence of parasites. Many of these infants had been treated before admission: if they had received antibiotics for less than three days, gastric contents were obtained at least 48 hours after their discontinuation; if the antibiotic therapy was longer, a delay of seven days was allowed before sampling.

Twelve of the infants were reevaluated after clinical recovery from protein calorie malnutrition and after recuperation of the alimentary tolerance for age. This occurred three to 20 weeks after the first sampling of gastric contents. The remaining two infants of the chronic diarrhoea group died.

METHODS

Intubation and sampling

To avoid possible effects of environmental factors on results (Arnold and Brody, 1927; Campos, Hoenen, Costa, Trabulsi, and Pontes, 1958) samples of all groups were collected simultaneously.

After the baby had fasted for two hours we passed a sterile nasogastric polyvinyl catheter through a segment of a rubber tube to avoid contamination from the nostrils, and a first sample of gastric contents was immediately obtained to ascertain the position of the catheter and to wash contaminants out from the oropharynx. The gastric contents which remained in the catheter were reinjected. The rubber tube was withdrawn along the catheter, which remained *in situ* for two hours. This time

interval allowed the infants' reactions to stabilize and also the interaction of the microflora (eventually introduced from proximal segments) with the gastric juice (Franklin and Skoryna, 1971).

Gastric contents for pH reading and quantitative cultures were collected after two hours with the baby still fasting; the first aspirate was discarded. Whenever there was doubt, the position of the catheter in the stomach was checked by fluoroscopy after collection.

In 12 infants with chronic diarrhoea, besides the study after recovery, we repeated the pH determination after varied time intervals but before clinical recovery, to confirm alterations in pH. We also took throat swabs from 36 infants of the various groups.

pH reading

The pH of the gastric contents was measured with a glass electrode pH meter (Metrohm, E 350 B) one to three days after sampling, aspirates being stored at -20°C . Preliminary studies showed that no significant changes in pH occurred during that storage time.

Microbiological techniques

Samples (0.1 ml) of undiluted gastric juice, and of tenfold dilutions with saline, up to 10^{-7} , were streaked in triplicate onto plates with Oxoid mannitol salt agar, McConkey's agar, 10% sheep's blood agar and Sabouraud's dextrose agar with neomycin, 200 μg ug/ml. No quantification was done for saliva which was streaked on the same media without triplicate.

Specimens were incubated for 30 to 60 minutes after collection. Mannitol, McConkey's and blood-agar plates were incubated for 24 hours at 37°C . If no growth was found, they were incubated again for another 24 hours, a half of the plates being tested with a standard culture. Sabouraud's agar plates were incubated for 48 hours at 37°C for a first reading, and for seven days for a second reading.

Microorganisms were identified after bacterioscopy of Gram-stained smears. Gram-negative bacteria were screened with triple iron-sugar and oxidase, and for biochemical identification standard procedures (Edwards and Ewing, 1972) were used. *E. coli* strains were typed serologically using polyvalent antisera prepared in our Microbiology Unit. Staphylococci were identified by the catalase reaction (Cowan and Steel, 1965) modified by Baird-Parker (1966), plasmacoagulase and desoxyribonuclease by the methods of Jeffries, Holtman, and Guse (1957). For the identification of catalase-negative Gram-positive cocci (streptococci) the activity on blood agar was observed. For alpha haemolytic colonies the bile solubility was tested. Yeast-like fungi were

identified by the production of germ tubes (Mackenzie, 1962) confirmed by the formation of clamydospores. For clamydospores forming yeast-like fungi fermentation and assimilation of sugars were tested (Bailey and Scott, 1970).

Statistics

Results were submitted to nonparametric tests (Siegel, 1956; Jones, 1973) at the 95% confidence level. We used Mann-Whitney's test for two independent samples (recovery group versus bottle-fed control) and the Kruskal-Wallis one-way analysis of variance for four independent samples (breast-fed control, bottle-fed control, acute and chronic diarrhoea groups). For association and contingency tables we used the χ^2 or Fisher's exact probability method (Cochran, 1954). For two related samples (chronic diarrhoea versus recovery) we employed the Wilcoxon matched pairs signed-ranks test, and McNemar's test for significance of changes.

Results

pH VALUES

Giannella, Broitman, and Zamcheck (1972) reported that no bactericidal or bacteriostatic activities are observed in gastric juice at or above pH 4.0. The frequency of pH values above 4.0 in our study was 14% in bottle-fed control and acute diarrhoea groups and 8.3% in the recovery group. This was statistically different from the great frequency with which these pH values occurred in the breast-fed control and chronic diarrhoea groups—57% in both (fig 1).

Fig 1 pH of gastric contents. The horizontal bars represent the mean values.

Fig 2 pH of gastric contents in different days in infants with chronic diarrhoea.

The pH determinations, repeated before clinical recovery from chronic diarrhoea, showed that pH values for individual cases were relatively constant, when we considered ranges of pH below 2.5, between 2.6 and 4.0 and above 4.0 (fig 2). From the bacteriological standpoint pH ranges seem to be more important than individual values (Giannella *et al*, 1972).

RELATION BETWEEN pH VALUES AND CULTIVATION OF MICROORGANISMS

A bactericidal effect of pH below 2.5 was observed in all groups of infants, for Gram-negative bacilli, for staphylococci and streptococci separately and for total bacteria. Cultures were positive for bacteria in all but two aspirates (pH 2.5 and 3.0) in which pH was equal to or above 2.5; below this level only one sample (pH 2.0) yielded more than 1.00 ($\log_{10}$) bacterium per ml. No statistically significant pH dependency was observed for yeast-like fungi (fig 3).

CONCENTRATIONS OF MICROORGANISMS Gram-negative bacilli (*Enterobacteriaceae* and *Pseudomonaceae*)

Bacilli concentrations were significantly higher in the chronic diarrhoea group (fig 4), this higher concentrations appearing to be related to the higher pH values in this group. It must be emphasized, however, that concentrations of bacilli were significantly lower in breast-fed infants when compared with the chronic diarrhoea group, although pH values were similar for both; we even had three samples from the

Fig 3 Logarithm_{10} of yeasts concentrations per millilitre of gastric contents in relation to its pH values.

The spots below the horizontal line at 1.00 ($\log_{10}$) represent the negative samples. For the horizontal line at 3.00 ($\log_{10}$) the readers are referred to the text.

Fig 4 Logarithm_{10} of Gram-negative bacilli concentrations per millilitre of gastric contents.

The length of the blocks represents the range and the vertical bars the logarithmic means of positive samples. The numbers to the left of the diagram represent the percentage of negative samples and to the right the percentage of samples with a concentration above 4.00 ($\log_{10}$) per ml.

breast-fed group which failed to grow Gram-negative bacilli, despite pH values between 5 and 6 (fig 5).

Table I shows the recovered bacilli. No enteropathogenic *E. coli* serotypes were identified.

Gram-positive cocci (staphylococci and streptococci)

No significant differences between concentrations of these cocci were found in the different groups (fig 6).

Coagulase-positive and coagulase-negative staphylococci were recovered in similar concentrations from each sample. Streptococci with partial haemoly-

Fig 5 Logarithm_{10} of Gram-negative bacilli concentrations per millilitre of gastric contents in relation to its pH values.

The spots below the horizontal line at 1.00 ($\log_{10}$) represent the negative samples. For horizontal line at 4.00 ($\log_{10}$) the readers are referred to the text.

Groups of Infants		Microorganisms					
		<i>Escherichia coli</i>	<i>Klebsiella</i> / <i>Enterobacter</i> sp	<i>Proteus mirabilis</i>	<i>Proteus vulgaris</i>	<i>Proteus morganii</i>	<i>Pseudomonas</i> sp
Breast-fed	(6)	5	4	—	—	—	1
Bottle-fed	(5)	5	1	2	1	—	1
Acute diarrhoea	(5)	2	2	2	—	1	—
Chronic diarrhoea	(10)	6	8	2	—	1	5
Recovery	(3)	2	2	—	—	1	1

Table I Occurrence of enterobacteriaceae and pseudomonaceae in gastric contents

() Number of samples which yielded Gram-negative bacilli.

Fig 6 *Logarithm₁₀ of Gram-positive cocci concentrations per millilitre of gastric contents.*

The length of the blocks represents the range and the vertical bars the logarithmic mean of positive samples. The numbers to the left of the diagram represent the percentage of negative samples.

tic activity (alpha) were present in all samples with streptococci strains.

Yeast-like fungi

The frequency of isolation of yeast-like fungi in control groups was significantly less than in the diarrhoeic and recovery groups (table II). Concentrations above 3.00 (log₁₀) per ml were significantly more frequent in the chronic diarrhoea group than in both control and in recovery groups, no differences being observed in relation to the acute diarrhoea group (table II).

In 94% of the samples with yeast-like fungi we identified *Candida albicans* and we also recovered other yeast-like fungi from 18% of the samples.

Groups of Infants	Percentage of Isolation	Percentage of Concentrations Above 3.0 (log ₁₀)/ml
Breast-fed control	62	31
Bottle-fed control	50	21
Acute diarrhoea	79	50
Chronic diarrhoea	100	91
After recovery	92	17

Table II *Occurrence of yeast-like fungi in gastric contents*

Total bacteria and total microorganisms

Total bacterial concentrations were significantly higher in the chronic diarrhoea group, being more frequently above 4.00 (log₁₀) per ml during diarrhoea than after recovery (fig 7).

Considering all recovered microorganisms (total

bacteria and yeast-like fungi), the chronic diarrhoea group again had higher concentrations than the other groups (fig 7), but in relation to the breast-fed control group this difference was at the limit of significance. Otherwise, concentrations of microorganisms above 4.00 (log₁₀) per ml were statistically more frequent in the breast-fed control and in the chronic diarrhoea groups (fig 7). These facts show that total count analysis is less sensitive than separate analyses of groups of microorganisms.

Fig 7 *Logarithm₁₀ of total bacterial and total microorganism concentrations per millilitre of gastric contents.*

The length of the blocks represents the range and the vertical bars the logarithmic mean of positive samples. The numbers to the left of the diagram represent the percentage of negative samples, and to the right the percentage of samples with a concentration above 4.00 (log₁₀) per ml.

CONCORDANCE BETWEEN MICROORGANISMS IN OROPHARYNX AND GASTRIC CONTENTS

We considered that there was concordance when at least one similar microorganism of the same group was found at both sites in the same infant. For analysis of bacterial concordance we excluded those 'sterile' (below 1.00 (log₁₀) per ml) cases with a pH of gastric contents below 2.5.

Gram-negative bacilli, staphylococci and streptococci were found at both sites in 80, 83 and 76% of the cases respectively; concordance was statistically significant. Yeast-like fungi were isolated more frequently from gastric contents than from the oropharynx and in only 59% was there concordance; McNemar's test was not significant.

Discussion

HYDROGEN-ION CONCENTRATION (pH)

As suspected, infants with chronic diarrhoea and protein calorie malnutrition had difficulty in acidify-

ing gastric contents, an ability that reappeared only after recovery from diarrhoea and protein calorie malnutrition. Well nourished infants with acute diarrhoea behaved like bottle-fed controls (low pH values), suggesting that the pH alterations occurred during evolution to a chronic state perhaps being related to protein calorie malnutrition.

The difficulty in acidification certainly was not related to the calcium carbonate, administered orally to 50% of the infants, because the effect of neutralizing substances disappears up to 60 minutes (Martinen and Visakorpi, 1968) and otherwise calcium carbonate presents the acid rebound effect (Fordtran, 1968; Barreras, 1970). Our infants received CaCO_3 four hours or more before sampling, and no differences were observed between results for infants with or without this medication. This was the case also for homatropine methylbromide, a drug that was discontinued eight hours or more before sampling; as this drug must be administered every four to six hours to have its action sustained, it seems to us that it had no influence on our results.

Also the milk substitute given to some of the infants with chronic diarrhoea seems not to explain the decrease in the gastric acid secretion, because, being a diet rich in proteins and poor in carbohydrates, it has (like cow's milk) a high buffering capacity (Bullen and Willis, 1971), and in consequence, greatly stimulates acid secretion (Saint-Hilaire, Lavers, Kennedy, and Code, 1960; Kotrba and Code, 1969).

Indeed, it may be that infants having difficulty in increasing the hydrogen-ion concentration in gastric juice could neither counteract the effect of alkalinizing substances nor of diets with high buffering capacity. The results with or without drugs and/or special diets reflect the difficulty in acidification presented by those infants.

Several hypotheses may be proposed to explain this difficulty: those infants may have a diminished parietal cell mass as a consequence of protein-calorie malnutrition, since a direct relationship between maximal acid output and parietal cell mass (Card and Marks, 1960) or infants' weight (Ghai, Singh, Walia, and Gadekar, 1965; Rødbro, Krasilnikoff, and Christiansen, 1967; Micheli, 1969; Adesola, 1970) has been described. A greater reabsorption of H ions through an altered mucous membrane or a greater secretion of nonparietal cells (Fordtran and Walsh, 1973) are other possibilities.

Strangely, the breast-fed controls had pH values as if they also had difficulty in acidifying the gastric contents. Mason (1962) found low pH values (below 2.5) in only 10% of newborn infants (5-13 days) four hours after a breast milk feeding. Some indirect evidence also favours the impression that breast-fed

infants do not acidify gastric contents to low pH values or for a long time: in breast-fed infants the human milk IgA resists the passage through the gastrointestinal tract (Kenny, Boesman, and Michaels, 1967; Gindrat, Gothefors, Hanson, and Winberg, 1972) although it does not resist the action of pepsin at pH 2.0 during 30 minutes (McClelland, Samson, Parkin, and Shearman, 1972); gastric protein hydrolysis is minimal in breast-fed infants (Berfenstam, Jagenburg, and Mellander, 1955; Mason, 1962); some animal species begin gastric acid secretion only after lactation (Deren, 1971).

CONCENTRATIONS OF MICROORGANISMS

The higher concentrations of Gram-negative bacilli in gastric contents of infants with chronic diarrhoea could be a consequence of diminished bactericidal and/or bacteriostatic activity related to high pH values. But, for similar high pH values, the breast-fed control group had low concentrations of Gram-negative bacilli, similar to the concentrations observed in the other control group with lower pH values.

Low bacilli concentrations in breast-fed infants may be related to lesser contamination, but human milk is contaminated by Gram-negative bacilli in 31% of the cases, sometimes up to 7.0 ($\log_{10}$) per ml of milk (Wyatt and Mata, 1969). Also, it may be that there are much lower pH values in the first hours after ingestion because of the poor buffering capacity of human milk (Bullen and Willis, 1971), but this hypothesis is hardly compatible with the maintenance of IgA activity.

Otherwise, there is strong evidence that human milk protects babies even in conditions of great contamination by enteropathogenic bacteria (Mata, Urrutia, Garcia, Fernández, and Béhar, 1969). Lysozyme (Chandan, Shahani, and Holly, 1964), poor buffering capacity (Harrison and Peat, 1972), lactoferrin (Bullen, Rogers, and Leigh, 1972) and secretory IgA (Ammann and Stiehm, 1966; Mata and Wyatt, 1972) are some of the factors involved in the greater resistance of these babies to infection.

It could be also that the great proliferation of Gram-negative bacilli in the chronic diarrhoea group with protein calorie malnutrition is propitiated by a decrease of the gastric secretory IgA (Fernandez, Averbach, Paolo, Delle Done, and Gonzalez, 1971), and/or of other factors, provided that the pH permits it. Unfortunately, studies of the gastrointestinal flora of patients with a- or hypogammaglobulinaemia do not help us, because so often these patients also have gastric acid deficiency (Hersh, Floch, Binder, Conn, Prizont, and Spiro, 1970; Parkin, McClelland, O'Moore, Percy-Robb,

Grant, and Shearman, 1972; Brown, Butterfield, Savage, and Tada, 1972).

Gram-positive cocci were isolated in similar concentrations in all groups of infants. It is as if there were a relative depression of them, when compared with Gram-negative bacilli, in the chronic diarrhoea group. This may be a consequence of previous antibiotic therapy.

The significantly lesser frequency of yeast-like fungi isolated in both control groups could be interpreted as a consequence of the fact that only these groups had never received broad-spectrum antibiotics before. If other factors are important they cannot be interpreted in our study. Bishop, Barnes, and Townley (1974) considered yeast-like fungi concentrations of 3.00 (log₁₀) per ml as the limit of normality. The significantly greater frequency of higher concentrations in infants with chronic diarrhoea may also be only a consequence of antibiotic therapy. The diminution of concentrations of yeast-like fungi after recovery from chronic diarrhoea also does not necessarily mean that diarrhoea was a consequence of, or maintained by, yeast-like fungi overgrowth as suggested by Bishop *et al* (1974); it can be explained by the longer time elapsing since antibiotic administration or by the effect of specific treatment with nystatin being coincidental with the recovery from diarrhoea. We do not know why yeast-like fungi were more often isolated from gastric contents than from the oropharynx, but the same fact was observed by others (Bishop *et al*, 1974).

Our microbiological data in gastric contents are similar to those reported by Gracey and Stone (1972), Mata *et al* (1972), and Challacombe *et al* (1974) in jejunal juice of diarrhoeic infants. Otherwise, when the microflora was determined in both, gastric and jejunal sites comparable results were frequently obtained (Drasar *et al*, 1969; Gorbach, Mahalanabis, Brayton, Jacobs, Chatterjee, and Neogy, 1970; Mata *et al*, 1972).

These considerations favour the impression that gastric functional alterations are important contributory factors to the small bowel overgrowth syndrome in infants. Certainly the gastric alterations and their relation to the small bowel overgrowth syndrome deserve further studies.

The authors are indebted to Dr G. Moreno and his staff for assistance in microbiological aspects and for permission to use the Microbiology Unit, to Dr N. F. Novo and Dr E. R. de Paiva for statistics and to Dr M. R. Montenegro for help in preparing the paper in English.

References

Adesola, A. O. (1970). Histamine or pentagastrin stimulation in

- the assessment of gastric secretion in children. *Brit. J. Surg.*, **57**, 854.
- Amman, A. J., and Stiehm, E. R. (1966). Immune globulin levels in colostrum and breast milk, and serum from formula- and breast-fed newborns. *Proc. Soc. exp. Biol. (N.Y.)*, **122**, 1098-1100.
- Arnold, L., and Brody, L. (1927). Influence of 'effective temperature' upon bactericidal action of gastro-intestinal tract. *Proc. Soc. exp. Biol. (N.Y.)*, **24**, 832-833.
- Bailey, W. R., and Scott, E. G. (1970). *Diagnostic Microbiology*, 3rd ed., pp. 266-269. Mosby, St. Louis.
- Baird-Parker, A. C. (1966). In *Identification Methods for Microbiologists*, edited by B. M. Gibbs and F. A. Skinner, pp. 59-64. Academic Press, New York.
- Barreras, R. F. (1970). Acid secretion after calcium carbonate in patients with duodenal ulcer. *New Engl. J. Med.*, **282**, 1402-1405.
- Berfenstam, R., Jagenburg, R., and Mellander, O. (1955). Protein hydrolysis in the stomachs of premature and full-term infants. *Acta paediat. (Uppsala)*, **44**, 348-354.
- Bishop, R. F., Barnes, G. L., and Townley, R. R. W. (1974). Microbial flora of stomach and small intestine of infantile gastroenteritis. *Acta paediat. scand.*, **63**, 418-422.
- Brown, W. R., Butterfield, D., Savage, D., and Tada, T. (1972). Clinical, microbiological, and immunological studies in patients with immunoglobulin deficiencies and gastrointestinal disorders. *Gut*, **13**, 441-449.
- Bullen, C. L., and Willis, A. T. (1971). Resistance of the breast-fed infant to gastroenteritis. *Brit. med. J.*, **3**, 338-343.
- Bullen, J. J., Rogers, H. J., and Leigh, L. (1972). Iron-binding proteins in milk and resistance to *Escherichia coli* infection in infants. *Brit. med. J.*, **1**, 69-75.
- Campos, J. V. M., Hoenen, W., Costa, A., Trabulsi, L., and Pontes, J. F. (1958). Changes in intestinal flora under tetracycline. *Gastroenterology*, **34**, 625-635.
- Card, W. I., and Marks, I. N. (1960). The relationship between acid output of the stomach following 'maximal' histamine stimulation and the parietal cell mass. *Clin. Sci.*, **19**, 147-163.
- Challacombe, D. N., Richardson, J. M., Rowe, B., Anderson, C. M. (1974). Bacterial microflora of the upper gastrointestinal tract in infants with protracted diarrhoea. *Arch. Dis. Childh.*, **49**, 270-277.
- Chandan, R. C., Shahani, K. M., and Holly, R. G. (1964). Lysozyme content of human milk. *Nature (Lond.)*, **204**, 76-77.
- Chievitz, I. (1921). Die Salzsäuresekretion bei Säuglingen unter normalen und pathologischen Bedingungen. *Acta paediat. (Uppsala)*, **1**, 416-437.
- Cochran, W. G. (1954). Some methods for strengthening the common χ^2 tests. *Biometrics*, **10**, 417-451.
- Coello-Ramirez, P., Lifshitz, F., and Zuniga, V. (1972). Enteric microflora and carbohydrate intolerance in infants with diarrhoea. *Pediatrics*, **49**, 233-242.
- Cowan, S. T., and Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*, p. 217. University Press, Cambridge.
- Deren, J. S. (1971). Development of structure and function in the fetal and newborn stomach. *Amer. J. clin. Nutr.*, **24**, 144-159.
- Drasar, B. S., Shiner, M., and McLeod, G. M. (1969). Studies on the intestinal flora. I. The bacterial flora of the gastrointestinal tract in healthy and achlorhydric persons. *Gastroenterology*, **56**, 71-79.
- Edwards, P. R., and Ewing, W. H. (1972). *Identification of Enterobacteriaceae*. 3rd ed., p. 362. Burgess, Minneapolis.
- Fernandez, L. B., Averbach, J., Paolo, M. I. L. de, Delle Done, M. E., and Gonzalez, F. (1971). La IgA en el contenido gástrico y el suero de lactantes eutróficos y desnutridos. *Arch. argent. Pediat.*, **69**, 377-381.
- Fordtran, J. S. (1968). Acid rebound. *New Engl. J. Med.*, **279**, 900-905.
- Fordtran, J. S., and Walsh, J. H. (1973). Gastric acid secretion rate and buffer content of the stomach after eating. Results in normal subjects and in patients with duodenal ulcer. *J. clin. Invest.*, **52**, 645-657.
- Franklin, M. A., and Skoryna, S. C. (1971). Studies on natural gastric flora: survival of bacteria in fasting human subjects. *Canad. med. Ass. J.*, **105**, 380-386.
- Ghai, O. P., Singh, M., Wallia, B. N. S., and Gadekar, N. G. (1965). An assessment of gastric acid secretory response with 'maximal' augmented histamine stimulation in children with peptic ulcer. *Arch. Dis. Childh.*, **40**, 77-79.

- Giannella, R. A., Broitman, S. A., and Zamcheck, N. (1972). Gastric acid barrier to ingested microorganisms in man: studies *in vivo* and *in vitro*. *Gut*, 13, 251-256.
- Gindrat, J. J., Gothefors, L., Hanson, L. Å., and Winberg, J. (1972). Antibodies in human milk against *E. coli* of the serogroups most commonly found in neonatal infections. *Acta paediat. scand.*, 61, 587-590.
- Gorbach, S. L., Mahalanabis, D., Brayton, J., Jacobs, B., Chatterjee, B. D., and Neogy, K. N. (1970). Intestinal microflora in Asiatic cholera. III. Studies in pediatric cholera. *J. infect. Dis.*, 121, 46-47.
- Gracey, M., and Stone, D. E. (1972). Small-intestinal microflora in Australian aboriginal children with chronic diarrhoea. *Aust. N.Z. J. Med.*, 2, 215-219.
- Harrison, V. C., and Peat, G. (1972). Significance of milk pH in newborn infants. *Brit. med. J.*, 4, 515-518.
- Hersh, T., Floch, M. H., Binder, H. J., Conn, H. O., Prizant, R., and Spiro, H. M. (1970). Disturbance of the jejunal and colonic bacterial flora in immunoglobulin deficiencies. *Amer. J. clin. Nutr.*, 23, 1595-1601.
- Jeffries, C. D., Holtman, D. F., and Guse, D. G. (1957). Rapid method for determining the activity of microorganisms on nucleic acids. *J. Bact.*, 73, 590-591.
- Jones, J. G. (1973). Use of nonparametric tests for the analysis of data obtained from preliminary surveys (a review). *J. appl. Bact.*, 36, 197-210.
- Kenny, J. F., Boesman, M. I., and Michaels, R. H. (1967). Bacterial and viral coproantibodies in breast-fed infants. *Pediatrics*, 39, 202-213.
- Klementsson, E. (1923). Recherches sur le suc gastrique chez les enfants de 1 à 12 ans. *Acta paediat. (Uppsala)*, 31, 136-158.
- Kotrba, C., and Code, C. F. (1969). Gastric acid secretory responses to some purified foods and to additions of sucrose or olive oil. *Amer. J. dig. Dis.*, 14, 1-8.
- McClelland, D. B. L., Samson, R. R., Parkin, D. M., and Shearman, D. J. C. (1972). Bacterial agglutination studies with secretory IgA prepared from human gastrointestinal secretions and colostrum. *Gut*, 13, 450-458.
- Mackenzie, D. W. R. (1962). Serum tube identification of *Candida albicans*. *J. clin. Path.*, 15, 563-565.
- Maffei, H. V. L. (1973). PhD Thesis, Faculty of Medical and Biological Sciences of Botucatu, São Paulo, Brazil.
- Marcondes, E., Berquó, E. S., Yunes, J., Luongo, J., Martins, J. S., Zacchi, M. A. S., Levy, M. S. F., and Hegg, R. (1971). Estudo antropométrico de crianças brasileiras de zero a doze anos de idade. *Anais Nestlé*, no. 84, 1-200.
- Marttinen, E., and Visakorpi, J. K. (1968). Measurement of gastric acidity by radiotelemetry in pediatric practice. *Ann. Paediat. Fenn.*, 14, 115-118.
- Mason, S. (1962). Some aspects of gastric function in the newborn. *Arch. Dis. Childh.*, 37, 387-391.
- Mata, L. J., and Wyatt, R. G. (1972). Amamantamiento y resistencia del huesped a la infección. OPAS, Publicación Científica no. 250, 11-23.
- Mata, L. J., Jiménez, F., Cordón, M., Rosales, R., Prera, E., Schneider, R. E., and Viteri, F. (1972). Gastrointestinal flora of children with protein-calorie malnutrition. *Amer. J. clin. Nutr.*, 25, 1118-1126.
- Mata, L. J., Urrutia, J. J., Garcia, B., Fernández, R., and Béhar, M. (1969). *Shigella* infection in breast-fed Guatemalan Indian neonates. *Amer. J. Dis. Child.*, 117, 142-146.
- Micheli, H. (1969). La stimulation gastrique maximale à l'histamine chez l'enfant normal. *Helv. paediat. Acta*, 24, 278-292.
- Muhl, G. (1925). The secretion of hydrochloric acid in normal conditions and in chronic gastric affections of children from 1 to 13 years of age. *Acta paediat. (Uppsala)*, 4, 356-379.
- Parkin, D. M., McClelland, D. B. L., O'Moore, R. R., Percy-Robb, I. W., Grant, I. W. B., and Shearman, D. J. C. (1972). Intestinal bacterial flora and bile salt studies in hypogammaglobulinaemia. *Gut*, 13, 182-188.
- Rødbrø, P., Krasilnikoff, P. A., and Christiansen, P. M. (1967). Parietal cell secretory function in early childhood. *Scand. J. Gastroent.*, 2, 209-213.
- Saint-Hilaire, S., Lavers, M. K., Kennedy, J., and Code, C. F. (1960). Gastric acid secretory value of different foods. *Gastroenterology*, 39, 1-11.
- Siegel, S. (1956). *Nonparametric Statistics for the Behavioral Sciences*. McGraw-Hill, New York.
- Wolman, I. J. (1943). Gastric digestive secretions in infancy and childhood: a review. *Amer. med. Sci.*, 206, 770-794.
- Wyatt, R. G., and Mata, L. J. (1969). Bacteria in colostrum and milk of Guatemalan Indian women. *J. trop. Pediat.*, 15, 159-161.