Kwashiorkor in western Nigeria:
a study of traditional weaning foods, with particular
reference to energy and linoleic acid*

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1. The fatty-acid composition of the plasma total lipids of children with kwashiorkor and of healthy infants was determined by gas–liquid chromatography. Breast milk from Yoruba mothers, and traditional weaning foods, were also analysed for fatty acids, and for protein, fat and carbohydrate.

2. Evidence of essential fatty acid (EFA) deficiency was obtained in the children with kwashiorkor. The proportions of linoleic and arachidonic acids were reduced in the plasma lipids, whereas the endogenous eicosatrienoic acid showed a marked increase. The triene:tetraene ratio had the abnormally high value of 1·08. These changes were consistent with the prolonged ingestion of suboptimal amounts of linoleic acid.

3. Breast milk was of good quality, with a particularly high concentration of retinol. The milk was also rich in linoleic acid, and was thus discounted as a factor in the development of EFA deficiency, but the weaning foods were found to provide substantially less than the minimum recommended intake of 1 % of the total energy as linoleic acid.

4. The maize pap with which the children with kwashiorkor had been fed for several months before the appearance of acute symptoms provided almost 7 % of the energy as protein, but only 1·21 MJ (290 kcal)/kg. To satisfy energy requirements, it would have been necessary to consume 3–4 kg of the pap each day.

5. It is concluded that the protein deficiency which leads to the development of kwashiorkor in the Yoruba community arises from a very severe restriction in energy intake rather than from the consumption of foods very low in protein at adequate or excessive levels of energy intake.

The development of kwashiorkor has long been attributed to the consumption of diets low in protein and rich in carbohydrate (Brock & Autret, 1952). The lack of fat in such diets, though occasionally remarked upon (Bronte-Stewart, 1961), has not, however, been considered as an important dietary factor in the aetiology of the disease. The human infant, like the young of other species, has been shown to have a requirement for fat by virtue of its content of linoleic acid – the essential fatty acid (EFA). Wiese, Hansen & Adam (1958) noted that a diet providing 1 % of the total energy as linoleic acid was just adequate to prevent the clinical manifestations of EFA deficiency, and concluded, on the basis of measurements of serum fatty acid ratios, that optimum consumption would represent 4 % of the energy intake.

In the infant with kwashiorkor, a deficiency of linoleic acid might have arisen in a number of ways: in the preweaning period from inadequate nutrition of the mother, resulting in the secretion of milk with a low concentration of linoleic acid, or, in the postweaning period, from feeding the children on traditional infant foods low in fat, or from an impaired absorption of fat.

* A preliminary account of part of this work has been published in abstract form (Naismith, 1964).

The purpose of this investigation was to seek biochemical evidence of EFA deficiency in patients with kwashiorkor. Blood samples were obtained for analysis of total plasma lipids, and the composition, with respect to fatty acids, was compared with that of plasma lipids obtained from normal healthy infants. Samples of breast milk and of the foodstuffs most commonly used in infant feeding were also analysed.

EXPERIMENTAL

Subjects

Twenty male infants with kwashiorkor were selected, during 1963, from the many who were brought each day from the city and from surrounding villages to the Out-patients Clinic of University College Hospital, Ibadan. None had suffered recently from measles or from other infections, and all had the symptoms that are characteristic of kwashiorkor in western Nigeria to a marked degree. These are extreme misery and apathy, diarrhoea, oedema, skin changes and low body-weight for age. The children were aged between 2 and 3 years, and their mean body-weight was 8·1 kg. A history of the disease and a detailed account of the nature of the diet offered to the child during the months preceding the appearance of acute symptoms were obtained from the mother of each child with the help of a paediatrician and a skilled hospital interpreter. For comparison, twenty healthy infants, aged between 12 and 18 months, who were taking part in the trial of a measles vaccine, were also studied.

Venous blood (4 ml) was drawn from each patient on admission and from the controls for determination of the composition of the total plasma lipids.

Milk samples

Breast milk was obtained from twenty-four healthy Yoruba women. Mid-stream samples of transitional milk, between the 4th and 10th days of lactation, were collected in the Department of Obstetrics from twelve mothers before they were released from hospital. Mature milk (from the 5th to 11th month of lactation) was obtained from twelve mothers who were living-in with infant patients in the paediatrics wards, and who were expressing milk in order to prevent the cessation of milk flow. The samples of transitional milk and mature milk were pooled and analysed for total solids, protein, fat and retinol, and the fatty acid composition of the total lipids was determined by gas–liquid chromatography.

Infant weaning foods

It was established from the interviews with the mothers that the patients with kwashi-orkor had been fed almost entirely on a starchy pap made from maize (eko), with, very occasionally, small amounts of a preparation of cooked cassava (amala) or yam (eba). Four samples of amala and of eba were purchased in the markets of Ibadan, and six samples of eko were obtained from the mothers of the patients at the time of interview. The foods were pooled and analysed for water, protein and fat. Carbohydrate was calculated by difference. The linoleic acid content of the fats was measured by gas–liquid chromatography.

Analytical methods

Lipids were extracted from 1·0 ml samples of blood plasma and milk by shaking with 10·0 ml ethanol–diethyl ether (3:1, v/v). The extract was separated by centrifugation, a few crystals of hydroquinone being added to minimize oxidation, and rapidly evaporated to dryness on a water-bath under reduced pressure. The foodstuffs were freeze-dried before extraction at room temperature with a 2:1 (v/v) mixture of chloroform and methanol. This extract was freed from protein by washing with a solution of calcium chloride (0·5 g/l) (Folch, Lees & Stanley, 1957) before evaporating to dryness in the presence of hydroquinone.

The lipids were saponified by refluxing for 2 h with 0·5 M-KOH in methanol. Nonsaponifiable material was removed by washing twice with light petroleum (b.p. 40°–100°) and the fatty acids were extracted with light petroleum after acidification with 2·5 M-H_2SO_4. Methyl esters were prepared by refluxing for 1 h with H_2SO_4 in methanol (20 ml/l). Gas–liquid chromatography was carried out on an argon gas chromatograph incorporating a strontium detector (W. G. Pye & Co. Ltd, Cambridge). The methyl esters were separated at 185° on a column packed with 15 % ethyleneglycol-adipate polyester (PEGA) on 100–200 mesh Celite. Individual fatty acids were identified by (*a*) carbon numbers (Woodford & van Gent, 1960) on PEGA and occasionally on Apiezon L columns and (*b*) comparison of retention times, relative to methyl stearate, with those of pure reference compounds or with published values. The chromatographs were compared with those obtained from analyses of the plasma lipids of rats reared on a fat-free diet for further confirmation of the identity of eicosatrienoic acid (20:3 ω9). The proportions of the fatty acids in the various lipids were calculated from the areas of the peaks, measured by triangulation.

The retinol concentration in the plasma was measured by the method of Paterson & Wiggins (1954). The total solids in milk and in the foodstuffs were estimated by freeze-drying weighed portions, and the concentration of fat was determined by extracting the dried material in a Soxhlet apparatus using a chloroform–methanol mixture (2:1, v/v). The extract was evaporated to dryness after washing as described above.

Fresh milk and the infant foods were analysed for nitrogen by the Kjeldahl procedure. Proteins were precipitated from the milk at 0° with trichloroacetic acid, and a value for milk protein was obtained by multiplying protein nitrogen by 6·38. The factor 6·25 was used for the estimation of protein in the foods.

The results were evaluated statistically by Student's *t*-test.

RESULTS

Plasma lipid analyses

The results of the analyses of the plasma lipids of infants with kwashiorkor and of healthy infants are summarized in Table 1.

Changes in the proportions of the major fatty acids that are characteristic of EFA deficiency were observed in the patients with kwashiorkor. The concentration of linoleic acid (18:2 ω6) in the fatty acids of the total plasma lipids was considerably

Table 1. *Percentage composition of the fatty acids of total plasma lipids from twenty healthy infants and from twenty children with kwashiorkor*

(Mean values with their standard errors)

Fatty acid		Healthy infants	Children with kwashiorkor
Designation	Trivial name		
14:0	Myristic	1·9±0·17	1·0±0·09
16:0	Palmitic	22·0±0·51	25·6±0·56
16:1	Palmitoleic	1·8±0·06	3·1±0·16***
18:0	Stearic	10·2±0·75	9·2±0·26
18:1	Oleic	24·4±0·91	32·0±0·80***
18:2	Linoleic	27·4±0·58	20·5±0·54***
18:3	Linolenic	1·7±0·20	1·1±0·11***
20:3	Eicosatrienoic	2·0±0·21	3·9±0·42***
20:4	Arachidonic	8·6±0·50	3·6±0·19***
20:3–20:4		0·23±0·02	1·08±0·15***

*** Value differs significantly from value for healthy infants ($P < 0.001$).

lower than the value found for healthy infants; the concentration of arachidonic acid (20:4 ω6), which is synthesized from linoleic acid in the tissues, was less than half that in the controls. The fall in the essential polyunsaturated fatty acids was compensated by a rise in the proportion of endogenous eicosatrienoic acid (20:3 ω9), and in the mono-unsaturated palmitoleic and oleic acids. These differences were all statistically significant.

Holman (1960) has suggested that the best measure of the extent of EFA deficiency is the triene:tetraene ratio (20:3 ω9 to 20:4 ω6) in the tissues or in the plasma. A ratio less than 0·4 is taken to indicate that the minimum dietary requirement for linoleate has been met. In none of the control subjects did the triene:tetraene ratio exceed 0·4, whereas in all the infants with kwashiorkor ratios greater than 0·4 were observed, with a mean value of 1·08.

Analyses of breast milk

The hypothesis that EFA deficiency might have been induced during the suckling period by the ingestion of milk with a low content of linoleic acid was not upheld by the results of the analysis of breast milk (Table 2).

No differences worthy of comment were found between the samples of transitional and mature milk in the composition of the fat. Linoleic acid made up between 15 % and 16 % of the total fatty acids of the milk lipids. For comparison, the composition of the milk fat of North American women, as reported by Macy & Kelly (1961) is included in Table 2. The composition is similar apart from the proportion of linoleic acid. The Yoruba mother provided almost twice as much linoleic acid in her milk as did her American counterpart.

The gross composition of the two Nigerian milk samples is given in Table 3. Lactose was not determined chemically, but was calculated by subtracting the values for fat and protein from the total solids. The value shown in the table includes, therefore, the minerals and the other minor constituents of milk. The concentrations of protein, fat and carbohydrate did not differ from those found in the milk of North

Table 2. *Percentage composition of the fatty acids of the total lipids of breast milk*

Fatty acid	Western Nigeria		Tanzania*	North America†
	Transitional	Mature		
12:0	4·7	4·0	16·5	5·8
14:0	7·4	6·5	12·7	8·6
16:0	19·5	25·0	17·5	22·6
16:1	1·4	2·5	1·4	2·9
18:0	6·0	5·8	6·0	7·7
18:1	41·9	39·5	39·6	36·4
18:2	16·3	15·1	1·0	8·3
18:3	1·1	0·6	NR	0·4
20:4	0·9	0·5	NR	1·0

NR, not recorded.   * Read, Lutz & Tashjian (1965).   † Macy & Kelly (1961).

Table 3. *Composition of the milk of healthy Yoruba mothers*

Milk sample	Total solids (g/l)	Fat (g/l)	Protein (g/l)	Lactose (g/l)	Retinol (mg/l)
Transitional (8 d)	134·5	42·2	20·3	71·8	2·57
Mature (7 months)	131·9	40·5	12·2	76·7	1·12

American women (Macy & Kelly, 1961). A striking difference was, however, found in the concentration of retinol, which, in the mature milk, was approximately double the average value reported by Macy & Kelly for the milk of healthy American women (0·53 mg/l).

Analyses of infant foods

The gross compositions of eko, the traditional infant food used in western Nigeria, and, for comparison, Nigerian whole maize are given in Table 4. Adult foods made from cassava and yam are also thought to be suitable for feeding to infants and are included in the diet in small amounts. The composition of these foods is shown in Table 4.

Eko, the major component of the diet of infants who develop kwashiorkor, contained little more than 70 g solids/kg, the bulk of which consisted of carbohydrate. Consequently this food would provide only 1·21 MJ (290 kcal)/kg. During the preparation of eko from maize, 81 % of the fat was lost, along with 41 % of the protein. The proportion of the total metabolizable energy available from fat was thereby reduced from 11 % in raw maize to 2 % in the infant food. Analysis of the fat extracted from eko showed that linoleic acid made up 41 % of the total fatty acids. Thus, when allowance was made for the glycerol component of the fat, the proportion of the total energy that could be derived from linoleic acid was found to be 0·74 %. The adult foods which were given occasionally to the infants contributed even less linoleic acid to the diet. Analyses of the lipids extracted from amala and eba revealed that linoleic acid made up 16 % and 27 % respectively of the total fatty acids. These foods could provide, therefore, no more than 0·13 % and 0·23 % of their total energy as linoleic acid.

Table 4. *Composition of traditional Yoruba weaning foods and uncooked Nigerian maize*

Foodstuff	Water (g/kg)	Energy		Dry matter (g/kg)		
		(MJ/kg)	(kcal/kg)	Protein	Fat	Carbohydrate
Eko (maize)	928	1·21	290	67	9	924
Eba (cassava)	765	3·93	940	10	4	986
Amala (yam)	682	5·30	1270	51	6	943
Maize	120			114	51	834

DISCUSSION

Kwashiorkor is a common disorder in western Nigeria, the maximal incidence occurring between the ages of 3 and 4 years. Most Yoruba children are weaned when 2–3 years old, which may account for the comparatively late onset of the disease. The characteristic features of kwashiorkor, as seen in Ibadan, are oedema of the face and limbs, persistent diarrhoea and loss of weight (the symptoms which usually persuade the mother to seek medical advice), extreme misery and apathy, a rough and scaly skin, loss of hair pigmentation, and a decline in appetite. Moderate liver enlargement is less common, occurring in about 40 % of the patients (unpublished study of 454 cases of kwashiorkor in Ibadan carried out in the Children's Department by Dr Mary A. le C. Hills).

The patients involved in the present investigation were between 2 and 3 years old, with an average age of 2 years and 9 months. Each one displayed all of the symptoms listed above. Although the healthy infants studied were not ideal control subjects, since they were, on average, 18 months younger, the choice was deliberate, because it allowed a comparison of children whose diet consisted very largely of breast milk with children who had received no breast milk for many months.

From the histories obtained from the mothers, a consistent picture emerged. The acute symptoms, usually diarrhoea and swelling of the limbs, had been noticed 2–4 weeks before the child was brought to hospital, but the mothers admitted that their children had been unwell for many weeks or months beforehand. The diet had consisted almost entirely of eko with occasional small amounts of amala and eba as used in the adult diet. Whereas eba and amala fall into the category of foods rich in carbohydrate and very low in protein that have come to be accepted as the prime dietary cause of kwashiorkor, eko, the major component of the diet, does not.

In the classical description of protein-energy malnutrition, the two clinically distinct forms of the disease, marasmus and kwashiorkor, are regarded as having different aetiologies. The former is attributed to 'an insufficiency of all food, however good', i.e. energy, whereas the latter is attributed to 'a deficiency of protein in a diet potentially high in calories' (McCance, 1968). Though it cannot be disputed that many of the foodstuffs used in the preparation of infant weaning foods, in areas of the world where kwashiorkor is endemic, are extremely low in protein, there is, however, no evidence from dietary studies to support the view that the energy intake of children with kwashiorkor has been adequate or more than adequate. On the contrary, when measurements of food consumption have been made, energy intakes have been found

to be far below the recommended levels. In a field study conducted among poor rural communities in southern India, Gopalan (1968) found no qualitative differences between the diets of children who developed marasmus and those who developed kwashiorkor. Furthermore, the children suffering from kwashiorkor had not been receiving more food than those with marasmus but had subsisted for some weeks before the appearance of the acute symptoms on even smaller quantities of the same cereal-based diets taken by the rest of the child population.

To provide for the full achievement of the potential capacity for growth in a 2- to 3-year-old child, the age-group of the subjects of the present study, the daily intake of energy recommended is 5·4 MJ (1300 kcal) (FAO, 1968). Since the weaning food eko provided only 1·21 MJ (290 kcal) per kg, the children would have had to consume 4·5 kg of this pap each day in order to meet their needs for energy, which is clearly impossible. It might be argued that the prediction of energy requirements should be based on the actual mean body-weight of the children rather than on the ideal body-weight corresponding to their age. Though such an exercise is valuable, since it serves to reinforce a point made earlier, it is of doubtful significance for the following reason: the body-weights at the time of admission had been profoundly influenced by two opposing factors, the magnitude of neither of which could be assessed, the retention of water, as evidenced by the presence of oedema, and a loss of body-weight over a period of months before the recognition of the oedema by the mother. The mean body-weight of the twenty patients was 8·1 kg, which is well below the median value of 12·1 kg given by Morley, Woodland, Martin & Allen (1968) for normal male children of the same age from a typical large Yoruba village. At this weight, the daily energy requirement of 3·7 MJ (884 kcal) could be met by the consumption of 3·1 kg pap, still a volume of food that would greatly exceed the appetite and stomach capacity of the child.

It may therefore be concluded that kwashiorkor in the Yoruba community is precipitated by the consumption of a diet which offers almost 7 % of the total energy as protein, albeit a protein of poor quality, but which can provide no more than a small fraction, probably less than a quarter, of the energy requirement.

The results of this investigation in no way deny the paramount role of protein deficiency in the causation of kwashiorkor, but, like those of Gopalan (1968), indicate that the condition of protein deprivation is reached as a result of a very severe restriction in energy intake rather than from the consumption of foods very low in protein at levels of energy intake approaching the normal.

The utilization of both energy and protein is known to be influenced by the concentrations of many of the minor nutrients in the diet, including linoleic acid. Wiese *et al.* (1958) observed that the voluntary food consumption of infants fed on different milk mixtures rose or fell sharply, without a corresponding change in the rate of gain in body-weight, following the removal of linoleic acid from, or the addition of methyl linoleate to, the diet and impaired nitrogen retention has been demonstrated in rats maintained on a diet devoid of linoleic acid, when compared with pair-fed controls that had received a polyunsaturated fat (Naismith, 1962).

From descriptions of the nature of the various weaning foods used in countries

where protein-energy malnutrition occurs (Brock & Autret, 1952), it seemed likely that the lack of fat in the diet might well lead to EFA deficiency. This impression was confirmed in the present study. In all twenty infants suffering from kwashiorkor there was a reduced proportion of linoleic and arachidonic acids in the total lipids of the plasma, and a rise in the proportion of the endogenous polyunsaturated eicosatrienoic acid. Using the ratio triene:tetraene as the criterion of EFA deficiency (Soderhjelm, Wiese & Holman, 1971), the abnormally high mean value of 1·08 was found for the patients, whereas for the breast-fed infants a value well below 0·4, the upper limit of normality, was found.

Much higher triene:tetraene ratios have been reported recently in the plasma lipids in children in whom an EFA deficiency was produced inadvertently by prolonged intravenous feeding with a fat-free preparation (Paulsrud, Pensler, Whitten, Stewart & Holman, 1972). In this instance, the response to the exclusion of linoleic acid from an otherwise adequate diet was found to be extremely rapid. The concentration of eicosatrienoic acid rose and those of the essential polyunsaturated acids fell markedly within a few days of initiating the intravenous feeding, indicating that the serum linoleic acid pool had been depleted. The dietary situation of the children with kwashiorkor differed in one important respect, in that the diet did provide some linoleic acid, although at a concentration substantially below the minimum recommended level of 1 % of the total energy. This difference undoubtedly accounts for the less dramatic changes that were observed in the pattern of fatty acids. A comparison of the present results with those obtained in animal experiments is instructive. Alterations in the composition of the plasma total lipids similar to those found in the infants with kwashiorkor have been noted in pigs reared from weaning on diets deficient in linoleic acid (Leat, 1963). In this animal, which has the same requirement for linoleic acid as the human infant, when the linoleic acid intake was reduced from optimum intake, i.e. greater than 1 %, to 0·5 % of the total energy, plasma linoleic acid showed a comparatively moderate decline of 24 %, which was accompanied by a threefold increase in eicostrienoic acid. The triene:tetraene ratio rose to 0·9. In the human infants, the fall in the proportion of linoleic acid in the plasma lipids was 25 %, and the rise in eicosatrienoic acid was twofold. The triene:tetraene ratio was similar.

These findings for the pig strongly suggest that the development of EFA deficiency in the children with kwashiorkor was also the long-term effect of consuming a diet which failed to provide the minimum requirement of linoleic acid rather than the acute effect of a more drastic reduction in linoleic acid intake, although impaired digestion and absorption of fat may also have contributed to the condition. Many of the children were observed to pass the food they had eaten still recognizable in their stools, which may have been due, *inter alia*, to a loss of lipase activity in the duodenal contents, which has been reported in patients with kwashiorkor in other parts of Africa (Brock & Autret, 1952).

Maize, the staple from which the traditional infant food was prepared, itself contains a highly unsaturated fat, but after the preparation of eko from maize, a process which involves steeping the grain in water for at least 48 h, the removal of most of the germ and husk, and partial fermentation of the starch, little of the fat remains.

The possibility of EFA deficiency having occurred before weaning was discounted when the results of the analyses of human milk were obtained. The milk of Yoruba mothers was found to be particularly rich in linoleic acid, which accounts for the low triene:tetraene ratios in the plasma lipids of the breast-fed infants. The concentration of linoleic acid in human milk has, however, been shown to depend on the quality of the dietary fats (Read, Lutz & Tashjian, 1965) and the Yoruba mother is fortunate in that she makes liberal use of groundnut oil and red palm oil in her cooking. The analysis of milk for retinol was prompted by the pale yellow colour of the milk, from which a layer of cream, vermillion in colour, separated on standing. The very high concentration of retinol in the milk was clearly the result of the ingestion of large amounts of carotene in the red palm oil.

In other regions of the continent where, for various reasons, highly unsaturated vegetable oils may not readily be available, the situation may be very different. The milk of apparently healthy mothers of the Sukuma and Nyamwezi Bantu tribes of the lake region of Tanzania has been shown to have fat containing only 1 % of linoleic acid (Read *et al.* 1965) compared with 15 % in the milk of Yoruba mothers, and increased proportions of the short-chain lauric and myristic acids (see Table 2), indicative of a diet very high in carbohydrate and low in fat (Insull, Hirsch, James & Ahrens, 1959). This milk would provide the suckling infant with less than 0·5 % of its total energy intake as linoleic acid. It is regrettable that an analysis was not made of the plasma lipids of unweaned infants from these tribes.

In other respects, the Yoruba milk was of good quality. No evidence was obtained of an abnormally low concentration of protein in the mature milk, as was found by Lutz & Platt (1958) to accompany a declining milk yield in poor mothers in other parts of Africa and in India, and the composition of the milk fat was consistent with the consumption of a balanced diet rather than of a diet of predominantly carbohydrate-rich foods.

In conclusion, it is suggested that the Yoruba infant, during the early months of life, receives adequate amounts of protein and linoleic acid, but that as breast milk fails to satisfy the infant's need for energy, and as maize-pap supplements then finally replaces milk a point may be reached when the mechanisms of adaptation cease to cope. The extent and duration of this replacement before diversification of the diet presumably determines which child will develop kwashiorkor and which child will not. Though EFA deficiency may not be a major dietary factor in the aetiology of kwashiorkor, experiments on human infants and on the rat suggest that the disease may be exacerbated by a failure to make optimum use of the severely limited amounts of both energy and protein that are provided by the diet.

REFERENCES

Brock, J. F. & Autret, M. (1952). *FAO nutr. Stud.* no. 8.
Bronte-Stewart, B. (1961). In *Recent Advances in Human Nutrition* p. 178 [J. F. Brock, editor]. London: J. and A. Churchill.
FAO (1968). *FAO nutr. Stud.* no. 15.
Folch, J., Lees, M. & Stanley, G. H. S. (1957). *J. biol. Chem.* **266**, 497.
Gopalan, C. (1968). In *Protein Deficiencies and Calorie Deficiencies* p. 49 [R. A. McCance and Elsie M. Widdowson, editors]. London: J. and A. Churchill.
Holman, R. T. (1960). *J. Nutr.* **70**, 405.
Insull, W. Jr, Hirsch, J., James, A. T. & Ahrens, E. H. Jr (1959). *J. clin. Invest.* **38**, 443.
Leat, W. M. F. (1963). *Biochem. J.* **89**, 44.
Lutz, P. & Platt, B. S. (1958). *Proc. Nutr. Soc.* **17**, iii.
Macy, I. G. & Kelly, H. J. (1961). In *Milk: The Mammary Gland and Its Secretion* Vol. 2, p. 275 [S. K. Kon and A. T. Cowie, editors]. New York and London: Academic Press.
McCance, R. A. (1968). In *Protein Deficiencies and Calorie Deficiencies* p. 1 [R. A. McCance and Elsie M. Widdowson, editors]. London: J. and A. Churchill.
Morley, D. C., Woodland, M., Martin, W. J. & Allen, I. (1968). *W. Afr. med. J.* **17**, 8.
Naismith, D. J. (1962). *J. Nutr.* **77**, 381.
Naismith, D. J. (1964). *Proc. Nutr. Soc.* **23**, viii.
Paterson, J. C. S. & Wiggins, H. S. (1954). *J. clin. Path.* **7**, 56.
Paulsrud, J. R., Pensler, L., Whitten, C. F., Stewart, S. & Holman, R. T. (1972). *Am. J. clin. Nutr.* **25**, 897.
Read, W. W. C., Lutz, P. G. & Tashjian, A. (1965). *Am. J. clin. Nutr.* **17**, 180.
Soderhjelm, L., Wiese, H. F. & Holman, R. T. (1971). *Progr. Chem. Fats* **9**, 555.
Wiese, H. F., Hansen, A. E. & Adam, D. J. D. (1958). *J. Nutr.* **66**, 345.
Woodford, F. P. & van Gent, C. M. (1960). *J. Lipid Res.* **1**, 188.