Department of Health and Social Security

Report on Health and Social Subjects

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THE COMPOSITION OF MATURE HUMAN MILK

Report of a Working Party of the Committee on Medical Aspects of Food Policy

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Preface

Following the report "Present-day practice in infant feeding" (Number 9 in this series) the Food Standards Committee announced a review of the composition, description, labelling and advertising of manufactured foods for infants and children up to the age of approximately three years. The Committee on Medical Aspects of Food Policy was asked for advice on all nutritional aspects of the review and we are grateful to Professor T. E. Oppé and the members of the Working Party for their willingness to undertake such an arduous task. The Working Party decided to begin by considering foods for healthy full-term young infants. As expected they agree that such foods should be as close to the average composition of mature human milk as is possible in the present state of scientific knowledge and technological expertise.

This report presents an account of the first stage of the Working Party's study. Samples of human milk from mothers living in different areas of Great Britain have been analysed in one laboratory by techniques which are considered to be more advanced than those employed by workers in the 1940s and 1950s in America and elsewhere. We thank the Laboratory of the Government Chemist for undertaking the analyses. It is both reassuring and salutary that the results are found to be in harmony with those achieved some 30–40 years ago before present refinements in technique were known.

We trust that these results may be of interest to others working in the field of infant feeding, and that publication of this report will act as a stimulus to further research into the composition and characteristics of human milk.

H. YELLOWLEES
Chairman,
Committee on Medical Aspects of Food Policy

Committee on Medical Aspects of Food Policy

Working Party on the Composition of Foods for Infants and Young Children

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Members of the Sub-Group who were especially concerned with organising the collection of samples, analysis of the milk and preparing the report.

Mr. A. A. Christie	Laboratory of the Government Chemist Department of Industry, London
Dr. S. J. Darke	Department of Health and Social Security London
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Dr. F. Cockburn	Simpson Memorial Maternity Pavilion The Royal Infirmary Edinburgh
Dr. B. D. Corner	Southmead Hospital Westbury-on-Trym Bristol
Dr. D. P. Davies[1]	St. David's Hospital Cardiff
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Dr. B. A. Wharton	The Sorrento Hospital Birmingham

The members of the Working Party also wish to record their thanks and their appreciation of the ready help given by the nursing sisters, midwives and nurses in arranging for and supervising the collection of the samples of milk, and to thank the mothers who lived within reach of the above hospitals and who willingly gave samples of milk.

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Contents

Introduction

1.1 An increasing interest in the importance of the composition of human milk for the growth and development of infants, and in the problems which may stem from artificial feeding with cows' milk foods, was recognized by the Department of Health and Social Security when, in May 1973, the Committee on Medical Aspects of Food Policy set up an expert Working Party to review present-day practice in infant feeding. The report of the Working Party was accepted by the Committee in December 1973 and was published in October 1974.

1.2 Recommendations in the report (Department of Health and Social Security, 1974) included para 6.3.1 (p 25) which stated that "the reconstituted artificial feed . . . should approximate to the composition of breast milk as nearly as is practicable", and para 6.7 (p 26) which recommended that "the legislation concerning the composition, labelling and advertising of infant foods be reviewed".

1.3 In April 1974 the Food Standards Committee[1] was invited by Ministers to advise on the need for standards or for other controls on the composition and description of foods for infants and young children, and a review of these foods was announced (Ministry of Agriculture, Fisheries and Food, 1974). Accordingly the Food Standards Committee sought advice on the nutritional aspects of their review from the Committee on Medical Aspects of Food Policy, which set up the present Working Party (membership p iv).

1.4 The Working Party decided to consider first those foods which are the sole source of nourishment for infants who are not breast fed. Human milk, provided that enough is available, is known to be nutritionally adequate for almost all normal healthy infants during the first 4–6 months of postnatal life. The composition of human milk must therefore be taken into account when suggesting nutritional guidelines for such foods.

1.5 The composition of human milk is by no means constant, even from the same individual, but differs at different stages of lactation and depends to some extent on the maternal diet. There are also differences in the relative concentrations of some nutrients in milk which has been secreted at different times during the day, and even in the milk produced at the beginning and towards the end of any one feed. The concentration of fat, for example, tends

[1]The Food Standards Committee advises the Minister of Agriculture, Fisheries and Food, the Secretary of State for Social Services, the Secretary of State for Scotland and the Head of the Department of Health and Social Services for Northern Ireland on the exercise of their powers, under the Food and Drugs Act 1955 and the corresponding enactments relating to Scotland and Northern Ireland, to control the composition and description of food.

to be greatest at about noon and least at about midnight, and increases during the course of a feed.

1.6 The variation in composition suggests that human milk cannot be used as a precise model in the formulation of infant milk foods. Nevertheless, the more an artificial milk food departs from the average composition of human milk, the greater is the possibility of untoward effects in the infants to whom it is fed. Adverse effects could be due to either deficiency or excess of nutrients, or to the interaction of one nutrient with another.

1.7 The importance of human milk to the young infant is not only a matter of nutrient composition. Human milk contains lactoferrin, an iron-binding protein which is of importance in the prevention of infection by *Escherichia coli*; a specific immunoglobulin (IgA) which acts in the gut to limit the growth and reproduction of bacterial and viral pathogens; enzymes with bacteriostatic properties; antibodies from the maternal blood; living cells—leucocytes, neutrophils and macrophages—and non-pathogenic bacteria. These components of human milk are outside the scope of this report.

2. Review of the Literature

2.1 Sources of information

2.1.1 Many analyses of human milk have been reported. Table 1 (p 7) summarizes only those values for mature[1] human milk which are found in the standard works of reference commonly used in the United Kingdom. The origins of the values given under the seven column headings are described below.

2.1.2 Macy, Kelly and Sloan (1953) reviewed more than 1500 publications from many countries on the composition of colostrum and of transitional and mature human milk, and selected 268 on the basis of "characteristics of the subjects, methods of collecting samples, analytical techniques, diet and environmental factors". The results reported in these publications showed that individual milk samples varied widely in composition. Macy *et al* (1953) reported the mean values obtained by the various selected authors for each nutrient, with the minimum and maximum figures given by each author, the numbers of samples which had been analysed and the references to the publications. Column 1 of Table 1 (p 7) shows the range of the mean values given by Macy *et al* (1953) from the work of the different investigators.

2.1.3 In 1961 Macy and Kelly wrote a chapter called "Human milk and cow's milk in infant nutrition" for the book entitled "Milk: the mammary gland and its secretion" edited by Kon and Cowie. In this chapter Macy and Kelly gave two different tables of nutrient composition of human milk. One was derived from the arithmetic mean of the mean values reported in the 268 publications which Macy *et al* had selected in 1953. These means of mean values are given in column 2 of Table 1 (p 7).

2.1.4 In their 1961 publication Macy and Kelly also report some previously unpublished analyses by Macy, together with the analyses, made by her in 1949, of 216 samples of mature human milk which she had collected under controlled conditions. The women were all living in the same geographical area in the United States of America and were known to have had an adequate diet. These results (mean values and ranges) are given in column 3 of Table 1 (p 7).

2.1.5 The values in column 4 of Table 1 (p 7) were published by Fomon (1974). Most of them were taken directly from Macy's (1949) analyses which were reported by Macy and Kelly in 1961 and are therefore the same as the

[1]By mature human milk is meant the secretion of the mammary gland from about the 14th day after the birth of the infant.

mean values which are given in column 3. The values for some inorganic nutrients and vitamins came from other sources, for example, Hartman and Dryden (1965).

2.1.6 Kon and Mawson (1950) analysed 600 samples of mature milk from mothers in Reading and 121 samples from mothers in Shoreditch. The samples were collected between 1942 and 1945. The mothers were eating a wartime diet. The report was primarily concerned with vitamins and provides information on the effect of the vitamin intake of the mother on the concentration of vitamins in her milk. The values given in column 5 of Table 1 (p 7) are taken from the summary on p 182 of the Kon and Mawson (1950) report. Two figures are quoted where the values for Reading and Shoreditch were different.

2.1.7 The figures in column 6 of Table 1 (p 7) are from a survey of the literature on the composition of human milk summarized by Morrison (1952). While the preparation of Morrison's review was in progress, Kon and Mawson (1950) published their review on vitamins in milk and Morrison did not duplicate this work. Morrison therefore only gave figures for the main energy producing nutrients and for the minerals.

2.1.8 The figures in column 7 of Table 1 (p 7) for protein, fat, carbohydrate and inorganic constituents were from the analyses of pooled milk from 9 mothers in Cambridge (England) who were 15–16 weeks post-partum (McCance and Widdowson, 1967). The figures for vitamins were derived from a review of the literature.

2.1 Nutrient constituents of milk

2.2.1 *Protein.* The values given in Table 1 (p 7) represent total nitrogen (g) × 6·38. There is little variation in mean values from one publication to another and 1·2 g per 100 ml may be considered as representative.

2.2.2 *Non-protein nitrogen.* Only about 80% of the nitrogen in mature human milk is protein nitrogen, and about 20% is present as urea, creatinine, uric acid and amino acids in approximately the same concentrations as in the mother's plasma (Ericson, Gulick and Hunscher, 1934). Urea is the main constituent and free amino acids contribute only about 6 mg N per 100 ml or 3% of the total nitrogen.

2.2.3 *Fat.* The concentration of fat in milk shows a diurnal variation and also a variation from the beginning to the end of a feed. The results of analyses reported by Macy and Kelly (1961) varied from 1·3 to 8·3 g per 100 ml with a mean of 4·5 g per 100 ml. The mean values given by other authors varied from 3·3 to 5·3 g per 100 ml.

2.2.4 *Carbohydrate.* All the mean values shown for carbohydrate in Table 1 are about 7 g per 100 ml, but Macy and Kelly (1961) found a variation of 5·0 to 9·2 g per 100 ml. McCance and Widdowson (1967) expressed their values for carbohydrate in milk as monosaccharide but, since all the other authors presumably expressed carbohydrate as disaccharide, McCance and Widdowson's values for carbohydrate in Table 1 (p 7) have also been expressed as disaccharide.

2.2.5 *Energy.* Figures for energy depend not only upon the amount of protein, fat and carbohydrate per 100 ml milk, but also upon the factors used to convert these to calories or joules. In Table 1 (p 7) all figures for energy have been calculated from the protein, fat and carbohydrate by using the modified Atwater factors of Southgate and Durnin (1970).

2.2.6 *Vitamins.* Values for vitamin A are given in a variety of ways in the earlier tables and, in order to simplify comparisons, all values in Table 1 (p 7) have been expressed as retinol equivalents[1]. The concentration of most vitamins in breast milk is largely influenced by the amounts in the maternal diet (Kon and Mawson, 1950). Even so, the range of mean values in Table 1 (p 7) is no greater than that for some other constituents, though individual women (column 3), produced milk with widely varying concentrations of thiamin, riboflavin and nicotinic acid and of vitamins A and C. There is little information about vitamin D; the value in columns 1 and 2 is based on the analysis of the fat fraction from one pooled sample of milk.

2.2.7 *Sodium.* The mean concentrations of sodium which have been reported by different authors are similar (15–17 mg per 100 ml). However, analyses of samples of milk from individual women gave results varying from 6 to 44 mg per 100 ml (Macy and Kelly, 1961).

2.2.8 *Potassium.* The mean figures do not vary greatly from one author to another. There does not seem to be the same wide individual variation for potassium as there is for sodium.

2.2.9 *Chloride.* While the means obtained by different authors are on the whole similar, the individual values obtained by Macy and Kelly (1961) show an eight-fold variation.

2.2.10 *Calcium, magnesium and phosphorus.* For each of these three nutrients the mean values reported by the different authors are similar, but Macy and Kelly (1961) found a wide variation in individual samples.

2.2.11 *Iron.* With one exception, the variation of the means shown in Table 1 (p 7) is from 30 to 50 μg per 100 ml. The exception is the value of 360 μg per 100 ml in column 1 from Macy *et al* (1953). It is the inclusion

[1]1 retinol equivalent = 1 μg retinol or 3·33 international units retinol or 6 μg β-carotene or 12 μg other biologically active carotenoids (Department of Health and Social Security, 1969).

of this high figure which raises the mean value to 150 μg per 100 ml quoted in column 2 of Table 1 (p 7).

2.2.12 *Copper.* The concentration of copper is similar to that of iron, that is to say, 30 to 50 μg per 100 ml.

2.2.13 *Zinc.* The concentration of zinc, as given by different authors, varies considerably. This variation may be real or may be due to technical problems: earlier workers used a chemical method which was laborious and not always precise. Moreover, the concentration of zinc varies with the stage of lactation and is five times greater in colostrum than in later milk (Berfenstam, 1952).

2.2.14 *Other trace elements.* There is very little information in the reports that were used to compile Table 1, or elsewhere in the literature, about the concentrations of other trace elements in human milk. Fomon (1974) quotes values of 0·7–1·5 μg manganese, 3 μg iodine and 1·3–5·0 μg selenium per 100 ml.

Table 1: *The composition of 100 ml mature human milk (values to be found in standard works of reference)*

		Column 1 Macy, Kelly & Sloan 1953 Range of mean values from the literature	Column 2 Macy & Kelly 1961 mean of mean values in column 1	Column 3 Macy & Kelly 1961 individual samples		Column 4 Fomon 1974	Column 5 Kon & Mawson 1950 Mean for each of 2 samples*	Column 6 Morrison 1952	Column 7 McCance & Widdowson† 1967
				Mean	Range	Mean		Mean	Mean
Water	g	87·1— 87·9	87·6	87·1	82·5— 89·7	87·1	86·7	—	89·8
Protein	g	0·9— 1.6	1·2	1·1	0·7— 2·0	1·1	1·2	1·3	1·2
Non-protein nitrogen	mg	29 — 62	39	32	17 — 60	32	—	—	—
Fat	g	3·1— 5·2	3·8	4·5	1·3— 8·3	4·5	4·8, 3·9	3·3	5·3
Carbohydrate	g	6·0— 7·6	7·0	6·8	5·0— 9·2	6·8	7·0	7·2	6·6
Energy	kcal	67 — 75	71	75	45 —119	75	76, 70	64	80
Sodium	mg	13 — 17	15	17	6 — 44	16	—	17	15
Potassium	mg	48 — 71	55	51	37 — 64	51	—	52	54
Chloride	mg	34 — 53	43	38	9 — 73	39	—	46	66
Calcium	mg	28 — 40	33	34	17 — 61	34	30	29	30
Magnesium	mg	3 — 5	4	4	2 — 6	4	—	3	3
Phosphorus	mg	13 — 18	15	14	7 — 27	14	13	14	14
Iron	µg	30 —360	150	30	20 — 90	50	—	—	50
Copper	µg	30 — 50	40	30	10 — 70	40	—	—	—
Zinc	µg	118 —350	530	118	17 —302	300—500	—	—	—
Thiamin	µg	9 — 22	16	14	8 — 23	16	17, 18	—	21
Riboflavin	µg	25 — 60	43	37	20 — 79	36	26, 22	—	31
Nicotinic Acid	µg	160 —183	172	183	66 —330	147	—	—	175
Ascorbic acid	mg	3·5— 5·6	4·3	5·2	0 — 11·2	4·3	3·6, 3·4	—	3.6
Vitamin A (retinol equivalents)	µg	52 — 65	57	65	15 —239	57	50, 45	—	52
Vitamin D	µg	0·01§	0·01	—	—	0·055	—	—	0·026

*Where one figure is given the means of each sample were the same.
†See para 8.2.1. (p. 23).
§See para 9.3. (p. 27).

3. Background to the Present Analyses

3.1 Many of the analyses to which reference has been made were carried out in countries other than the United Kingdom and were made before the introduction of techniques such as atomic absorption spectrophotometry, gas liquid chromatography, ion exchange chromatography or microbiological assay. The Working Party therefore decided that samples of mature human milk should be collected in different parts of Great Britain and that the Laboratory of the Government Chemist should be asked to undertake the analyses.

3.2 The samples were analysed for the following nutrients.

water	retinol	sodium
nitrogen	β—carotene	potassium
amino acids	vitamin E (α—tocopherol)	chloride
fat	vitamin C	calcium
fatty acids	thiamin	magnesium
lactose	riboflavin	phosphorus
cholesterol	nicotinic acid	iron
	vitamin B_6	copper
	vitamin B_{12}	zinc
	folic acid	selenium
	pantothenic acid	fluoride
	biotin	iodide

Dr. E. M. Widdowson of the Department of Medicine, University of Cambridge, agreed to undertake the estimations of vitamin D sulphate. Too little milk was available for determination of the fat-soluble vitamin D.

3.3 It would clearly have been ideal to analyse samples that were collected, at different stages of lactation, from individual women so that mean values and standard deviations for the concentrations of the known nutrients could be obtained. To do so, however, would have required much larger volumes of milk and would have taken a much longer period of time. The Working Party aimed to obtain values which could be used as nutritional guidelines in their deliberations on the composition of infant milk foods, and the decision was therefore made, for practical reasons, to select 4 to 6 weeks as the stage of lactation and to analyse pooled samples of milk obtained from women living in as many areas of Great Britain as possible. Although many areas were willing to cooperate, difficulties were encountered in obtaining the promise of sufficient milk. Eventually five centres were chosen: in England—Birmingham, Bristol and Newcastle-upon-Tyne; in Scotland—Edinburgh; and in Wales—Cardiff.

4. Obtaining the Samples

4.1 The samples were obtained at each centre under the supervision of an experienced member of the hospital nursing staff. Transport was provided for the mothers to and from the hospital.

4.2 The infants were fully breast fed, that is to say, were receiving no complementary or supplementary feeds or cereals. Samples were obtained only from mothers whose infants appeared to be healthy and thriving and were not in need of any special medical attention.

4.3 A protocol was devised for the collection of the samples of milk (Appendix A, p 33). In practice there were some deviations from the protocol and these are recorded below and in Table 2 (p 12).

4.4 A pilot study was undertaken in Birmingham to ascertain how many samples would be needed to make up a pool of one litre, which was the volume of milk required for the complete programme of analyses. In this study the average volume of milk obtained from the mothers was 60–70 ml and it was therefore estimated that about 15 mothers would be needed at each centre. In the main study there was considerable variation in yield from individual mothers and the number of mothers who gave milk therefore varied from one centre to another.

4.5 The intention was to collect the milk at 4–6 weeks post-partum, but the fact that this was achieved only in Bristol and Cardiff shows that some difficulty was experienced in obtaining enough samples.

4.6 From each mother the maximum amount of milk was collected from one breast only, usually between the hours of 9 a.m. and 2 p.m., about 3–4 hours after the infant's previous early morning feed.

4.7 Milk was collected by hand expression or by the use of an electric pump. In Bristol and Newcastle all the milk samples were expressed by hand. In the other areas some samples were obtained entirely by hand expression, others entirely by breast pump. Five mothers in Cardiff used both methods.

4.8 Originally it had been proposed that samples would be collected at the different centres during the same 2 weeks but in practice this proved to be impossible. The period in which samples were collected was between April and August 1975. The total volume of milk varied from 1284 ml in Edinburgh to 1590 ml in Bristol.

4.9 Care was taken to ensure that the milk was not contaminated by contact with plastic, metal or rubber. Glass bottles, of capacity 200 ml, were provided by the Laboratory of the Government Chemist. The bottles were cleaned with dilute hydrochloric acid, thoroughly washed with hot water, rinsed with deionized water, dried[1] and issued with a firmly adherent white label. Special care to avoid contamination was taken when a breast pump was used for the collection.

4.10 The separate milk samples from individual mothers were kept as cool and dark as possible. Each was frozen and stored at approximately $-20°C$ within an hour of commencing the collection.

4.11 When each hospital had collected enough milk the bottles were taken from the deep freeze and placed in insulated wooden boxes. Arrangements were successfully made for rapid transport by rail to London. The samples were still frozen on arrival at the Laboratory of the Government Chemist. These arrangements ensured that changes in nutrient composition from the time of collection to analysis were minimal.

[1]Tests were made to ensure that these glass bottles did not cause any chemical or other contamination of the milk.

5. Information about the Mothers and their Infants

5.1 The questionnaires (Appendix B, p 36), which were completed by the nursing sister or the midwife at the same time as the samples were obtained, supplied some details about the mothers and their infants. These are summarized in Table 2 (p 12).

5.2 There were 96 mothers and about half of them were primiparae. There were no statistically significant differences between the mean heights, weights and ages of the mothers in the five areas. In Birmingham, Bristol, Edinburgh and Newcastle over 70%, but in Cardiff only 36%, of the mothers who supplied milk samples belonged to Social Classes I and II. During pregnancy 84% of the mothers said that they had taken vitamin and/or iron supplements, and 53% said that they were continuing to do so while breast feeding.

5.3 There were 97 infants, including one set of twins. The mean age was 36 days (range 14–63 days). The mean birth weight was 3·4 kg (range 1·7–4·6 kg). Three infants had birth weights which were less than 2·5 kg; one in Cardiff (2·25 kg), one in Edinburgh (1·70 kg) and one in Newcastle (2·38 kg).

Table 2: *Information derived from the completed questionnaires*

		Birmingham	Bristol	Cardiff	Edinburgh	Newcastle	All Centres
MOTHERS							
Total number		19	18	11	23	25	96
Number of primiparae		14	8	5	12	12	51
Age years	mean ±SD	26 ± 3	26 ± 3	25 ± 3	27 ± 3	29 ± 4	27 ± 4
	range	21— 30	22— 33	20— 28	19— 32	21— 42	19— 42
Height cm	mean ±SD	164 ± 8	166 ± 4	165 ± 4	165 ± 6	162 ± 8	164 ± 7
	range	151—182	157—175	159—173	150—175	147—180	147—182
Weight kg	mean ±SD	63 ± 9	63 ± 7	59 ± 7	62 ± 8	61 ± 11	62 ± 9
	range	51— 91	52— 80	51— 76	49— 84	43— 85	43— 91
Percentage in Social Classes I and II		80	72	36	83	76	73
Number who took supplements of vitamins and/or iron							
	during pregnancy	17	17	10	14	23	81
	during lactation	12	13	5	10	13	53
Milk collection							
	by hand expression	0	18	0	1	25	44
	by breast pump	19	0	6	22	0	47
	by hand expression and breast pump	0	0	5	0	0	5
Volume of milk ml							
	mean ±SD	74 ± 21	93 ± 38	110 ± 38	58 ± 38	53 ± 26	73 ± 38
	range	35—105	50—165	50—166	10—145	20—105	10—166
INFANTS							
Number		19	18	11	24	25	97
Age days	mean ±SD	37 ± 8	34 ± 5	33 ± 5	34 ± 11	38 ± 11	36 ± 9
	range	25— 52	29— 43	29— 44	24— 57	14— 63	14— 63
Percentage of infants aged 4 weeks 0 days to 5 weeks 6 days		68	89	91	33	64	53
Birth weight kg	mean ±SD	3·5 ±0·5	3·5 ±0·4	3·3 ±0·7	3·4 ±0·6	3·4 ±0·2	3·4 ±0·5
	range	2·8—4·6	2·6—4·3	2·2—4·6	1·7—4·6	2·4—4·6	1·7—4·6

6. Analysis of the Milk

6.1 Preparation of the samples for analysis

6.1.1 On arrival at the Laboratory the samples of human milk were placed, still frozen, in a cabinet kept at a temperature of about −15°C. The day before the samples were required for analysis they were put in a refrigerator at +4°C to thaw.

6.1.2 The volume of milk donated by each mother was measured and the means, standard deviations and ranges are shown in Table 2 (p 12). Fourteen of the individual samples exceeded 100 ml in volume and, from these 14 samples, 100 ml portions were set aside for the main analyses and the remainder used for the estimation of vitamin D sulphate. The 100 ml portions from any one centre were pooled with the rest of the samples from the same centre. The average volume of the five pooled samples was 1280 ml. After gentle mixing, 500 ml portions were removed for the determination of vitamins A and C, folic acid and fatty acids. The remainder of each sample was immediately freeze dried, gently crushed and passed through a 1 mm mesh plastic sieve. The powders were thoroughly mixed and were stored in screw-capped containers at a temperature of about –15°C until required for analysis.

6.1.3 The period between the collection of the first samples of milk in any one area and the arrival of all the samples at the Laboratory varied from one week to 4 months. The additional time for preparation of the freeze dried powders after the arrival of the samples was less than one week for four of the centres, but for one centre was five weeks because the volume of milk first collected was less than one litre and a second collection had to be made.

6.2 Analytical methods

The methods used for the determination of the amounts of each nutrient in the analysis of the 5 pooled samples and the relevant references are given in Appendix C (p 37).

7. Results of the Analyses

7.1 The results of the analyses are presented in Tables 3–9 (pp 16 to 22) using the metric system per 100 ml of milk. Appendix D contains the results shown in Tables 3–5 expressed in S.I. units.

7.2 Where a mean value in the tables does not agree with the numerical average of the results for the five centres, this is because results have been rounded.

Table 3: *The amounts of water, total nitrogen[1], protein[2], fat, carbohydrate[3], energy[4], non-protein non-amino acid nitrogen[5] and cholesterol in 100 ml of pooled mature human milk*

Centre	Water	Total[1] nitrogen	Protein[2]	Fat	Carbohydrate[3]	Energy[4]	Non-protein[5] non-amino acid nitrogen	Cholesterol
	g	g	g	g	g	kcal	mg	mg
Birmingham	90·1	0·19	0·95	3·9	7·3	66	44	16
Bristol	89·3	0·22	1·20	4·8	7·1	75	41	23
Cardiff	89·7	0·21	1·10	4·4	7·3	71	41	12
Edinburgh	90·1	0·21	1·00	3·7	7·3	65	51	20
Newcastle	89·3	0·22	1·10	4 2	7·8	71	55	10
Mean values	89·7	0·21	1·07	4·2	7·4	70	46	16

(1) Determined by Kjeldahl method.
(2) Calculated from total amino acid nitrogen (ie free amino acids plus those derived by hydrolysis of milk protein) multiplied by the factor 6.38.
(3) Expressed as monosaccharide.
(4) Calculated by applying the modified Atwater factors (Southgate and Durnin, 1970) ie protein 4 kcal per g; fat 9 kcal per g: carbohydrate (as monosaccharide) 3.75 kcal per g.
(5) See para 8.2.3.

Table 4: *The amounts of different vitamins in 100 ml pooled mature human milk*

Centre	Retinol	α-tocopherol	Vitamin[1] C	Thiamin	Riboflavin	Nicotinic acid	Nicotinic[2] acid equivalents	Vitamin B_6 (pyridoxal)	Vitamin B_{12}	Folic[3] acid (total)	Pantothenic acid	Biotin
	μg	mg	mg	μg	μg	mg	mg	μg	μg	μg	mg	μg
Birmingham	56	0·35	4·1	16	31	0·21	0·58	5·8	0·01	3·1	0·27	0·72
Bristol	66	0·38	4·5	14	31	0·22	0·67	5·1	0·01	5·2	0·23	0·52
Cardiff	40	0·29	3·1	14	31	0·21	0·61	5·8	0·01	6·2	0·22	0·72
Edinburgh	76	0·39	3·8	21	31	0·24	0·57	5·8	0·01	6·2	0·33	1·13
Newcastle	62	0·36	3·6	13	31	0·27	0·65	7·2	0·01	5·2	0·25	0·72
Mean values	60	0·35	3·8	16	31	0·23	0·62	5·9	0·01	5·2	0·26	0·76

(1) Ascorbic acid plus dehydroascorbic acid.
(2) 1 nicotinic acid equivalent = 1 mg available nicotinic acid or 60 mg tryptophan.
(3) Conjugated plus non-conjugated folic acid.

Table 5: *The amounts of some inorganic nutrients[1] in 100 ml of pooled mature human milk (expressed as mg or μg per 100 ml, or as meq per litre)*

Centre	Na mg	K mg	Cl mg	Ca mg	Mg mg	P mg	Fe μg	Cu μg	Zn μg	Se μg	F μg	I μg	Na meq/l	K meq/l	Cl meq/l
Birmingham	11	60	35	36	2·8	15	62	37	290	1·1	6·2	2	4·8	15	10
Bristol	16	61	45	36	2·8	15	93	40	290	1·6	5.2	11	7·0	16	13
Cardiff	11	59	36	32	2·6	15	62	37	330	1·9	2·1	2	4·8	15	10
Edinburgh	20	62	55	35	3·0	14	82	39	260	0·8	9·3	12	8·7	16	15
Newcastle	15	57	44	34	2·9	14	82	43	300	1·5	15·5	7	6·5	15	12
Mean values	15	60	43	35	2·8	15	76	39	295	1·4	7·7	7	6·4	15	12

[1] The milks were examined for their content of chromium, cobalt, manganese and molybdenum but the amounts present were below the limits of the analytical methods employed.

Table 6: *Amino acid composition of pooled mature human milk (expressed as mg per g N)*

Centre	Ile	Leu	Lys	Met	Cys	Phe	Tyr	Thr	Trp	Val	Arg	His	Ala	Asp	Glu	Gly	Pro	Ser
Birmingham	330	590	425	90	115	220	180	265	140	405	225	150	235	530	1090	145	595	240
Bristol	325	575	460	95	125	(290)	190	285	150	435	250	155	260	545	1130	160	575	260
Cardiff	345	610	440	100	115	270	(235)	285	145	(325)	235	155	255	555	1110	155	600	265
Edinburgh	295	555	400	90	120	215	175	270	135	(450)	225	135	240	525	990	150	575	255
Newcastle	310	570	420	85	120	220	180	265	135	400	225	135	240	530	1050	145	540	255
Mean values	320	580	430	90	120	230	180	275	140	415	230	145	245	535	1075	150	575	255

Values given in brackets represent unsatisfactory determinations and have been omitted in calculating the means.

Key to abbreviations

Ile Isoleucine
Leu Leucine
Lys Lysine
Met Methionine
Cys Cystine
Phe Phenylalanine
Tyr Tyrosine
Thr Threonine
Trp Trytophan
Val Valine
Arg Arginine
His Histidine
Ala Alanine
Asp Aspartic acid
Glu Glutamic acid
Gly Glycine
Pro Proline
Ser Serine

Table 7: *Amino acid composition of pooled mature human milk (expressed as mg per 100 ml milk)*

Centre	Ile	Leu	Lys	Met	Cys	Phe	Tyr	Thr	Trp	Val	Arg	His	Ala	Asp	Glu	Gly	Pro	Ser
Birmingham	63	110	81	17	22	42	34	50	27	77	43	28	45	100	205	28	115	46
Bristol	72	125	100	21	28	(64)	42	63	33	96	55	34	57	120	250	35	125	57
Cardiff	72	130	92	21	24	57	(50)	60	30	(68)	49	33	54	115	235	33	125	56
Edinburgh	62	115	84	19	25	45	37	57	28	(94)	47	28	50	110	210	32	120	54
Newcastle	68	125	92	19	26	48	40	58	30	88	50	30	53	115	230	32	120	56
Mean values	67	120	90	19	25	48	38	58	30	87	49	31	52	110	225	32	120	54

Values given in brackets represent unsatisfactory determinations and have been omitted in calculating the means.

For key to abbreviations see footnote to Table 6.

Table 8: *Fatty acid composition[1] of pooled mature human milk (expressed as g per 100 g total fatty acids)*

Centre	saturated					mono-unsaturated				poly-unsaturated	
	10:0	12:0	14:0	16:0	18:0	16:1	17:1	18:1	20:1	18:2	18:3
Birmingham	1·6	6·0	7·9	26·5	8·5	4·2	0·5	35·0	0·5	7·7	0·8
Bristol	1·2	4·4	6·9	26·5	9·4	4·1	0·7	36·0	0·5	7·6	0·9
Cardiff	1·4	5·7	7·4	26·0	10·5	3·9	0·6	35·5	0·6	6·4	0·7
Edinburgh	1·3	5·6	7·6	27·0	9·3	4·2	0·6	34·5	0·5	7·2	0·7
Newcastle	1·3	5·3	6·9	26·5	10·0	3·7	0·5	36·0	0·6	7·1	0·9
Mean values	1·4	5·4	7·3	26·5	9·5	4·0	0·6	35·5	0·5	7·2	0·8

[1] Traces (less than 0·5 p per 100 g total fatty acids) of other fatty acids were also present, as follows;
- Bristol —8:0, 15:1, 15:br, 17:0, 20:3, 20:4, 20:5.
- Birmingham —8:0, 15:0, 15:1, 15:br, 17:0, 20:0, 20:3, 20:4, 20:5.
- Cardiff —8:0, 15:0, 15:1, 15:br, 17:0, 20:0, 20:2, 20:3, 20:4, 20:5.
- Newcastle —8:0, 15:0, 15:1, 17:0, 20:3, 20:4, 20:5.
- Edinburgh —8:0, 14:1, 15:0, 15:1, 17:0, 20:2, 20:3, 20:4, 20:5.

Notation —acids are represented by numbers indicating chain length and the number of double bonds, branched chains are indicated by 'br'.

Table 9: *Fatty acid composition[1] of fat in pooled mature human milk (expressed as mg per 100 ml milk[2])*

Centre	saturated					mono-unsaturated				poly-unsaturated	
	10:0	12:0	14:0	16:0	18:0	16:1	17:1	18:1	20:1	18:2	18:3
Birmingham	59	220	290	975	315	155	19	1290	18	285	29
Bristol	54	200	315	1200	425	185	32	1630	23	345	41
Cardiff	58	235	310	1080	435	160	25	1480	25	265	29
Edinburgh	45	195	265	950	325	145	21	1210	17	250	24
Newcastle	52	210	275	1050	395	145	20	1430	24	280	36
Mean values	54	210	290	1050	380	160	23	1410	21	285	32

(1) Traces of other fatty acids were also present (see footnote to Table 8).
(2) In the calculation it was assumed that 1 g of milk fat (triglyceride) is equivalent to 0·945 g fatty acids.

8. Discussion of Results

8.1 Comparison of results for the pooled samples from different centres

8.1.1 The values shown in Tables 3–9 give the composition of a pooled sample of human milk from each of the 5 centres. There was some variation in composition between the samples, although this was considerably less than the variation which has been reported between milk samples from individual women (Macy and Kelly, 1961).

8.1.2 The water content and the concentration of the chief energy-giving nutrients of the 5 samples (Table 3, p 16) showed relatively little variation, although the differences, especially in the fat content, resulted in energy values of from 65 to 75 kcal (272 to 314 kJ).

8.1.3 The concentrations of retinol, α-tocopherol and vitamin C (Table 4, p 17) were lower in Cardiff than in the other areas, although the concentrations of B vitamins in the milk from Cardiff were similar to those from other areas.

8.1.4 The concentration of sodium (Table 5, p 18) varied from 11 mg per 100 ml in Birmingham and Cardiff to 20 mg per 100 ml in Edinburgh. The concentration of chloride varied in a corresponding way. The concentrations of both fluoride and iodide varied at least six-fold from one area to another. The concentration of fluoride was particularly low in Cardiff and high in Newcastle. Fluoridation of water supplies to a concentration of 1 mg fluoride per litre is practised in Birmingham and Newcastle, but not in Bristol, Cardiff or Edinburgh, where the natural concentration of fluoride in domestic water supplies does not exceed 0·2 mg per litre (Department of the Environment, 1975).

8.2 Comparison of the results with those of other reported analyses

8.2.1 The results of the present analyses have been expressed in appropriate units per 100 ml milk, which weigh 103 g. Other values to which reference has been made, that is to say, those of Macy *et al* (1953), Macy and Kelly (1961), Fomon (1974), Kon and Mawson (1950) and Morrison (1962), were also expressed per 100 ml, but those of McCance and Widdowson (1967) per 100 g. In Table 1 (p 7) all the values of McCance and Widdowson have been multiplied by 1·03 to bring them into line with the rest.

8.2.2 *Water.* The mean value for the amount of water obtained in the analyses made by the Laboratory of the Government Chemist was 89·7 g per 100 ml milk (Table 3, p 16). This figure is higher than all the previously reported values (Table 1) except that of McCance and Widdowson with which it agrees when the McCance and Widdowson analysis per 100 g is adjusted to 100 ml milk. McCance and Widdowson (1967) reported 87·1 g water per 100 g milk which is similar to the values per 100 ml reported by other workers.

8.2.3 *Protein.* If the total nitrogen found in the milk in the present study is multiplied by the conventional factor 6·38, the result is 1·3 g protein per 100 ml. This is the same as the mean of the values for European and American samples (Morrison, 1952) shown in Table 1, column 6. In the results presented in this report the protein has been calculated from total amino acids, that is to say, free amino acids and those derived from the hydrolysis of protein. The nitrogen in urea and other non-protein nitrogenous compounds derived from blood was excluded, but is shown separately in the penultimate column in Table 3 (p 16). Lönnerdal, Forsum and Hambraeus (1976) also measured protein from the amino acids in milk and found the results to be similar to the calculated difference between total and non-protein nitrogen multiplied by 6·38.

8.2.4 *Fat.* The mean value (4·2 g/100 ml) is within the range of results of earlier analyses. Care was taken to collect all the milk from one breast, so as to minimize errors due to changes in fat content during the course of a feed, but it was not possible to obtain more than one sample from each woman. Gunther and Stanier (1949) showed that the concentration of fat in breast milk tends to be highest at noon and lowest at midnight, so in the present study the time of day chosen for the collection, between 9 a.m. and 2 p.m., was when the fat content might have been higher than at other times. However, in a study in which all the milk was collected throughout 72 hours from 10 women (Southgate and Barrett, 1966), the concentration of fat was 4·03 $\pm$ 0·43 (range 3·51 – 4·83) g/100 ml (Southgate, personal communication) which is not statistically significantly lower than the mean value in Table 3 (p 16).

8.2.5 *Lactose.* The values in the present study have been expressed as monosaccharide and the mean concentration was 7·4 g per 100 ml. This corresponds with the value obtained by earlier investigators of 7 g per 100 ml expressed as disaccharide in Table 1 (p 7).

8.2.6 *Energy.* Previous analyses (Table 1, p 7) showed that there is considerable individual variation in the energy value of human milk. The mean values given by different authors are close to the mean obtained in this study of 70 kcal per 100 ml, shown in Table 3 (p 16).

8.2.7 *Vitamins.* The concentrations of retinol, vitamin C, thiamin and riboflavin in the present study are of the same order as those reported by earlier investigators; those for nicotinic acid are a little higher. Values for biotin, pantothenic acid and vitamin B_{12} fall within the ranges quoted by Macy and

Kelly (1961); those for vitamin B_6 are appreciably lower. Concentrations of folic acid agree well with those reported in more recent publications (Karlin, 1967; Ford and Scott, 1968). No β-carotene was found in the milk. Vitamin D sulphate is discussed in Section 9 (p 27).

8.2.8 *Minerals.* Concentrations of sodium, potassium, chloride, calcium, magnesium, phosphorus, iron, copper and zinc all lie with the ranges given by previous authors.

8.2.9 The concentrations of selenium (0·8 – 1·9 μg per 100 ml) correspond with the lower end of the range of values reported by Hadjimarkos (1963) and Hadjimarkos and Shearer (1973) for samples of human milk collected in the United States; they are also similar to values reported by Millar and Sheppard (1972) for milk from New Zealand women.

8.2.10 The low concentration of fluoride in Cardiff is similar to the value obtained by Ericsson and Ribelius (1970), who reported 2·5 μg per 100 ml in the milk of Swedish mothers. The pooled samples from the other areas all contained more than this (para 8.1.3, p 23).

8.2.11 Salter (1950) reported mature human milk to contain 4–8 μg iodide per 100 ml. The values in the present study are of this order but the range is wider, the milk from Birmingham and Cardiff having the lowest concentration and Edinburgh the highest. The concentration of iodide in cows' milk, and presumably also in human milk, is influenced by the iodide intake of the mother. This may be the explanation for the area differences reported here.

8.2.12 Many analyses have been made for iron in human milk besides those used in compiling Table 1 (p 7). There are variations in the average figures given for mature milk by different authors, but most of the accepted values fall between 30 μg and 80 μg per 100 ml. The mean for milks included in the present study, 76 μg per 100 ml, is within this range. Picciano and Guthrie (1976) have recently analysed 7 samples of milk from each of 50 American women between the 6th and 12th week of lactation. They obtained a low mean value of 20 μg per 100 ml, but with a wide range, from < 10 to 160 μg per 100 ml.

8.2.13 The mean value for copper in the present series of mature milks (39 μg per 100 ml) is similar to the results of Macy and her co-workers (Table 1, Columns 1 and 2, p 7). Picciano and Guthrie (1976) determined copper as well as iron in their samples of human milk and obtained a mean value of 24 μg per 100 ml with a range of from 9 to 63 μg per 100 ml.

8.2.14 Since the concentration of zinc in human milk is so dependent on the stage of lactation (para 2.2.13) comparison of the present results with those reported in the literature is not helpful unless the stage of lactation is known. The present results fall between the mean values given by Macy *et al* (1953)

and Macy and Kelly (1961). Picciano and Guthrie (1976) obtained their samples of milk at a later stage of lactation than that of the present study. This may explain why their mean value (163 μg per 100 ml) is lower than that shown in Table 5 (p 18). They found very little variation in the concentration of zinc in the milk secreted by one woman from day to day, but a wide range, from 140 μg to 3,950 μg per 100 ml in the milk of different women.

8.2.15 *Amino acids.* Values for amino acids in human milk were given by Macy *et al* (1953), Macy and Kelly (1961), McCance and Widdowson (1967) and Fomon (1974). Some of these are clearly the same figures used by more than one author. The present results (Tables 6 and 7, p 19 and p 20) agree in general with these earlier values.

8.2.16 *Fatty acids.* The fatty acid composition of the fat in the samples of human milk collected for the present study (Tables 8 and 9, p 21 and p 22) was on the whole similar to that previously reported (Macy *et al*, 1953). No quantitative estimations of long chain polyunsaturated fatty acids were made.

9. Vitamin D Sulphate

9.1 It has been reported by Japanese and French workers (Sahashi, Suzuki, Higaki and Asano, 1967; 1969; Le Boulch, Gulat-Marnay and Raoul, 1974) that the aqueous fraction of human milk contains vitamin D sulphate. The amount is of the order of 2 μg per 100 ml in the early stages of lactation (Sahashi *et al*, 1967; 1969), which can be compared with the value of 0·01 μg per 100 ml usually quoted for the fat-soluble vitamin D in human milk Table 1, p 7).

9.2 Chemical determination of vitamin D sulphate (para 3.2, p 8) was made on the milk of three of the women in Birmingham, of six in Bristol and of five in Cardiff. The pooled samples from Edinburgh and Newcastle were also analysed. The results are shown in Table 10 (p 28).

9.3 These values for vitamin D sulphate in mature milk are lower than those obtained for colostrum (Lakdawala and Widdowson, 1977), and lower than those of the Japanese investigators for milk collected between the fourth and fifteenth day post-partum. This suggests that the concentration of vitamin D sulphate falls during the course of lactation. If the biological activity is similar to that of fat soluble vitamin D, a breast-fed infant would receive on average about 0·8 μg biologically active vitamin D per 100 ml milk in addition to the 0·01 μg of fat soluble vitamin. This would explain why healthy breast-fed babies do not show signs of vitamin D deficiency, a problem posed by Harris and Bunker (1939) when they made the first estimation of vitamin D in the lipid fraction of a composite sample of human milk and found 0·42 i.u. (0·01 μg) per 100 ml of milk—the figure quoted from Macy and Kelly (1961) in Table 1 (p 7).

Table 10: *The amount of vitamin D sulphate in the aqueous fraction of 100 ml mature human milk*

Centre	Number of samples	μg Vitamin D sulphate	
		Mean	Range
Birmingham	3	0·57	0·50—0·66
Bristol	6	0·75	0·45—1·05
Cardiff	5	0·99	0·91—1·05
Edinburgh	pooled samples	0·75	—
Newcastle	pooled samples	0·95	—
Mean value	—	0·80	—

10. Conclusions

10.1 Although there are variations in the composition of human milk from one area of Great Britain to another the differences, with the exception of the amounts of fluoride and iodide, are small. It is reassuring that the results of the present analyses differ little from those of earlier workers in spite of advances in analytical techniques.

10.2 The analyses of earlier workers together with those presented in this report can therefore be used with some confidence in suggesting nutritional guidelines for the composition of infant milk foods.

10.3 Members of the Working Party hope the publication of these results will stimulate further analyses of human milk, especially for other trace elements, long chain polyunsaturated fatty acids and vitamin K.

References

For references to analytical methods see Appendix C (pp 37 to 43).

Berfenstam, R., 1952.
Studies on blood zinc. A clinical and experimental investigation into the zinc content of plasma and blood corpuscles with special reference to infancy.
Acta Paediatrica Scandinavica, **41,** Supplement 87, 3–97.

Department of the Environment, 1975.
(unpublished information).

Department of Health and Social Security, 1969.
Recommended intakes of nutrients for the United Kingdom.
Reports on Public Health and Medical Subjects, No. 120.
London, H.M.S.O.

Department of Health and Social Security, 1974.
Present-day practice in infant feeding.
Reports on Health and Social Subjects, No. 9.
London, H.M.S.O.

Ericson, B. N., Gulick, M., Hunscher, H. A. and Macy, I. G., 1934.
Human milk studies. xv. The non-protein nitrogen constituents.
Journal of Biological Chemistry, **106,** 145–159.

Ericsson, Y. and Ribeleus, U., 1970.
Increased fluoride ingestion by bottle-fed infants and its effect.
Acta Paediatrica Scandinavica, **59,** 424–426.

Fomon, S. J., 1974.
Infant Nutrition. (2nd edition).
Philadelphia, W. B. Saunders Company.

Ford, J. E. and Scott, K. J., 1968.
The folic acid activity of some milk foods for babies.
Journal of Dairy Research, **35,** 85–90.

Gunther, M. and Stanier, J. E., 1949.
Diurnal variation in the fat content of breast-milk.
Lancet, ii, 235–242.

Hadjimarkos, D. M., 1963.
Selenium content of human milk: possible effects on dental caries.
Journal of Pediatrics, **63,** 273–275.

Hadjimarkos, D. M. and Shearer, T. R., 1973.
Selenium in mature human milk.
American Journal of Clinical Nutrition, **26,** 583–585.

Harris, R. S. and Bunker, J. W., 1939.
Vitamin D potency of human breast milk.
American Journal of Public Health, **29,** 744–747.

Hartman, A. M. and Dryden, L. P., 1965.
Vitamins in milk and milk products.
American Dairy Science Association.

Karlin, R., 1967.
Folic acid levels in human and cow's milk.
Internationale Zeitschrift für Vitaminforschung, **37,** 334–342.

Kon, S. K. and Cowie, A. T., 1961.
Milk: The mammary gland and its secretion.
New York, Academic Press.

Kon, S. K. and Mawson, E. H., 1950.
Human milk.
Special Report Series of the Medical Research Council, No. 269.
London, H.M.S.O.

Lakdawala, D. R. and Widdowson, E. M., 1977.
Vitamin D in human milk.
Lancet, i, 167–168.

Le Boulch, N., Gulat-Marnay, C. and Raoul, Y. 1974.
Dérivés de la vitamine D3 des laits de femme et de vache: ester sulfate de cholecalciferol et hydroxy-25-cholecalciferol.
International Journal for Vitamin and Nutrition Research, **44,** 167–169.

Lönnerdal, B., Forsum, E. and Hambraeus, L., 1976.
The protein content of human milk—a transverse study of Swedish normal material.
Nutrition Reports International, **13,** 125–134.

McCance, R. A. and Widdowson, E. M., 1967.
The composition of foods.
Special Report Series of the Medical Research Council, No. 297.
London, H.M.S.O.

Macy, I. G., 1949.
Composition of human colostrum and milk.
American Journal of Diseases of Children, **78,** 589–603.

Macy, I. G., Kelly, H. J. and Sloan, R. E., 1953.
The composition of milks.
National Research Council Publication, No. 254.
Washington D.C., National Academy of Sciences.

Macy, I. G. and Kelly, H. J., 1961.
Human milk and cows' milk in infant nutrition.
In: Milk: the mammary gland and its secretion.
Volume II, pp 265–304. Editors S. K. Kon and A. T. Cowie.
New York, Academic Press.

Millar, E. R. and Sheppard, A. D., 1972.
α-tocopherol and selenium levels in human and cows' milk.
New Zealand Journal of Science, **15,** 3–15.

Ministry of Agriculture, Fisheries and Food, 1974.
Food Standards Committee Review of Infant Foods.
Food Facts.
London, H.M.S.O.

Morrison, S. D., 1952.
Human Milk.
Commonwealth Bureau of Animal Nutrition. Technical Communication No. 18.
Aberdeen, University Press.

Picciano, M. F. and Guthrie, H., 1976.
Copper, iron, and zinc contents of mature human milk.
The American Journal of Clinical Nutrition, **29,** 242–254.

Sahashi, Y., Suzuki, T., Higaki, M. and Asano, T., 1967.
Metabolism of vitamin D in animals. 5. Isolation of vitamin D sulphate from mammalian milk.
Journal of Vitaminology, Japan, **13,** 33–36.

Sahashi, Y., Suzuki, T., Higaki, M. and Asano, T., 1969.
Antirachitic potency of vitamin D sulphate in human milk.
Journal of Vitaminology, Japan, **15,** 78–82.

Salter, W. T., 1950.
The chemistry and physiology of the thyroid hormones.
In: The hormones: physiology, chemistry and applications, Vol. 2, p. 18, editors G. Pincus and K. V. Thimann.
New York, Academic Press.

Southgate, D. A. T. and Barratt, I. M., 1966.
The intake and excretion of calorific constituents of milk by babies.
British Journal of Nutrition, **20,** 363–372.

Southgate, D. A. T. and Durnin, J. V. G. A., 1970.
Calorie conversion factors. An experimental reassessment of the factors used in the calculation of the energy value of human diets.
British Journal of Nutrition, **24,** 517–535.

The Royal Society, 1972.
Metric units, conversion factors and nomenclature in nutritional and food sciences.
Report of The Sub-Committee on Metrication of the British National Committee for Nutritional Sciences.
Cambridge, University Printing House.

Appendix A

PROTOCOL FOR THE COLLECTION OF THE SAMPLES OF MILK

General Information

1. The *mothers* from whom breast milk is to be collected should be:

   (a) 'white' (i.e. not Asian or other dark-skinned races—merely in order to standardise, at least in one way, the women from whom milk is collected).

   (b) 4–6 weeks post-partum.

   (c) breast feeding fully, i.e. not giving any complementary or supplementary feeds.

2. The *baby* should be thriving i.e. not requiring medical attention, and the weight at birth should be more than 2·5 kg.

3. The *breast milk* should be collected between 9.00 am and 2.00 pm, and about 3–4 hours after the previous feed. One breast should be emptied completely.

4. The milk must not be contaminated in any way, e.g. by contact with plastic, metal or rubber. All glass-ware used must have been cleaned with deionized or distilled water and thoroughly dried. Glass funnels and bottles with glass stoppers in which the milk is to be collected and stored will be supplied clean and dry and *ready for use.*

5. The milk is best expressed by hand. If a breast pump is to be used, the glass of the collection funnel and jar must be washed and dried (as above) before each collection, and the collecting tube into the glass jar must be *well below* the level of the rubber stopper (see diagram). The collecting jars will be supplied ready for use.

6. The milk should be kept as cool and dark as possible, and transferred to the deep freeze as quickly as possible, and certainly *within one hour of commencing the collection.*

7. Expression of the breast and collection of the milk should therefore be made *under supervision at the hospital.*

8. If possible breast milk should be obtained from *at least* 15 *mothers.* This is to ensure that the total volume of milk obtained from any one area is sufficient to provide enough milk solids for complete analysis.

9. The collections of breast milk should be made within a period of 2 weeks. Transport to London will then be arranged. Somebody will collect the milk and bring it by hand direct to the Laboratory of the Government Chemist where it will be freeze dried ready for analysis.

Detailed instructions for the collection of breast milk—from each of 15 *mothers*

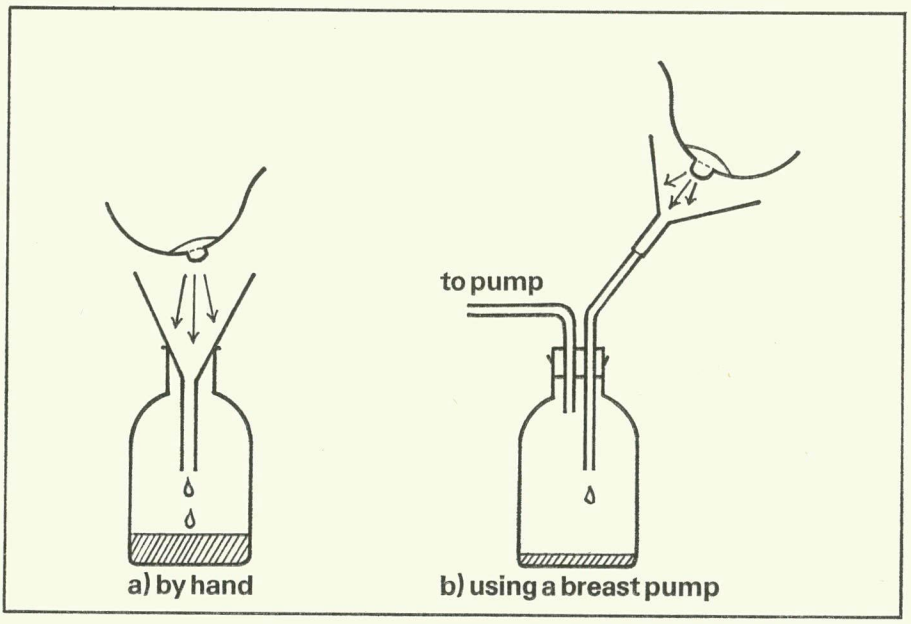
1. At some stage, please complete the questionnaire for each mother.

2. The mother should be white, 4–6 weeks post partum, breast feeding fully and with a thriving healthy baby.

3. The collection should be made between 9.00 am and 2.00 pm, 3–4 hours after the previous feed, at hospital.

4. The breast and nipple should be washed with deionized or distilled water, and thoroughly dried with a 'non-fluff' sterile towel.

5. One breast should be completely emptied, preferably by hand or by using an electric breast pump. Expression of the milk should be *supervised* to ensure that there is *no contamination*. The specially cleaned and sterilised bottles and funnels which are provided should be used (see diagrams).



6. When the contents of one breast have been collected, the milk should be mixed by gently rotating the bottle. The stoppers should be securely replaced.

7. If a breast pump has been used, the milk in the collecting bottle must be poured (using the funnel) into the glass stoppered bottle. Make sure that the stopper is replaced securely.

8. Make sure that the name of your area and of the mother are written on the label.

9. Place the labelled bottle of milk in the deep freeze at $-20°C$. This should be done within one hour of commencing the collection of the milk. The bottles should be left in the deep freeze until they are collected.

Appendix B

QUESTIONNAIRE TO BE COMPLETED AND SENT WITH EACH SAMPLE OF BREAST MILK.

1. Area where milk is collected .....

2. Date of collection .....

3. Name of mother .....

4. Mother's height ft inches, or cms

   weight st lbs. or kg

5. Mother's date of birth .....

6. How many children (live births) including the breast fed infant .....

7. How many miscarriages (including abortions and still births) .....

8. What is the occupation of the head of the household? (give in detail, e.g. whether foreman or not, whether self-employed or not)

   .....

   .....

   .....

9. Date of birth of the breast fed infant .....

10. Birth weight of infant .....

11. Present weight of infant .....

12. Has the milk been collected by hand expression — Yes .....

    No .....

    or by breast pump — Yes .....

    No .....

Appendix C

DETAILED ANALYTICAL METHODS (WITH REFERENCES)

These are described in the order in which the nutrients are given in the tables of results (pp 16–22).

Water

The water content was calculated from the sum of the loss in weight on freeze-drying and the water content of the freeze-dried solids, the latter being determined by drying 2 to 3 g at 100°C for 3 hours.

Nitrogen

Total nitrogen was determined by the Kjeldahl method.

Reference:
British Standard No. 1743, 1968.
Methods for the Chemical Analysis of Dried Milk pp 13, 14.
London, British Standards Institution.

Fat

Fat was determined by the Röse-Gottlieb procedure.

Reference:
British Standard No. 1743, 1968.
Methods for the Chemical Analysis of Dried Milk pp 6–12.
London, British Standards Institution.

Carbohydrate

Lactose was determined by the constant volume modification of the method of Lane and Eynon (1923) devised by the Laboratory of the Government Chemist (Nicholls, 1952).

References:
Lane, J. H. and Eynon, L., 1923.
Determination of reducing sugars by means of Fehling's solution with methylene blue as an internal indicator.
Journal of the Society of Chemical Industry, **42**, 32T–37T.

Nicholls, J. R., 1952.
Aids to the analysis of food and drugs. (7th edition.)
London, Baillière, Tindall and Cox.

Cholesterol

Dried powdered milk (1–2g) was saponified with ethanolic potassium hydroxide solution for 1 hour and the unsaponifiable material was extracted with diethyl ether. The ether extract was washed with water and the traces of water were removed from the ether by rotary evaporation in the presence of ethanol. After removal of traces of ethanol by a further evaporation with light petroleum, the residue was dissolved in 10 ml iso-octane (2, 2, 4, -trimethylpentane). An appropriate amount of an internal standard (α-tocopheryl propionate) was added to the extract and cholesterol was determined by gas-liquid chromatography on 2 metre glass columns, internal diameter 2 mm, containing 3% *OV17* on *Gas Chrom Q* at a temperature of 220°C.

Vitamin A (Retinol) and β-carotene

Liquid milk (30 ml) was saponified with ethanolic potassium hydroxide solution and the unsaponifiable matter, which contained retinol and β-carotene, was extracted with diethyl ether. Retinol was then separated from β-carotene sterols and vitamins D and E by partition chromatography between a mobile phase of 2, 2, 4-trimethylpentane and a stationary phase of methanol containing 10% water, using *Sephadex LH20* as the inert support for the stationary phase (Bell, 1971). Measurement of the absorbance of the retinol fraction in isopropyl alcohol (propan-2-ol) at 325 nm and of the β-carotene fraction in cyclohexane at 455 nm enabled the vitamin content to be calculated. Any irrelevant absorption in the ultra-violet region of the spectrum was allowed for by applying the correction of Wilkie (1964).

References:
Bell, J. G., 1971.
Separation of oil-soluble vitamins by partition chromatography on Sephadex LH20.
Chemistry and Industry, pp 201–202.
Wilkie, J. B., 1964.
Corrections for background in spectrophotometry using difference-in-absorbance values. Application to vitamin A.
Analytical Chemistry, **36**, 896–900.

α-tocopherol

Freeze-dried human milk (5g) was saponified with ethanolic potassium hydroxide solution under nitrogen. The unsaponifiable matter was extracted with diethyl ether and the extract was purified on a column (25 cm) of neutral alumina deactivated with 10% water. Tocopherols were then determined colorimetrically by reaction with ferric chloride and 4, 7-diphenyl-1, 10-phenanthroline reagents. Confirmation of the result, and evidence that only α-tocopherol was present, was obtained by gas-liquid chromatography on 2 metre glass columns of 3% *OV17 on Gas Chrom Q* at 235°C after removing cholesterol by precipitation from 72% ethanol, digitonide precipitation and passage through a column of *Celite* impregnated with digitonin. The internal standard was α-tocopherol propionate.

Reference:
Christie, A. A., Dean, A. C. and Millburn, B. A., 1973.
The determination of vitamin E in food by colorimetry and gas-liquid chromatography.
Analyst, **98**, 161–7.

Vitamin C

Preparation of extract:
Two hundred ml of an extracting solution consisting of 3% acetic and 8% metaphosphoric acids were added to 50 g of liquid milk. The vitamin was extracted by mixing in a *Sunbeam* blender for 2 minutes, centrifuging the mixture at 3000 revolutions per minute for 10 minutes and filtering the supernatant liquid through a filter paper.

Determination of ascorbic acid:

50 ml of the prepared extract were titrated with a solution of 2, 6-dichlorophenolindophenol which had been standardized against pure ascorbic acid.

Determination of ascorbic acid plus dehydroascorbic acid:

Ascorbic acid was converted to dehydroascorbic acid by shaking an aliquot of the prepared extract with *Norit* charcoal. The solution was then filtered and an aliquot was reacted with o-phenylenediamine reagent to form a fluorescent compound which was measured on a fluorometer at 455 nm using an exciting wave-length of 365 nm. Any fluorescence due to interfering substances was determined after complexing the dehydroascorbic acid with boric acid to prevent the formation of the fluorescent vitamin compound.

Reference:
Association of Official Analytical Chemists, 1975.
Official Methods of Analysis of the Association of Official Analytical Chemists. (12th edition), pp 829–831.
Washington, Association of Official Analytical Chemists.

Thiamin

Thiamin was determined by the fluorometric method recommended by the Sub-Committee on Vitamin Estimations. The stage involving isolation of the thiamin on a zeolite column was omitted and a range of standards was used to prepare a calibration graph.

Reference:
Society of Public Analysts and other Analytical Chemists.
Analytical Methods Committee, 1951.
Analyst, **76**, 127–133.

Vitamins of the B group other than thiamin

Riboflavin, nicotinic acid, vitamin B_6, vitamin B_{12}, folic acid, pantothenic acid and biotin were determined by microbiological assay. This involved liberation of the vitamin from the sample by acid or enzymatic hydrolysis; dilution with nutrient medium; sterilization of the solutions, inoculation and estimation of the growth of the micro-organisms by turbidimetry. A detailed description of all the techniques employed has been given by Bell (1974).

Vitamin	Micro-organism	American Type Culture Collection No.
Nicotinic acid	*Lactobacillus plantarum*	8014
Riboflavin	*Streptococcus zymogenes*	10100
Vitamin B_6	*Saccharomyces carlsbergensis*	9080
Vitamin B_{12}	*Lactobacillus leichmannii*	7830
Folic acid	*Lactobacillus casei*	7469
Pantothenic acid	*Lactobacillus plantarum*	8014
Biotin	*Lactobacillus plantarum*	8014

Reference:
Bell, J. G., 1974.
Microbiological assay of vitamins of the B group in foodstuffs.
Laboratory Practice, **23**, 235–242.

Sodium, potassium, calcium, magnesium, iron, copper, and zinc

Dried milk (5 g) was weighed into a silica dish and most of the organic matter was destroyed at a relatively low temperature either on a hot plate or under an infra-red lamp. The dish was then placed in a muffle furnace, the temperature was brought slowly to 550°C and the sample allowed to ash overnight. After cooling, 5 ml 1 : 1 hydrochloric acid solution were added and evaporated almost to dryness. The residue was heated with 5 ml of water and 5 ml 1 : 1 hydrochloric acid solution and filtered into a 100 ml graduated flask. The filter paper was ashed overnight in the same silica dish at 550°C and the residue was treated in the same way as before. The combined extracts in the graduated flask were diluted to 100 ml, thoroughly mixed and the solution was used either directly or after dilution for the determination of the metals by atomic absorption spectrometry in a *Perkin-Elmer* instrument, *model 403*. A portion of the extract was also used for the colorimetric determination of phosphorus.

Phosphorus

A suitable aliquot of the extract was transferred to a 50 ml graduated flask and 2 ml of ammonium molybdate reagent were added. The mixture was shaken well, allowed to stand for a few minutes and 2 ml of hydroquinone solution were added. The mixture was again shaken, 2 ml of sodium sulphite solution were added immediately and the solution diluted to volume. After standing for 1 hour the absorbance of the molybdenum blue complex was determined by means of a spectrophotometer at 650 nm and the phosphorus determined by comparison with a range of standards treated similarly.

Chloride

Dried milk (3 g) was moistened with a solution of sodium carbonate and heated first under an infra-red lamp and then in a muffle furnace at a temperature not exceeding 500°C. The residue was extracted with water,

acidified and diluted to about 100 ml. Chloride was then determined according to the Volhard procedure by treating this solution with an excess of 0·1N silver nitrate solution, adding 2 ml of nitrobenzene and determining the residual silver nitrate by titration with 0·1N potassium thiocyanate solution using ferric alum as indicator.

Selenium

Dried milk (1 g) was placed in a silica Kjeldahl digestion flask, a few ml of water, 5 ml nitric acid (sp. gr. 1·42) and 10 ml perchloric acid (sp. gr. 1·54) were added and the mixture allowed to stand overnight to allow some predigestion to take place. The following day the flask was gently heated and nitric acid added as required during the combustion to prevent charring. When the combustion was complete 10 ml sulphuric acid (sp. gr. 1·84) were added and the solution was evaporated, until fumes of sulphur trioxide were evolved, in order to remove all nitric and perchloric acids. Care was taken at this stage to avoid any trace of charring which would lead to loss of selenium as the hydride. After cooling, hydrogen peroxide solution was added to reduce Se^{VI} to Se^{IV} and the excess of hydrogen peroxide was destroyed by boiling. After cooling and diluting with water the solution was transferred to a conical flask and further diluted to about 100 ml. 25 ml of a glycine buffer solution and 10 ml of 0·02M ethylene diamine tetra-acetic acid solution were added and the pH was adjusted to 2·4 by the addition of 40% ammonia solution. Two ml of a freshly prepared and purified solution of 2, 3-diaminonaphthalene in 10% v/v sulphuric acid were added and the mixture heated for 1 hour. After cooling to room temperature the selenium-diaminonaphthalene complex was extracted with cyclohexane. The extract was filtered through siliconized filter paper to remove traces of water and the fluorescence measured at 518 nm, using an exciting wavelength of 378 nm. The method is a modification of that described by Ihnat (1974).

Reference:
Ihnat, M., 1974.
Fluorometric determination of selenium in food.
Journal of the Association of Official Analytical Chemists, **57,** 368–372.

Fluoride

Dried milk (3 g) was ashed at 500°C using magnesium succinate as an ashing aid. The residue was moistened with water and transferred to a 30 ml screw-capped polythene bottle with the aid of a solution of 4% silver sulphate in 60% perchloric acid. A piece of fluoride-free filter paper, which had been impregnated with magnesium succinate, was fitted inside the screw-cap lid which was securely closed. After heating in an oven at 60°C for 17 hours the paper was removed and placed in a 25 ml calibrated flask. To each flask 20 ml of water were added followed by 2·0 ml acidic zirconium reagent and 2·0 ml of *Solochrome cyanine R* reagent. After diluting to 25 ml with

water and thoroughly mixing the solutions, the fluoride was determined by its bleaching effect on the colour of the zirconium-solochrome cyanine R complex.

References:
Dixon, E. J., 1970.
Determination of microgram amounts of fluoride with zirconium and solochrome cyanine R.
Analyst, **95,** 272–277.

Sawyer, R., Grisley, L. M. and Cox, G. B., 1967.
Separation, identification and determination of fluoroacetamide residues in water, biological materials and soil, Part I.
Journal of the Science of Food and Agriculture, **18,** 283–286.

Iodide

Dried milk (0·1 g) was weighed into a crucible and ashed at 480°C using potassium carbonate as an ashing aid. The residue was extracted with water, centrifuged and the supernatant liquid diluted to 10 ml. To an aliquot of this solution potassium thiocyanate and ferric ammonium sulphate solutions were added, resulting in the formation of the red ferric thiocyanate complex. A solution of sodium nitrite was then added to oxidize the complex, a reaction catalysed by ionic iodine. The resulting diminution in colour, which is dependent on the amount of iodine present, was measured at 430 nm by spectrophotometry. Results were calculated using a calibration line constructed from standard solutions of potassium iodide.

Reference:
Shveikina, R. V., 1975.
Determination of iodine in milk.
Gigiyena-i-Sanitariya, **1,** 80–71 (in Russian).

Amino acids

A sample of the freeze-dried milk was defatted in a Soxhlet apparatus with light petroleum (b.p. 40°–60°C) for 2 hours and then thoroughly mixed. 200 mg of the defatted material were hydrolysed with 5 ml 6 N hydrochloric acid in an evacuated sealed tube for 16 hours at 125°C. The liberated amino acids were separated by ion-exchange chromatography on a *Technicon* autoanalyser and determined by colorimetric reaction with ninhydrin. Cystine and methionine were determined on pre-oxidized samples by the method of Moore (1963); tryptophan was determined after alkaline hydrolysis by the method of Miller (1967). Results were corrected for hydrolytic losses.

References:
Miller, E. L., 1967.
Determination of the tryptophan content of feeding stuffs with particular reference to cereals.
Journal of the Science of Food and Agriculture, **18,** 381–386.

Moore, S., 1963.
On the determination of cystine as cysteic acid.
Journal of Biological Chemistry, **238,** 235–237.

Fatty acids

The fat was isolated by a chloroform-methanol-water extraction process (Bligh and Dyer, 1959) from 16 ml of human milk. Solutions of the methyl esters of the fatty acids were prepared from the fat and the methyl esters of the fatty acids were separated and determined by gas-liquid chromatography (International Union of Pure and Applied Chemistry, 1976 a and b).

References:
Bligh, E. G. and Dyer, W. J., 1959.
A rapid method of total lipid extraction and purification.
Canadian Journal of Biochemistry, **37,** 911–917.

International Union of Pure and Applied Chemistry, 1976a.
In: Standard Methods for the Analysis of Oils, Fats and Soaps—4th supplement to 5th edition, Method II D.19. Preparation of Fatty Acid Methyl Esters.
Oxford, Pergamon Press.

International Union of Pure and Applied Chemistry, 1976b.
In: Standard Methods for the Analysis of Oils, Fats and Soaps—4th supplement to the 5th edition, Method II D.25. Gas liquid chromatography of the Fatty Acid Methyl Esters.
Oxford, Pergamon Press.

Vitamin D sulphate

The isolation and determination of vitamin D sulphate in the aqueous fraction of milk was made according to the method of Sahashi *et al* (1967). The procedure can be described in three steps.

1. Preliminary isolation of vitamin D sulphate.
2. Liberation of vitamin D from the sulphate complex.
3. Spectrophotometric determination of liberated vitamin D.

Vitamin D sulphate was isolated by shaking a portion of milk with ethanol acidified with acetic acid, centrifuging and separating the aqueous phase from the lipid fraction and the precipitated protein. The aqueous phase was treated with saturated barium hydroxide and centrifuged. The precipitate contained the vitamin as a complex of barium and vitamin D sulphate.

Vitamin D was liberated from the complex by saponifying with alcoholic potassium hydroxide and extracted with diethyl ether. The ether was removed under reduced pressure and the vitamin D was dissolved in chloroform.

The chloroform solution was treated with antimony trichloride reagent containing acetyl chloride (Nield's modification of the antimony trichloride reaction as described by Kodicek and Lawson, 1967). The difference between the reading at 500 and 550 nm gave the true absorbance for vitamin D, corrected for interfering substances.

References:
Kodicek, E. and Lawson, E. M., 1967.
In: The Vitamins (2nd edition), volume VI, chapter 4.
Editors P. György and W. N. Pearson.
New York, Academic Press.

Sahashi, Y., Suzuki, T., Higaki, M. and Asano, T., 1967.
Metabolism of vitamin D in animals. 5. Isolation of vitamin D sulphate from mammalian milk.
Journal of Vitaminology, Japan, **13,** 33–36.

Appendix D

TABLES OF RESULTS EXPRESSED IN S.I. UNITS.

Table 3D: *The amounts of water, total nitrogen[1], protein[2], fat, carbohydrate[3], energy[4], non-protein non-amino acid nitrogen[5] and cholesterol in one litre of pooled mature human milk*

Centre	Water	Total[1] nitrogen	Protein[2]	Fat	Carbohydrate[3]	Energy[4]	Non-protein[5] non-amino acid nitrogen	Cholesterol
	g	g	g	g	g	MJ	mg	mmol
Birmingham	901	1·9	9·5	39	73	2·8	440	0·42
Bristol	893	2·2	12·0	48	71	3·1	410	0·60
Cardiff	897	2·1	11·0	44	73	3·0	410	0·31
Edinburgh	901	2·1	10·0	37	73	2·7	510	0·52
Newcastle	893	2·2	11·0	42	78	3·0	550	0·26
Mean values	897	2·1	10·5	42	74	2·9	460	0·42

(1) Determined by Kjeldahl method.
(2) Calculated from total amino acid nitrogen (i.e. free amino acids plus those derived by hydrolysis of milk protein) multiplied by the factor 6·38.
(3) Expressed as monosaccharide.
(4) Calculated by applying the conversion factors advoctated by the Royal Society (1972), protein 17 kJ per g; fat 37 kJ per g; carbohydrate (as monosaccharide) 16 kJ per g.
(5) See paragraph 8.2.3.

Table 4D: *The amounts of different vitamins in one litre of pooled mature human milk*

Centre	Retinol	α-tocopherol	Vitamin[1] C	Thiamin[2]	Riboflavin	Nicotinic acid	Vitamin[3] B_6 (pyridoxal)	Vitamin[4] B_{12}	Folic[5] acid (total)	Pantothenic acid	Biotin
	μmol	μmol	mmol	μmol	μmol	μmol	μmol	pmol	nmol	μmol	nmol
Birmingham	2·0	8·1	0·23	0·60	0·82	17	0·35	70	70	12	29
Bristol	2·3	8·8	0·26	0·53	0·82	18	0·30	70	120	10	21
Cardiff	1·4	6·7	0·18	0·53	0·82	17	0·35	70	140	10	29
Edinburgh	2·7	9·1	0·22	0·79	0·82	20	0·35	70	140	15	46
Newcastle	2·2	8·4	0·20	0·49	0·82	22	0·43	70	120	11	29
Mean values	2·1	8·2	0·22	0·59	0·82	19	0·36	70	120	12	31

(1) Ascorbic acid plus dehydroascorbic acid.
(2) Thiamin converted to SI units using the molecular weight of thiamin hydrochloride—337·3.
(3) Vitamin B_6 converted to SI units using the molecular weight of pyridoxal—167·2.
(4) Vitamin B_{12} converted to SI units using the molecular weight of cyanocobalamin—1355.
(5) Folic acid (conjugated plus non-conjugated) converted to SI units using the molecular weight of pteroylglutamic acid—441·4.

Table 5D: *The amounts of some inorganic nutrients*[1] *in one litre of pooled mature human milk*

Centre	Na mmol	K mmol	Cl mmol	Ca mmol	Mg mmol	P mmol	Fe μmol	Cu μmol	Zn μmol	Se μmol	F μmol	I μmol
Birmingham	4·8	15	10	9·0	1·2	4·8	11	5·8	44	0·14	3·3	0·16
Bristol	7·0	16	13	9·0	1·2	4·8	17	6·3	44	0·20	2·7	0·87
Cardiff	4·8	15	10	8·0	1·1	4·8	11	5·8	51	0·24	1·1	0·16
Edinburgh	8·7	16	15	8·8	1·3	4·5	15	6·1	40	0·10	4·9	0·94
Newcastle	6·5	15	12	8·5	1·2	4·5	15	6·8	46	0·19	8·2	0·55
Mean values	6 4	15	12	8·7	1·2	4·7	14	6·2	45	0·17	4·0	0·54

(1) The milks were examined for their content of chromium, cobalt, manganese and molybdenum but the amounts present were below the limits of the analytical methods employed.

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