Changes in serum β-lipoprotein concentration during the development of kwashiorkor and in recovery

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(*Received 27 May 1971 – Accepted 2 September 1971*)

1. Serum β-lipoprotein and cholesterol have been measured in children 'at risk' to severe protein-calorie malnutrition and in others with kwashiorkor or marasmus. β-Lipoprotein was estimated by an immunological technique. In children recovering from kwashiorkor, serum triglyceride estimations and lipoprotein electrophoretic separations were also carried out.

2. β-Lipoprotein concentrations did not fall significantly until serum albumin concentration was less than 2·5 g/100 ml; cholesterol concentration fell before β-lipoprotein.

3. In frank kwashiorkor, serum β-lipoprotein concentration was reduced by about 30%, whereas cholesterol and triglyceride concentrations were reduced by about 50% in comparison with apparently normal children. Electrophoretic evidence showed that serum α-lipoprotein concentration was also reduced or absent altogether. Marasmic children had normal serum concentrations of β-lipoprotein and the other lipid components measured.

4. The metabolic significance of this degree of reduction in serum β-lipoprotein concentration in the pathogenesis of the fatty liver of kwashiorkor has been discussed. It was concluded that, in Ugandan children with serum albumin concentrations below 2·50 g/100 ml, the β-lipoprotein concentration was probably insufficient for normal mobilization of fat from the liver and the children could therefore be considered susceptible to the development of a fatty liver.

5. The recovery from kwashiorkor was marked by a rapid rise in serum β-lipoprotein concentration and hypertriglyceridaemia but a slower rise in cholesterol concentration. This confirmed the results of previous investigations.

A fatty liver in children suffering from kwashiokor is a well-known feature of the disease and many attempts have been made to define the cause; triglyceride is the main lipid fraction that accumulates (Iturra, 1947; Macdonald, 1960). Another biochemical characteristic of kwashiorkor is a reduction in the concentration of serum triglyceride (Schwartz & Dean, 1957; Macdonald, Hansen & Bronte-Stewart, 1963; Lewis, Hansen, Wittman, Krut & Stewart, 1964; Monckeberg, 1966, 1968; Flores, Pak, Maccioni & Monckeberg, 1967; Truswell, Hansen, Watson & Wannenburg, 1969). The usual interpretation has been that there must be a block in the release of hepatic triglycerides into the serum.

It now seems unlikely that dietary deficiencies of the lipotropic factors, choline and methionine, are responsible since there is no specific reduction in the concentrations of choline containing phospholipids in the serum (Truswell *et al.* 1969). The present hypothesis is that fat may accumulate in the liver because of a reduction in the concentration of circulating β-lipoprotein, the protein responsible for the transport of triglyceride from the liver to the fat depots (Truswell *et al.* 1969; Flores, Pak, Maccioni & Monckeberg, 1970; Flores, Sierralta & Monckeberg, 1970).

It was shown many years ago (Dean & Schwartz, 1953) that serum total cholesterol concentration was low in kwashiorkor and it is now known that most of this cholesterol is associated with β-lipoprotein. This early finding has led to detailed investigations

by various authors, but although serum lipoproteins have been measured after separation both by electrophoresis (Cravioto, de la Pena & Burgos, 1959; Chatterjee & Chaudhuri, 1961; Truswell *et al.* 1969) and by ultracentrifugation (Flores, Pak *et al.* 1970) there is no consistent agreement on the amount by which serum β-lipoprotein concentration actually is reduced. There is also controversy about the relative effects of malnutrition on the α- and β-lipoproteins. Clearly further information was required on the subject.

In terms of defining the course of events during the pathogenesis of the fatty liver, one limitation of previous work is that only children in hospital with frank kwashiorkor or marasmus have been investigated. The changes in lipid metabolism which accompany recovery have been studied and it has been assumed that these must represent a reversal of the processes involved in the development of a fatty liver, but this need only be true in a general sense.

In the present study we have attempted to provide new information by investigating a series of children at different stages of nutritional risk to kwashiorkor as well as others showing definite pathological signs of protein-calorie malnutrition.

EXPERIMENTAL

Children investigated

This investigation was carried out on two types of children – a group attending an out-patient clinic at Namulonge, which is 20 km north of Kampala, and cases of kwashiorkor and marasmus being treated in our metabolic ward. All the children were 0·5–3 years old.

None of the out-patients exhibited serious clinical signs of kwashiorkor or marasmus although many gave some indication that they might be subclinically malnourished. For the specific purposes of this study, eighty-six of these children were examined once only; many were underweight for their age, and serum albumin concentrations ranged from 2·06 to 4·21 g/100 ml. A smaller number of the clinic children were already being investigated as part of a prospective longitudinal study and thus additional serial analyses were possible in these subjects.

Of fifty-six hospitalized children, fifty had been diagnosed as cases of oedematous malnutrition (kwashiorkor) and the remaining six as cases of nutritional marasmus. All were investigated on admission and in eleven of them investigations were continued at regular intervals throughout treatment. Initial serum albumin concentrations ranged from 1·00 to 1·94 g/100 ml in the kwashiorkor group and from 2·09 to 4·35 g/100 ml in the marasmic group.

The in-patients were treated according to Staff (1968); children attending the clinic were given dietary and other therapies when necessary.

Blood samples and their analysis

Blood samples were obtained by venepuncture; with in-patients they were collected after an overnight fast but with the out-patients this precaution was not possible. However, local dietary habits are such that little or no breakfast is eaten and thus most of

the samples, which were collected during the morning, can be regarded as virtually fasting.

Determinations of β-lipoprotein were made using an immunological procedure based on that described by Heiskell, Fisk, Florsheim, Tachi, Goodman & Carpenter (1961) and available as the Beta-L-Test (Travenol Laboratories, Inc., Costa Mesa, California, USA). In this method a serum sample, in which β-lipoprotein has been precipitated by a specific antiserum, is drawn into a standard diameter capillary tube to a height of 60 mm. The tube is then sealed and centrifuged and the height of the column of precipitate is measured. The reading, expressed in mm, is termed the 'immunocrit'. In some serum samples β-lipoprotein was also determined by the method of Walton & Scott (1964). β-Lipoprotein was selectively precipitated by dextran sulphate, molecular weight 5×10^5 (Pharmacia, Uppsala, Sweden), and the resulting turbidity measured at 630 nm. There was good agreement between the two analytical methods; for forty-eight paired assays the linear correlation coefficient was 0·81 ($P < 0{\cdot}001$). Only values for the immunological method are reported in detail.

Electrophoretic separation of lipoproteins was performed only when fresh serum samples were available, using strips of cellulose acetate (Sepraphore III; Gelman Instrument Co., Ann Arbor, Michigan, USA). The individual lipoproteins were detected by staining with Oil Red O (Gelman Instrument Co., 1968). The electrophoretic strips were scanned by double reflectance using a Chromoscan Mk II (Joyce, Loebl and Co. Ltd, Gateshead, England).

Serum triglycerides were estimated using the enzymic procedure described by Eggstein & Kreutz (1966) and total serum cholesterol by the method of Huang, Chen, Wefler & Raftery (1961) as modified by Postma & Strocs (1968).

Concentrations of serum albumin were estimated by an automated colorimetric micromethod (Coward, Sawyer & Whitehead, 1971).

RESULTS

Changes during the development of kwashiorkor

Table 1 shows the concentrations of serum β-lipoprotein and total cholesterol which were found in the clinic children and in the children with kwashiorkor and marasmus admitted to the ward. The children have been subclassified on the basis of serum albumin concentration. There is evidence to suggest that this measurement provides an indication of the degree of risk of a subclinically malnourished child to a more acute episode of clinical kwashiorkor (Hansen, 1968; Whitehead, Poskitt & Frood, 1971) and the results have been expressed in this way to give an impression of the possible sequence of events during the development of kwashiorkor among Ugandan children. In frank kwashiorkor it is not necessarily true that the severity of malnutrition can be judged by the serum albumin level, but in the group of children studied the concentration of the lipid components did seem to change with albumin concentration and, for this reason, the classification system was extended into the kwashiorkor range.

The relationship between the β-lipoprotein immunocrit and serum total cholesterol concentration is illustrated in a different form in Fig. 1, which shows the mean percentage change in these two components at diminishing levels of serum albumin concentration, as compared with group A (serum albumin above 3·50 g/100 ml). The immunocrit did not decrease significantly until albumin concentration had fallen to below 2·50 g/100 ml, but between group A and groups H, J and K the difference became more significant. Although these results indicated that in general β-lipoprotein

Table 1. *Serum albumin (g/100 ml), β-lipoprotein (immunocrit, mm) and total cholesterol (mg/100 ml) in children attending an out-patient clinic and others admitted to hospital with kwashiorkor or marasmus*

Group	No. of children	Albumin		β-Lipoprotein		Cholesterol	
		Range	Mean	Mean	SE	Mean	SE
			Clinic children				
A	17	> 3·50	3·75	1·8	0·1	179	9
B	13	3·25–3·49	3·37	1·7	0·1 NS	174	12 NS
C	16	3·00–3·24	3·14	1·8	0·1 NS	159	5**
D	16	2·75–2·99	2·91	1·8	0·1 NS	144	6**
E	8	2·50–2·74	2·65	1·7	0·1 NS	130	14***
F	5	2·25–2·49	2·36	1·4	0·1*	105	6***
G	11	2·00–2·24	2·13	1·5	0·2*	108	8***
			Kwashiorkor admissions				
H	13	1·75–1·99	1·84	1·3	0·1***	89	11***
J	9	1·50–1·74	1·60	1·4	0·1**	99	8***
K	15	1·25–1·49	1·38	1·3	0·1**	94	6***
L	13	1·00–1·24	1·10	1·5	0·2 NS	109	11***
			Marasmus admissions				
M	6	2·90–4·53	3·47	2·0	0·3 NS	171	14 NS

NS, not significant.
Values significantly different from those in group A by the t test: * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

concentrations were low when albumin concentrations were also low, relatively high immunocrit values were found in some of the kwashiorkor children, particularly those with the lowest serum albumin concentrations (group L). In the children with severe marasmus the mean immunocrit was not reduced beyond the value found in group A.

As Fig. 1 shows, the pattern of change in serum cholesterol concentration was not identical with that of the immunocrit. Cholesterol had fallen significantly at quite mild degrees of hypo-albuminaemia (below 3·25 g/100 ml) and continued to do so steadily until the kwashiorkor values were reached, whereas the immunocrit fell precipitously only between stages E and F. In the marasmic children quite normal cholesterol concentrations were recorded, but again some of the children with severe kwashiorkor exhibited relatively high values.

In an attempt to explain the higher immunocrit values in group L, serum triglyceride concentration was measured in some of the samples. In one child with an immunocrit of 2·3 mm and a serum cholesterol of 131 mg/100 ml, the triglyceride concentration was 396 mg/100 ml; in another with an immunocrit of 2·0 mm and a

cholesterol of 218 mg/100 ml, the value was 429 mg/100 ml. These values are to be compared with an average triglyceride concentration of 108 mg/100 ml in another group of ten untreated cases of kwashiorkor with a mean immunocrit of 1·2 mm (Table 2). It would seem, therefore, that in a few children fat mobilization had probably started even before treatment began.

To see if relating changes in β-lipoprotein to albumin concentration revealed the sequence of events during the development of kwashiorkor, various individual

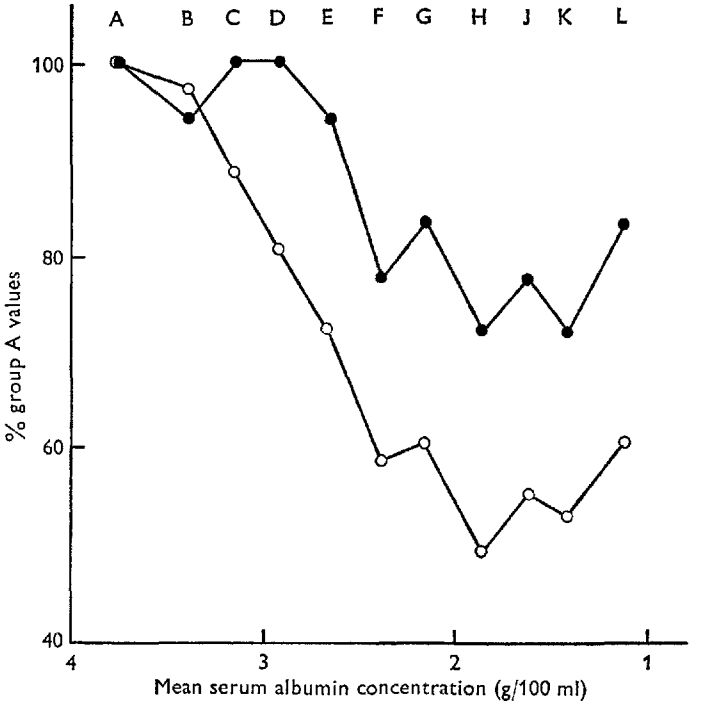

Fig. 1. Relationships of serum β-lipoprotein (●) and cholesterol (○) concentrations, expressed as percentages of group A values, to mean serum albumin concentrations in children at risk to kwashiorkor (groups A–G) and those with kwashiorkor (groups H, J–L) (see Table 1).

Table 2. *Serum albumin (g/100 ml), β-lipoprotein (immunocrit, mm), cholesterol (mg/100 ml) and triglycerides (mg/100 ml) in children recovering from kwashiorkor*

Time (weeks)	No. of children	Albumin Mean	Albumin SE	β-Lipoprotein Mean	β-Lipoprotein SE	Cholesterol Mean	Cholesterol SE	Triglycerides Mean	Triglycerides SE
Admission	10	1·52	0·12	1·2	0·1	86	4	108	16
0·5	11	2·05	0·14**	1·7	0·1**	110	6**	238	34**
1·0	10	2·59	0·21***	1·8	0·1**	122	8***	238	50*
1·5	10	2·84	0·20***	2·0	0·2**	137	9***	211	33*
2·0	7	2·67	0·28***	1·7	0·3 NS	140	13***	201	48 NS
2·5	8	3·07	0·17***	1·9	0·1**	163	12***	215	44*

NS, not significant.

Values significantly different from those on admission by the *t* test: * $P < 0.05$; ** P 0·01; *** $P < 0.001$.

clinic children were investigated on a longitudinal basis. Figs. 2 and 3 show the serial changes in the immunocrit, total cholesterol and albumin in two of the children. These examples were selected for inclusion in this paper because both had exhibited progressive hypo-albuminaemia and deterioration in clinical condition in spite of outpatient therapy. They were eventually admitted to the ward although neither had

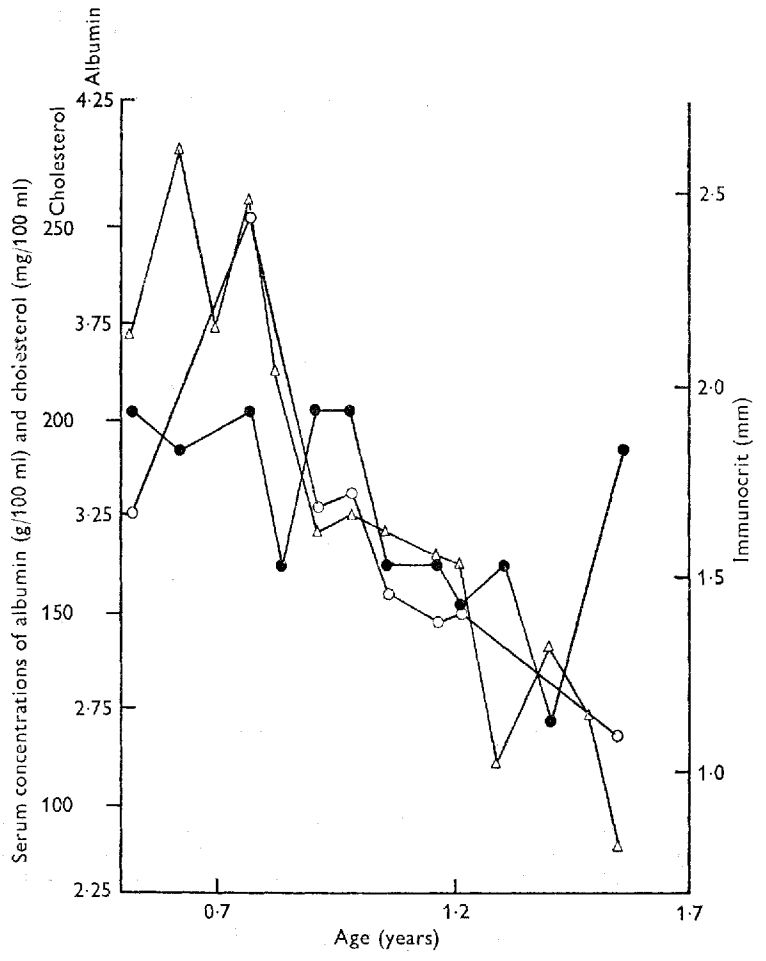

Fig. 2. Changes in serum concentrations of β-lipoprotein (●), cholesterol (○) and albumin (△) in a child, Kyasi, studied longitudinally.

gross signs of kwashiorkor; subsequent treatment resulted in a normal recovery. Both children confirmed the impression gained from the analysis of the cross-sectional results. In the child described in Fig. 2 there was a steady fall in serum albumin and after a few weeks this was followed by a reduction in the concentration of β-lipoprotein. The case illustrated in Fig. 3 is especially interesting since there was a partial recovery in serum albumin concentration at about 1·4 years and an improvement of the immunocrit occurred at the same time. In each case there was a definite rise in β-lipoprotein concentration towards the terminal stage but no rise in albumin took

place; by then both children were rather ill and anorexic. The existence of this final metabolic response had also been suggested by the cross-sectional results. In general, the serum total cholesterol concentrations changed in a manner similar to those of β-lipoprotein but, as with the cross-sectional results, there were some differences.

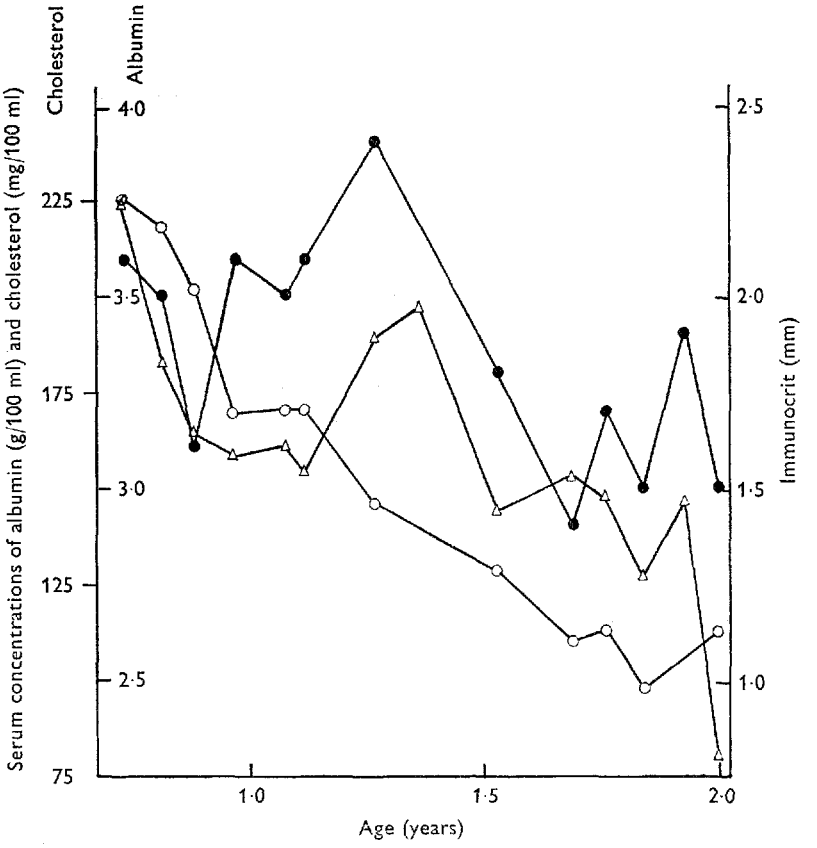

Fig. 3. Changes in serum concentrations of β-lipoprotein (●), cholesterol (○) and albumin (△) in a child, Minani, studied longitudinally.

Changes during recovery from kwashiorkor

In the group of children followed during recovery it was possible to take blood samples more frequently and in slightly greater quantities, and thus the analysis could be extended to include triglyceride estimations and electrophoresis of the lipoproteins. Details are given in Tables 2 and 3; the interrelationships between changes in cholesterol, β-lipoprotein, albumin and triglyceride concentrations are illustrated in Fig. 4.

The results for changes in triglyceride concentration are similar to those reported by other workers (Schwartz & Dean, 1957; Macdonald *et al.* 1963; Lewis *et al.* 1964; Monckeberg, 1966, 1968; Flores *et al.* 1967; Truswell *et al.* 1969). There was a rapid and significant rise in triglyceride only during the first half-week of treatment, after which the concentrations fell slightly. The major rise in the immunocrit also occurred during this initial period and there was no significant rise from then onwards. As in

the investigations carried out in the children becoming clinically malnourished, serum total cholesterol concentration responded more uniformly than β-lipoprotein and followed the changes in serum albumin more closely than the immunocrit.

The lipoprotein electrophoretograms of the untreated children indicated a reduced amount of each of the three main fractions. In five out of ten children α-lipoprotein could not be detected at all and only two of the ten children showed any pre-β-lipoprotein. On treatment the situation changed markedly. As with the serum triglyceride and immunocrit measurements, the main response in β-lipoprotein took place

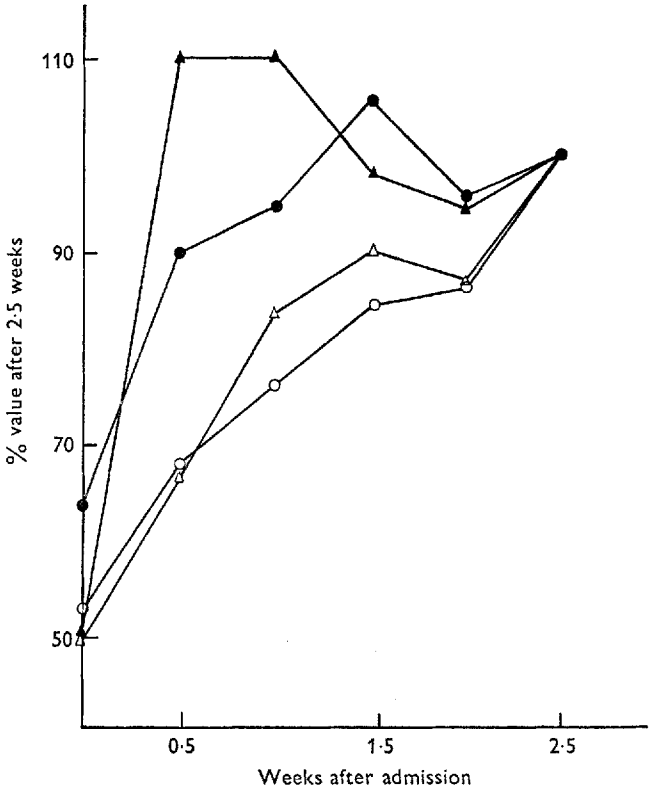

Fig. 4. Changes in serum concentrations of β-lipoprotein (●), cholesterol (○), triglyceride (▲) and albumin (△), expressed as percentages of the values after 2·5 weeks, in children recovering from kwashiorkor (see Table 2).

over the first few days; pre-β-lipoprotein was found in seven out of eleven children after 3 d and in all children by the 2nd week. Although α-lipoprotein had reappeared in all the children after 1 week, maximum colour intensity of this band was not reached until the later stages of the investigation.

DISCUSSION

From the scientific point of view it was unfortunate that no actual measurements of liver fat content could be made. It was considered unethical to perform liver biopsies on the relatively healthy children attending the clinic and serial biopsies to

complement the biochemical findings were quite out of the question. However, a fatty liver is invariably present in Ugandan children with kwashiorkor and there is no reason to believe that the cases described here were atypical. A parallel investigation is being made on experimentally malnourished baboons and in these animals both serial biopsies and biochemical studies are performed. The findings will be described in a future paper.

The present results showed that a group of Ugandan children with severe kwashiorkor, in contrast to others with severe marasmus, usually had low serum β-lipoprotein and cholesterol concentrations. The concentrations of α- and pre-β-lipoprotein were also reduced. During the development of kwashiorkor, β-lipoprotein concentration fell as hypo-albuminaemia became more marked. Although lipoprotein electrophoresis could not be carried out on the samples obtained at the clinic, the cholesterol measurements indicated that α-lipoprotein concentrations were probably affected even before the β-lipoprotein concentration. Fredrickson, Levy & Lees (1967*a*) stated that, on average, α- and β-lipoproteins account for 90% of the total serum cholesterol, and the findings of Truswell *et al.* (1969) showed that in African children about 100% of the cholesterol is in these fractions. Since total serum cholesterol had decreased markedly before there were any significant changes in β-lipoprotein concentrations it must be concluded that α-lipoprotein concentration was probably affected at a very early stage.

These findings are at variance with those of Truswell *et al.* (1969), who found that in South African children with kwashiorkor only β-lipoprotein concentration was reduced; α-lipoprotein concentrations were unaltered. On the other hand, Cravioto *et al.* (1959) in Mexico and Chatterjee & Chaudhuri (1961) in India reported that in kwashiorkor α-lipoproteins were more severely affected than β-lipoproteins; in fact the latter workers claimed that β-lipoprotein concentrations were virtually unaffected. It would seem that some of the biochemical features of kwashiorkor may vary in different parts of the world. The β-lipoprotein levels reported from India could have arisen after the onset of severe anorexia, as they did in both the Ugandan children studied longitudinally. Since, in marasmus, serum β-lipoprotein concentrations appeared not to be affected, a period of anexoria before kwashiorkor might have resulted in a switch of the metabolic adaptative mechanisms towards a starvation response. The way in which this might take place has been discussed by Whitehead & Alleyne (1972). Such a response might be particularly expected in the type of malnutrition which terminates in marasmic kwashiorkor. The lack of change in the α-lipoprotein concentrations in the South African children is more difficult to explain, but it could be related to the different functions of the two main lipoprotein fractions. β-Lipoprotein is concerned mainly with the transport of fat from the liver into the serum, whereas α-lipoprotein may be associated with the removal of lipid from the serum into the fat depots (Fredrickson, Levy & Lees, 1967*b*).

On recovery, β-lipoprotein concentration increased towards normality much more rapidly than cholesterol and from this it might be inferred that α-lipoproteins were regenerated relatively slowly. Examination of the lipoprotein electrophoretograms confirmed this impression. All investigators have noted a marked hypertriglyceri-

daemia early in treatment. This suggests that triglycerides have been released from the liver into the serum at a rate in excess of the child's capacity for their transfer into the fat depots. A low α-lipoprotein concentration could contribute to this phenomenon; serum triglycerides are elevated in familial α-lipoprotein deficiency (Fredrickson *et al.* 1967*b*).

There has been only one previous investigation in which changes in pre-β-lipoprotein concentrations were included (Truswell *et al.* 1969) and there is general agreement between the two sets of results. Pre-β-lipoprotein may be regarded as lipoprotein very heavily loaded with triglyceride (Levy, Lees & Fredrickson, 1966), and the absence of this band in kwashiorkor and its enhanced appearance during treatment would have been expected.

The apparently gross depletion in serum lipoproteins indicated by routine electrophoresis and noted by various workers is misleading since in this system of analysis the staining is for total lipid. This does not adequately represent the plasma concentration of the carrier moiety. The fact that only moderate reductions in β-lipoprotein concentration were found by direct measurement both in this investigation and in that of Truswell *et al.* (1969) raises the question whether this could account for the accumulation of substantial amounts of fat in the liver. There is another complication; the classical concept is that the metabolic precursors of the accumulated fat are the excessive calories in the diet. However, recent unpublished work from this unit and the studies of Gopalan (1968) and Sukhatme (1970) in India have shown that kwashiorkor develops in many children who have been living on a quite inadequate intake of total energy.

To some extent these problems have been resolved by recent fundamental studies into the biochemical mechanisms involved in fatty infiltration. The work of Lombardi & Ugazio (1965) on carbon tetrachloride-intoxicated rats demonstrated that reductions in the serum lipoprotein concentrations of the order found in kwashiorkor did give rise to substantial fatty infiltration after only 4 h. Furthermore, it is not necessary to assume that either an excessive calorie intake on a low-protein diet or an episode of acute starvation, resulting in increased mobilization of depot fat which exceeds the metabolic capacity of the liver, is essential for the development of a fatty liver in kwashiorkor. In the normal individual depot fat is continually being broken down and released into plasma as free fatty acids, some of which are used as direct sources of energy by various body tissues. The remainder is re-esterified in the liver and released into the plasma on carrier β-lipoprotein for transport back to the fat depots. The magnitude of this free fatty acid flux is greatly in excess of the metabolic needs of the liver (Farquhar, Gross, Wagner & Reaven, 1965). Even an individual in perfect metabolic balance between energy intake and expenditure would be susceptible to a fatty liver if a reduction occurred in the rate of synthesis of β-lipoprotein.

Truswell *et al.* (1969) have proposed that 'low serum triglyceride, low cholesterol (especially the β-lipoprotein fraction), and low albumin singly or, better, combined, have some value in making a clinical diagnosis of the severity of fatty liver in a patient with kwashiorkor'. In general we would agree with these conclusions but the present results indicate the possibility of misleadingly normal or high values for β-lipoprotein

and cholesterol during the terminal acute episode. The marked responsiveness of serum β-lipoprotein concentration in comparison with albumin to changes in diet may be explained in various ways. β-Lipoprotein turns over far more quickly than albumin. In a comparative study in man, Volwiler, Goldsworthy, MacMartin, Wood, Mackay & Fremont-Smith (1955) showed that the half-life of β-lipoprotein was 3·5 d as compared with 21·5 d for albumin. Furthermore, albumin represents about 50% of the total serum protein, whereas β-lipoprotein is only 5%. Thus replenishment and loss of the latter substance could occur more rapidly. Because of the greater stability of serum albumin concentrations and because of the reasonable relationship between albumin and β-lipoprotein concentrations, at least in Uganda, an albumin concentration of less than 2·5 g/100 ml might be considered indicative, not only of susceptibility to oedema (Whitehead *et al.* 1971) but also to the development of a fatty liver.

We would like to acknowledge the valuable discussions we had with Professor John Hansen and Dr Stewart Truswell on the value of cholesterol measurements in kwashiorkor. The children investigated were under the care of Drs J. D. L. Frood, E. M. E. Poskitt and J. P. Stanfield and their nursing staff. Mr D. M. Katwire and Mr J. B. Kiwanuka helped with the chemical analyses.

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Printed in Great Britain