The Serum Chemistry in Uncomplicated Kwashiorkor

By R. F. A. DEAN and RUTH SCHWARTZ

*Medical Research Council Department of Experimental
Medicine, University of Cambridge*

Although the literature of kwashiorkor is now reaching large dimensions, few studies of the serum chemistry have so far appeared. The published results are difficult to summarize. There has been up to now no general agreement on diagnostic criteria, and the name kwashiorkor or one of its synonyms has undoubtedly been applied to diverse conditions arising from various causes in different parts of the world (Water-low, 1951). A biochemical investigation was undertaken to characterize the condition of children admitted to Mulago Hospital, Kampala, between May and December 1951 with the diagnosis of kwashiorkor and to follow the effect of treatment. As already indicated (Trowell, Davies & Dean, 1952) the disease as it is seen at Mulago is often complicated by other illness. It was, however, possible to select a group of children in whom the state of malnutrition was dominant, and the results to be described were obtained from this group.

Values found by previous investigators

Serum proteins. There is general agreement that the total serum proteins are reduced in active kwashiorkor, whatever the home diet of the children may have been. Uncertainties and variations of method reduce the comparative value of reports which do not contrast findings at the beginning and end of treatment.

The fourteen children with fatty-liver disease investigated by Waterlow (1948) in the West Indies had total serum proteins ranging from 3·1 to 6·4 g/100 ml., with an average of 4·5 g. After about 5 weeks' treatment with milk, the totals reached between 6·0 and 7·0 g/100 ml. in four children. Altmann (1948), working in Johannesburg, found an average of 4·2 g total protein/100 ml. in twenty freshly admitted children, and an albumin : globulin ratio of unity. Eleven children had on discharge serum proteins which averaged 7·0 g/100 ml., with an average ratio of 1·5. In a later series (Anderson & Altmann, 1951) these findings were on the whole confirmed, and further improvements of total protein and albumin : globulin ratio were noted after successful treatment. An electrophoretic examination of serums taken from children who were acutely ill showed that the γ-globulin was absolutely and relatively high, and albumin was low; α- and β-globulins were perhaps slightly higher than normal. Janssen & le Roux (1950) found an average of about 4·1 g total protein/100 ml. and an albumin : globulin ratio very near unity in forty-one children admitted to a Pretoria hospital. Treatment raised the average value for twenty-four children to about 6·5 g/100 ml. and the albumin : globulin ratio to about 1·5. Thirty-two children in Curaçao investigated by Van der Sar (1951) had an average of 4·2 g total

protein/100 ml. when they were first admitted to hospital, and on discharge the average value was 6·6 g. Albumin rose from 2·2 to 4·5 g and globulin from 2·0 to 2·1 g. The albumin : globulin ratio therefore changed from 1·1 to 2·2.

Other biochemical investigations. Very few other estimations have been attempted, and the effect of treatment has not usually been followed.

It has only recently been realized that protein deficiency plays a large part in kwashiorkor, and this must explain the lack of attention paid to protein metabolism. Chaudhuri (1948) in Calcutta and Delon (1951) in Morocco both found low levels of blood urea, of the order of 12–15 mg/100 ml., and Delon said that a level of 30 mg/100 ml. had a serious importance, as it indicated 'une rétention azotée paradoxale'. Chaudhuri found non-protein nitrogen ranging from 28 to 45 mg/100 ml., which seems to be more than might be expected from his figures for blood urea.

Chaudhuri (1948) found cholesterol concentrations (he was presumably estimating free cholesterol, but did not specify his method) of 28–45 mg/100 ml., Carvalho, Pinto, Schmidt, Potsch & Costa (1945) found an average of 88·5 mg/100 ml. (total cholesterol?) for children in Rio de Janeiro, and Delon (1951) found an average of 103 mg/100 ml. (total cholesterol) for five children. A series of Delon's estimations on one child gave results of 75, 185, 190 and 165 mg/100 ml., a rise and fall which was apparently related to treatment, and in another child, after a month's treatment, the value was 120 mg/100 ml. Sixteen estimations of total cholesterol made by Van der Sar (1951) averaged 151 mg/100 ml. with a ratio of free to total cholesterol of about 0·4. 'Total lipoid' was estimated at the beginning and end of treatment, but there was no significant alteration. 'Phospholipids' were said by Carvalho *et al.* (1945) to be at the low level of 6·1 mg/100 ml.

Serum enzymes have not been extensively studied. Waterlow (1948) found in eight children values for alkaline phosphatase ranging from 7·7 to 33·2 King-Armstrong units, with an average of 19·7 units. The children were investigated on only one occasion. He also reported (Waterlow, 1950) that large increases in serum pseudo-cholinesterase occurred in children who were successfully treated.

Calcium and phosphorus were estimated by Carvalho *et al.* (1945) and chlorides by Chaudhuri (1948). Low values were reported. A few estimations of sodium by Stransky & Dauis-Lawas (1950) suggested that levels in kwashiorkor might be slightly lowered, but the same authors' results for potassium were so variable that no conclusions could be drawn. It seems likely that all these estimations of electrolytes and minerals really indicate the presence of greater or lesser degrees of water retention or dehydration, and have therefore only a limited significance.

EXPERIMENTAL

Details of the children investigated

Dietary history. The circumstances in which kwashiorkor occurs vary from place to place, and one bar to progress in the understanding of the development of the disease has been the lack of exact data concerning diets. The data are usually very difficult to obtain. For the Mulago children, a well-educated African who thoroughly under-

stood the purpose of the investigation, talked with the relatives who accompanied the children to hospital, and completed a questionary for each child. The answers showed in nearly every case an identical history. The child had been entirely breast-fed for 6–8 months and had then been given the ordinary adult diet, at first as a supplement to the waning breast milk, and later as its only food. No tradition or practice of special feeding for small children exists amongst these people. It was not possible to relate other variations in diet to the time of onset or degree of severity of the disease, but the histories usually indicated the recognition of ill-health early in the 2nd year of life.

The children were either of the Baganda tribe or belonged to other tribes who, settled in Kampala or its vicinity, had adopted the feeding habits of the Baganda. In this tribe the most popular food is the cooked banana (*Musa balbisiana*). The staple next in popularity is the sweet potato (*Ipomoea batatis*). If this cannot be obtained maize flour (*Zea mais*) or cassava (*Manihot utilissima*) may be eaten. All these foods are cooked with water. They are usually eaten with a sauce made from one of a large number of local varieties of dried bean, which include the groundnut (*Arachis hypogea*), the butter bean (*Phaseolus vulgaris*) and the cow pea (*Vigna sinensis*) but not the soya bean (*Glycine hyspida*). The green leaves of *Amaranthus* and other plants, usually referred to under the collective name of 'spinach', are often cooked with the other ingredients of the diet. The beans must make a valuable contribution to the total protein of the diet, but the supply is seasonal and the price is high in relation to that of the other foods. The value of the green leaves must lie almost entirely in their vitamins and minerals, not in their protein.

The staple foods, which according to the family income probably provide from 85 to 95% of the total calories, are all very poor in total protein and there are indications that the protein of the cooked banana is deficient in one or more of the essential amino-acids (Dean, 1952*a*). It seems that usually the staple foods are eaten with about one-twentieth to one-fortieth of their weight of the foods richer in protein. It is difficult to see how a child in the first years of life, whose capacity for bulky food is necessarily small, can possibly obtain from such a diet more than 10–15 g protein daily, and there can be no guarantee that the mixture of proteins in the diet will provide an adequate mixture of amino-acids.

Clinical history. The children conformed to the clinical description of the disease, as it appears in Uganda, which has already been given (Trowell *et al.* 1952). They were stunted, oedematous and querulous, and they had in varying degree the characteristic skin lesions.

As already indicated, the children were believed to owe their condition almost entirely to their defective diet, and not to any concomitant disease, in either the past or the present. The ages, weights and bone ages of the children, and the state of the blood, have already been discussed (Trowell *et al.* 1952). The criteria for admission to the biochemical investigation included absence of enlargement of liver* and spleen, absence of pyrexia, blood slides that were negative on examination for malaria

---

* Kwashiorkor as it is seen in Kampala is not usually associated with enlargement of the liver and in this respect differs from the disease as described in many other parts of the world.

parasites, a negative Mantoux reaction, an X-ray picture of the chest that had no sign of tuberculosis or other infective illness, urine that contained neither red cells nor casts, stools that contained no cellular exudate or other evidence of an inflammatory condition, and, at the most, only a very few hookworm eggs, absence of any evidence of nephritis, and a negative Kahn reaction in the blood of the mother.

None of the children was being breast fed.

Treatment. The children were treated with a low-fat, high-protein diet, which provided only 2–3 g fat and between 60 and 100 g cow's-milk protein daily. This was achieved by mixing dried skim milk with a commercial preparation containing 70% milk protein. The diet and its variations will be described in full in a later paper. The diet was adopted, as a logical measure, as soon as it was realized that the chief deficiency in the home diets of the children must have been a shortage of protein. It was the most successful diet for promoting rapid recovery that had been tried at Mulago. Similar diets have been used elsewhere with good results (Altmann, 1948; Walt, Wills & Nightingale, 1950). The duration of treatment was usually about 28 days.

Analytical methods

Blood samples were obtained from a neck vein or the superior sagittal sinus, on admission and at approximately weekly intervals afterwards. The times between taking the samples were sometimes varied to coincide with planned changes of diet. The blood was allowed to clot and was ringed and spun at once. The serum was pipetted off immediately, and was stored at 4°. Serum amylase was always estimated within a few hours of taking the sample. The other estimations were usually made within a few days.

The greatest importance was attached to serial estimations on the same child, who thus acted as his own 'control'. Ideally, healthy African children of comparable ages, whose physique and clinical condition conformed to British and U.S. standards of excellence, would have been used to provide 'controls'; no such children could however be found. This is not an unusual experience in communities where there is much malnutrition (cf. Jannsen & le Roux, 1950; Walt *et al.* 1950).

For various reasons, the whole set of estimations could not be done on every serum. The usual practice was to stop the routine performance of an estimation as soon as a trend under treatment became clear.

Serum proteins. For total proteins the micro-Kjeldahl procedure after precipitation of the proteins with sodium tungstate or zinc hydroxide was used for about two-thirds of the estimations and, for the other one-third, the copper-sulphate specific-gravity method. The two methods, tested side by side, gave results that agreed within about 0·25 g/100 ml. protein.

The globulins were precipitated with 26% sodium sulphate (Majoor, 1947), and the albumin in the supernatant fluid was estimated by the micro-Kjeldahl procedure. The globulin figure was obtained by difference.

Some of the serums were fractionated by electrophoresis on filter-paper, using the apparatus described by Flynn & de Mayo (1951). The staining and elution of the

strips were carried out according to the instructions given by Kunkel & Tiselius (1951–2). Various difficulties at present prevent this method from being fully quantitative, but, as Flynn & de Mayo (1951) say, it is valuable for following changes in a series of blood samples.

Blood urea. Archibald's (1945) method was used, on a filtrate obtained by precipitation of the proteins with zinc hydroxide. Tungstic-acid and trichloroacetic-acid precipitation could not be used because of the production of colours or turbidity which interfered with the estimation.

Cholesterol. The free and total cholesterol were estimated by Sobel & Mayer's (1945) modification of the method of Schoenheimer & Sperry (1934).
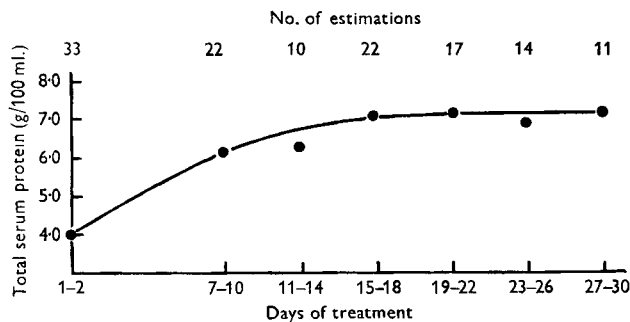

Fig. 1. Total serum protein in children treated for kwashiorkor.

Alkaline phosphatase. King's (1951) method was used, the phenol liberated from disodium-phenyl-phosphate being estimated with the Folin-Ciocalteu reagent.

Pseudo-cholinesterase. Michel's (1949) electrometric method was adapted for this estimation. Benzoylcholine chloride was used for the substrate and 0·2 ml. serum was used for each estimation. The results were expressed as depression of pH units/h.

The serums of six normal European adults, estimated by this method, gave results ranging from 0·60 to 0·95 pH units.

Amylase. The method described by King (1951) was used. It has the advantages of simplicity and rapidity. The estimation was stopped if the starch was still unchanged after 65 min, and the activity of the serum was then recorded as <25 Somogyi units.

Esterase. This was estimated by a modification of the method of Gomori (1949). Phenylbenzoate was used as the substrate and the liberated phenol was estimated by the Folin-Ciocalteu reagent. The results were expressed as mg phenol liberated/ 100 ml. serum in 15 min, i.e. in King-Armstrong units.

Chlorides. These were estimated by the direct titration method of Schales & Schales (1941).

RESULTS

The results obtained by chemical methods are presented as a series of diagrams (Figs. 1, 2 and 4–9) in which the values obtained have been shown in relation to the length of treatment. In each diagram a curve has been drawn which follows values obtained by averaging estimations made in successive 4-day periods. The number of estimations averaged is given.

A pair of electrophoretic patterns, obtained from serums taken from the same child at the beginning and end of treatment, is also given (Fig. 3).

Total protein (Fig. 1). The average of thirty-three estimations made on the 1st and 2nd days was 3·98 g/100 ml. The range was from 2·90 to 5·1 g/100 ml. By the 8th day a rise to about 6·0 g/100 ml. had occurred, and the final figure of 7·0 g/100 ml. was reached about the 21st day. This is almost exactly the value given by Behrendt (1949) as the average for normal children.
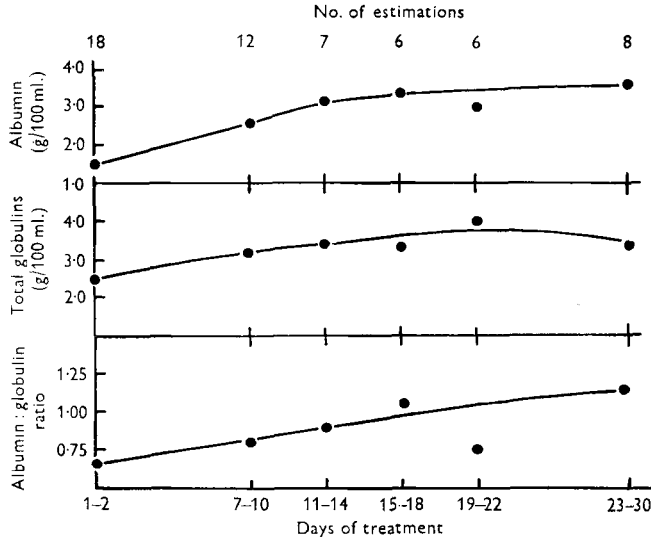
Fig. 2. Serum albumin, total globulins, and albumin : globulin ratio in children treated for kwashiorkor.

Albumin (Fig. 2). The low starting level of albumin concentration (1·51 g/100 ml. for eighteen estimations, with a range of 0·76–2·17 g/100 ml.) must have been a contributory factor to the oedema, and it was found that there was usually, although not invariably, some correlation between the concentration of albumin in the serum and the amount of weight lost by the removal of the oedema. Thus eleven of the eighteen children whose weight loss was 10% or less of their weight when the oedema had disappeared, had albumin values averaging 1·84 g/100 ml. The average value for the other seven children, whose weight losses were between 12 and 32%, was only 1·30 g/100 ml.

The rise from the 1st to the 8th day, a little more than 1·0 g, accounted for slightly more than half of the rise in total protein in that period (Fig. 1). The final average value of 3·6 g was well below the range of 4·4–5·5 g usually accepted as normal.

Total globulins (Fig. 2). The initial level of total globulins was 2·44 g/100 ml. It was the average of eighteen estimations, with a range of from 1·68 to 3·62 g/100 ml. As this is the value usually accepted as normal for British and American children the subsequent rise (to about 3·6 g/100 ml.) was unexpected. As with albumin, much of the rise took place in the 1st week.

Electrophoretic fractionation of the proteins (Fig. 3). The electrophoretic patterns shown were obtained from the serums of a child whose response to the high-protein

diet was extremely satisfactory. The change shown by the patterns is dramatic, and confirms the chemical findings.

Albumin : globulin ratio (Fig. 2). The ratio was at first 0·67. It was the average of eighteen estimations, with a range of from 0·25 to 1·26. The individual variations
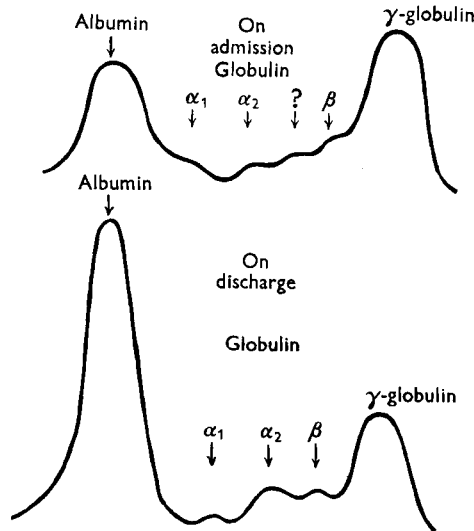

Fig. 3. Patterns obtained by the paper electrophoresis of serums from a child successfully treated for kwashiorkor. A component that has often been observed in such patterns is shown between the α_2- and γ-globulins in the serum taken on admission. An unknown component was found in a similar position by Viollier (1951) in his electrophoretic studies of serum from cases of advanced cirrhosis of the liver and liver atrophy. The globulin peaks in both patterns are probably too small, as no allowance was made for the lower uptake of dye by these fractions (see Flynn & de Mayo, 1951).
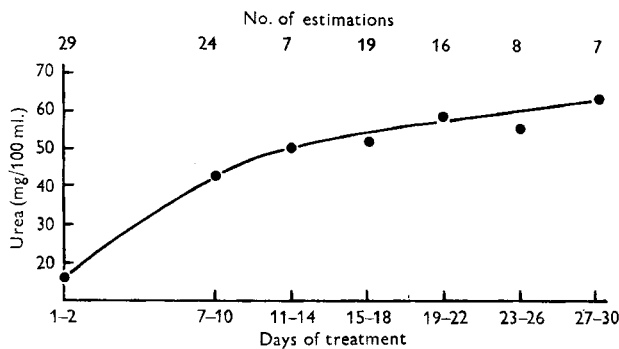

Fig. 4. Blood urea in children treated for kwashiorkor.

during treatment were rather large, indicating some variation in the rates of re-generation of the two classes of protein. The average ratio finally attained was a little over unity. This is only about half that found in normal children (Behrendt, 1949).

Urea (Fig. 4). The low average level at the beginning of treatment, 16·2 mg/100 ml. for twenty-nine estimations with a range of from 4·7 to 38·2 mg/100 ml., was suc-ceeded by a rise which far exceeded the normal level of 20–25 mg./100 ml. The peak

seems to have been reached about the 21st day, and afterwards there was no further increase. The peak values, of the order of 60 mg/100 ml. with a maximum of 90 mg/ 100 ml. in one child, were not accompanied by any sign of uraemia or dehydration, and the children seemed to be quite unaffected. The urine volume, so far as it could be judged in the absence of any method of ensuring complete collections, was satisfactorily high.
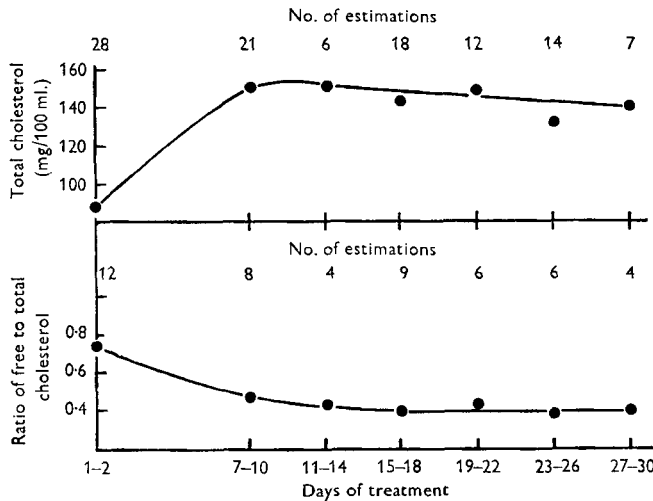

Fig. 5. Total serum cholesterol and ratio of free to total cholesterol in children treated for kwashiorkor.

Cholesterol (Fig. 5). The starting level of cholesterol was remarkably low, an average of 87 mg/100 ml., but within about 8 days the level was nearly twice as high. There was subsequently a slight fall, and at 28 days the average was about 140 mg/100 ml.

The ratio of free to total cholesterol showed a rapid fall from the high initial average level of 0·76. The range was from 0·97 to 0·64 in nine of the ten children. By the 8th day the ratio was about 0·48, and by the 14th day the final level of 0·40 had been reached. The ratios, and their changes, were remarkably consistent.

According to Behrendt (1949) the normal child of the same age as the kwashiorkor patients has 160–170 mg total cholesterol/100 ml. serum, with a ratio of free to total cholesterol of 0·2–0·3. In 'diffuse hepatic damage' the ratio is said to be >0·32. The ratio depends on the activity of an enzyme, a cholesterol-esterase, and the change in ratio therefore measures indirectly the activity of that enzyme (Sperry, 1935).

Alkaline phosphatase (Fig. 6). The average of twenty-three estimations of alkaline phosphatase at the start of treatment was 11·8 King-Armstrong units, which is below the normal range of 15–20 units quoted for children by Behrendt (1949). There was a rather wide range in the starting level, the two highest values being 23·1 and 19·6 units, and the two lowest 4·7 and 7·6 units. Nearly all the other values were between 10 and 15 units.

By the 8th day the enzyme levels fell in ten of the twenty children, and the average

was then 9·5 units. There was subsequently a fairly steady rise, and by the 28th day the average was 18·0 units.

Pseudo-cholinesterase (Fig. 7). The average Δ pH of twelve estimations done on the first 2 days of treatment was 0·24 unit, the range being 0·10–0·41 unit. The subsequent rise was very consistent, and by the 28th day the average was about 0·60 unit.
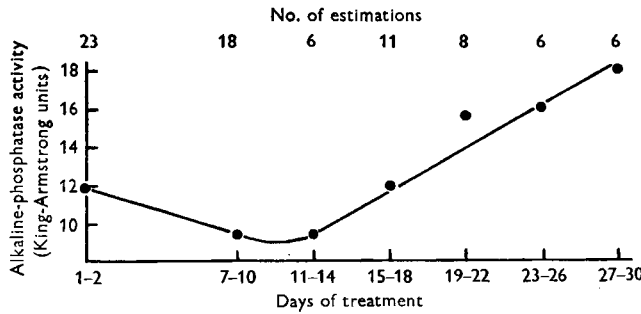

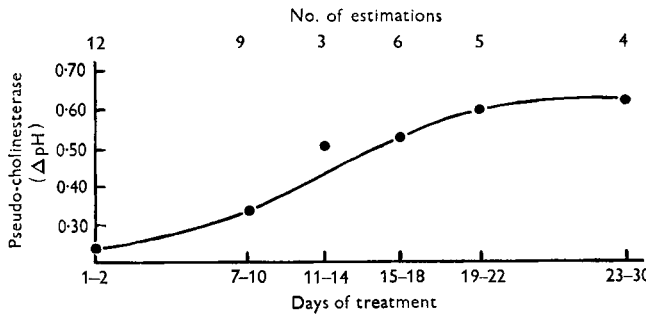Fig. 6. Alkaline-phosphatase activity of serum in children treated for kwashiorkor.

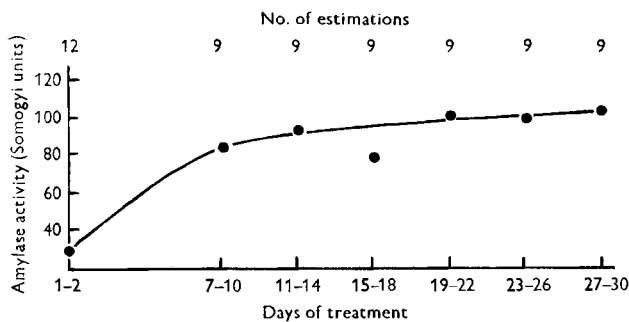Fig. 7. Pseudo-cholinesterase activity of serum in children treated for kwashiorkor.

Fig. 8. Amylase activity of serum in children treated for kwashiorkor.

On the basis of the rather inadequate trial of the method with adults (see above) and the known tendency for children's cholinesterase levels to be rather higher than those of adults, it seems that the value of 0·60 unit was lower than would have been expected in normal children.

Amylase (Fig. 8). In twelve of fourteen children in the first 2 days of treatment, the activity of serum amylase was < 25 Somogyi units; it was 40 and 55 units in the other two. There was a consistent tendency for the values to rise rapidly for the first 8 days or so, by which time the average reached about 90 units: afterwards there was a further slight rise to about 100 units on the 28th day.

At the end of treatment several children had values under 50 units, and it seemed that the values in good health probably had a wide range. Véghelyi (1949) gave the normal range in children as 60–200 units.
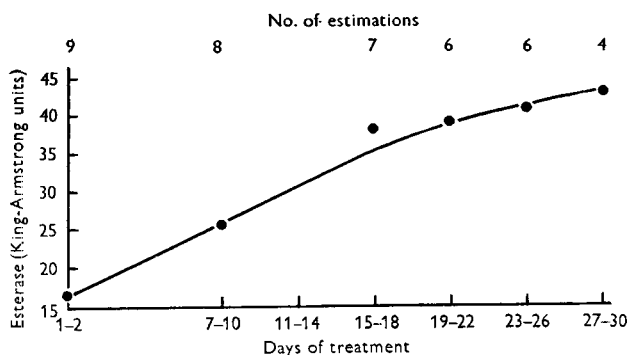

Fig. 9. Esterase activity of serum in children treated for kwashiorkor.

Esterase (Fig. 9). The average of nine estimations at the start of treatment was 16·8 units. The range was 4·7–26·2 units. There was a steep rise until the 15th day, when the average was about 40 units. Afterwards this value was maintained.

This estimation produced very consistent results, only one child failing to conform to the general pattern. That child had the highest initial level (26·2 units). On the 8th day the value was 48·4 units, and there was a further rise to 76 units on the 28th day.

We have found in the literature no values for the serum esterase of normal children.

Chlorides. Nineteen estimations of chlorides expressed as NaCl made at the beginning of treatment ranged from 547 to 701 mg and averaged 620 mg/100 ml. Afterwards, an average value of about 605 mg/100 ml. was maintained.

Normal sodium-chloride values for children are said to be between 590 and 620 mg/100 ml. serum (Behrendt, 1949).

DISCUSSION

The outstanding features of the results seem to be the low levels of albumin and blood urea in the serum of the children on admission, the low levels of enzyme activity at that time and the rapid rise of all these levels towards normal when therapy with large amounts of milk protein was begun.

The results, compared with those obtained elsewhere, show a close agreement in total protein, a somewhat greater abnormality of the albumin : globulin ratio, and similar levels of urea and total cholesterol. The rise and fall of total cholesterol found in one child by Delon (1951) was observed in many children. There was also a

tendency for the recovery of enzyme activity during treatment similar to that found by Waterlow (1948).

Protein metabolism and water balance. No estimations of extracellular fluid or of plasma volume were carried out, and it is, therefore, impossible to state exactly the extent to which the rises in the concentrations of the constituents of the blood were due to the loss of oedema fluid. Extracellular-fluid volume and plasma volume probably rise and fall together (McCance, 1951) and weight loss may serve as a rough guide to alterations in hydraemia. From the weight loss of the children with kwashiorkor during the first few days of treatment, it seems unlikely that the plasma volume decreased by more than one-eighth to one-ninth when the oedema fluid was removed. The changes in concentration observed at the end of the first 10 days, by which time the oedema had disappeared, were nearly all greater than this fraction and almost certainly represented the active regeneration of the substances estimated. As shown for example by the albumin and the total globulins, the regeneration of different substances proceeded at different rates.

The apparent increase in globulins was noticed by Anderson & Altmann (1951) who found that the thymol turbidity-test and the flocculation test frequently became positive during recovery. As these authors said, this probably indicated a rise in the γ-globulin (Kunkel, 1947; Maclagan, 1948). The electrophoretic patterns shown in Fig. 3, which are typical of many patterns obtained in Mulago, do not appear to confirm that the greater part of the increase in total globulins is due to an increase in this fraction, but further work will be required to decide the exact nature and extent of the changes that occur.

It is generally supposed that the γ-globulins carry the immune bodies. There is an impression among clinicians that children with kwashiorkor are at least as susceptible to infections as normal children, so that it is unlikely that their high level of γ-globulin in the serum confers a correspondingly high degree of immunity. A recent observation made by Holmes, Stanier, Semambo & Jones (1952) may be pertinent. The proportion of γ-globulin in the serum proteins was found to be much greater in healthy African adults than in Europeans.

It is known that a reduction in serum albumin increases the rate of formation of tissue fluid, and that 'the plasma is only one of the tissues participating in the general anasarca' (McCance, 1951). There seems to be little doubt that the low level of serum albumin found in the children at the beginning of treatment played a considerable part in the production of the oedema, and it may appear logical to assume that the rapid increase in the albumin caused, to some extent at least, the loss of the oedema. It was usual for this loss to occur within a very few days of the child's admission to hospital. The loss was not, so far as could be ascertained by questioning the mothers, due to a change in posture or to increased rest—factors of importance in the removal of hunger oedema. The loss was not so rapid if the protein in the diet was kept at a low level (Dean, 1952*b*) and it was therefore almost certainly the high level of dietary protein that brought about, directly or indirectly, the reduction in oedema. The mechanism may not have been simple; for instance, in the presence of a damaged liver, urinary output may be reduced (Shay, Kolm & Fels, 1945). This may

be due to failure of the liver to destroy anti-diuretic substances (Ralli, Robson, Clarke & Hoagland, 1948; Leslie & Ralli, 1947; Shorr, Zweifach & Baez, 1948; Baez, Mazur & Shorr, 1949) and it might well be profitable to investigate the occurrence of these substances in children with kwashiorkor. There may have been abnormal sodium retention (Faloon, Eckhardt, Cooper & Davidson, 1949). It is obvious that the adrenal cortex, which controls the retention of sodium and of water, should be studied.

Apart from slight variations probably caused by different degrees of hydration, the chloride values showed little change during treatment. Nevertheless, the need for complete studies of electrolyte movement in kwashiorkor is clear and such studies will no doubt be undertaken as soon as a successful standard form of treatment has been evolved.

Urea. The large rises in blood urea which have been described seemed to be related to the amounts of protein in the diet. This point has been discussed in another communication (Dean, 1952 b). The rises themselves were somewhat unexpected. Although it has been found in some mammals—the dog is a good example—that the concentration of blood urea is highly labile, depending on the amount of dietary protein, it is usually assumed that in the human species the level is not subject to much variation, whatever the diet may be. It is probable that very few children in Europe or America have been maintained on diets that contained so little protein as the home diets of the Mulago children, and no data from European or American sources are available for comparison. If the banana treatment of children with coeliac disease ever regains its past popularity it would be of interest to follow the blood urea of the children, especially if the amount of protein in the diet were varied widely. It appears that in the Mulago children, the mechanism regulating the level of urea in the body fluids was not sufficiently active to prevent abnormal changes. The nature of the defect causing the failure in regulation is unknown. It was presumably a renal defect, but even that will not be established until a full study of renal function has been made. The slight albuminuria found in kwashiorkor is one other detail of evidence for a renal lesion. It is not accompanied by the passage of red cells and casts and nothing else is known about it. Histological changes in the kidney are usually slight (Davies, 1948).

Digestive enzymes. The amylase and esterase which were estimated in the serums were probably mostly of pancreatic origin, although extra-pancreatic sources, including the liver, may normally exist (Nothman, Pratt & Benotti, 1948; Nothman, 1951). The amylase method used is not highly accurate and the esterase method, although it gave accurately reproducible results, measures anything in the serum that can split phenylbenzoate. The amount of splitting performed by lipase is undetermined; it is in fact considered doubtful if serum contains any lipase at all (Seligman & Nachlas, 1950). In spite of these uncertainties, the curves obtained for amylase and esterase activity demonstrated the recovery of enzyme systems as treatment progressed. Clinical observation showed that, parallel to that recovery, there was some recovery of ability to digest, and it was fortunately possible to obtain also estimations of the enzymic activity of the duodenal contents. The estimations were most kindly

performed by Dr M. D. Thompson, whose full results are being reported elsewhere (Thompson & Trowell, 1952).

Fig. 10 shows the results of parallel estimations of serum amylase and lipase and duodenal amylase, lipase and trypsin in a child who was given at first moderate amounts of protein, and later, large amounts. Some other data obtained at the same times have been included. The serum and duodenal enzyme concentrations obviously rise together.

Pseudo-cholinesterase and alkaline phosphatase. Pseudo-cholinesterase activity varies in relation to so many different states—undernutrition, thyroid activity, acute infections, liver disease—that it can hardly be expected to indicate anything specific in such a condition as kwashiorkor where all parts and systems of the body are probably affected. There is also some uncertainty in the correct interpretation of the results for the estimation of alkaline phosphatase. The effects of undernutrition on this enzyme have been summarized by Sherlock & Walshe (1951). The level of activity may be raised if the liver is damaged, and depressed if there has been a caloric deficiency in the diet. The Mulago children had damaged livers, and at least some of them, those who had passed into the stage of anorexia and poor absorption of food, had probably been receiving insufficient calories. The plurality of causes may have accounted for the wide range of starting levels already mentioned and a certain variability which was observed in the response to treatment.

Cholesterol. The low total cholesterol levels found in many of the children on admission may also have been due to a number of conditions. General undernutrition may have been partly responsible (Entenman, Changus, Gibbs & Chaikoff, 1940; Man & Gildea, 1936) and it was likely that the home diet, from which animal products were almost completely excluded, contained very little cholesterol. Groundnuts were almost the only discernible dietary source. The formation of cholesterol from acetate—itself derived from various amino-acids (Bloch & Rittenberg, 1944; Bloch, 1944)—may have been faulty and the extrahepatic, as well as the hepatic, sources of synthesis may have failed (Srere, Chaikoff, Treitman & Burstein, 1950).

The high ratio of free to total cholesterol may have been an indication of liver disease (Man, Kartin, Durlacher & Peters, 1945; Albrink, Man & Peters, 1950) or of undernutrition (Hodges, Sperry & Anderson, 1943). The rise in total cholesterol and the fall in the ratio were dramatic soon after treatment began. The diet contained dried skim milk, but since even full cream cow's milk contains very little cholesterol (Nataf, Mickelsen, Keys & Petersen, 1948) it was probable that alterations within the body, rather than alterations in the diet, were responsible for the changes.

Cholesterol may eventually be discovered to occupy a position of considerable importance, not only in kwashiorkor, but in other conditions found in Africans. It is, for instance, a precursor of adrenal cortical hormones (Conn, Vogel, Louis & Fajans, 1950) and of other steroid hormones (Bloch, 1945). There is evidence of many hormonal abnormalities in African adults (Davies, 1948, 1951) and probably in adolescents (Dean, 1952a) for which upsets in sterol formation may be responsible. The need for investigation of sterol metabolism in all age groups is evident.
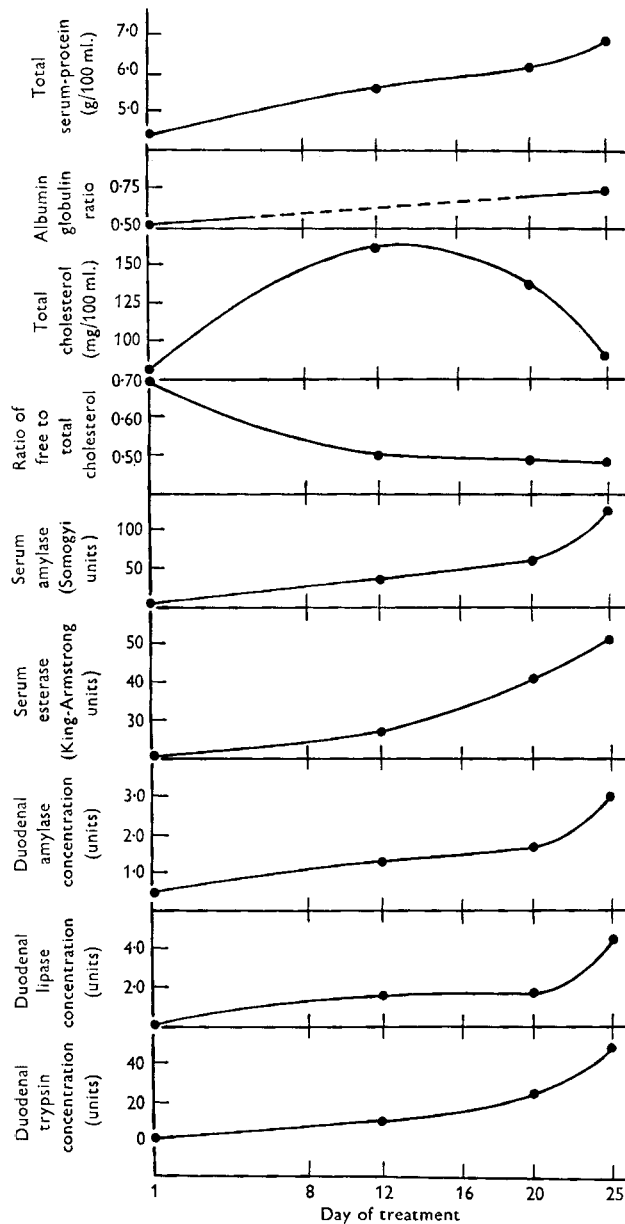
Fig. 10. Simultaneous investigation of enzymes of serum and duodenal contents in a child treated for kwashiorkor. This child was given a moderately high protein diet (35 g/day) until the 20th day, when the protein was increased (to 60 g/day). The response to the treatment was slow at first, but more rapid later. Serum and duodenal enzymes obviously improved together. Trypsin in the duodenal contents was estimated by the method of Charney & Tomarelli (1947) and the results were expressed in their units. The methods and units used for the other duodenal enzymes were those described by Thompson & Trowell (1952).

Conclusions

It was hoped that the investigation described might serve a number of purposes. These included the providing of evidence for or against the current theory of the cause of kwashiorkor, and a preliminary description of the disease in biochemical terms. The experience gained might also make possible the recommendation of a set of biochemical tests suitable for use in following the course of treatment.

The data presented confirm the theory that a lack of protein is responsible for the disease. The particular quality of protein which is involved cannot at the moment be named, but work is in progress at Mulago in which milk proteins, and proteins from other sources, are being compared in their ability to cause the changes in serum chemistry that have been described. Since these changes coincided with the clinical improvement of the children, they are regarded as being fundamental to cure. It is impossible to say, however, to what extent they occur in other disease conditions in African children, or whether the high levels achieved should be regarded as 'normal' or 'supernormal' for those children. It is therefore impossible to offer at the moment a biochemical definition of kwashiorkor, that would distinguish it from any other condition. Tentatively, it is suggested that the diagnosis and the assessment of the value of treatment can be aided by serial estimations of the concentrations in the serum of total proteins, albumin : globulin ratio, amylase and esterase. If suitable methods are chosen, none of these estimations calls for special laboratory apparatus or skill.

The regeneration of protein, the reduction of the ratio of free to total cholesterol, and most of the other changes that have been described, must involve various aspects of a general alteration in enzyme activity. The alteration seems to occur suddenly; it is as though a missing substance is provided and at once enzyme production is restarted. One of the conditions for the synthesis of proteins, and therefore of enzymes, is the simultaneous presence of all the required amino-acids in their optimum proportions. The lack of an amino-acid in the diet would therefore prevent enzyme production. It is unlikely, however, that all the diets that lead to kwashiorkor are equally deficient in the same amino-acid, and the search for the missing component, although it must consider the individual amino-acids, must undoubtedly go farther. It might be found that some abnormality in the absorption or utilization of one or more amino-acids was more important than the dietary supply.

SUMMARY

1. The serum chemistry of children suffering from kwashiorkor was studied. In the acute stage of the disease total serum proteins were reduced, chiefly because the level of albumin was low. Enzyme activity was also much reduced.

2. Treatment with large amounts of milk protein rapidly caused alterations in the blood, which coincided with alterations in the contents of the duodenal juice, and with improvements in the clinical condition of the children.

Permission to investigate the children in Mulago Hospital was very kindly granted by the Director of Medical Services for Uganda (Dr R. F. S. Hennessey). The

REFERENCES

Albrink, M. J., Man, M. B. & Peters, J. P. (1950). *J. clin. Invest.* **29**, 781.
Altmann, A. (1948). *Clin. Proc., Cape Town*, **7**, 32.
Anderson, C. G. & Altmann, A. (1951). *Lancet*, **260**, 203.
Archibald, R. M. (1945). *J. biol. Chem.* **157**, 507.
Baez, S., Mazur, A. & Shorr, E. (1949). *Fed. Proc.* **8**, 7.
Behrendt, H. (1949). *Diagnostic Tests for Infants and Children.* New York: Interscience Publishers Inc.
Bloch, K. (1944). *J. biol. Chem.* **155**, 255.
Bloch, K. (1945). *J. biol. Chem.* **157**, 661.
Bloch, K. & Rittenberg, D. (1944). *J. biol. Chem.* **155**, 243.
Carvalho, M., Pinto, A. G., Schmidt, M. M., Potsch, N. & Costa, N. (1945). *J. Pediat., Rio de J.*, **11**, 395.
Charney, J. & Tomarelli, R. M. (1947). *J. biol. Chem.* **171**, 501.
Chaudhuri, K. G. (1948). *Acta paediat., Stockh.*, **36**, 110.
Conn, J. W., Vogel, W. C., Louis, L. H. & Fajans, S. S. (1950). *J. Lab. clin. Med.* **35**, 504.
Davies, J. N. P. (1948). *Lancet*, **254**, 317.
Davies, J. N. P. (1951). *Transactions of the Ninth Conference on Liver Injury.* New York: Josiah Macy Jr. Foundation,
Dean, R. F. A. (1952*a*). *E. Afr. med. J.* **29**, 1.
Dean, R. F. A. (1952*b*). *Brit. med. J.* ii, 791.
Delon, J. (1951). *Maroc méd.* **6**, 30.
Entenman, C., Changus, G. W., Gibbs, G. E. & Chaikoff, I. L. (1940). *J. biol. Chem.* **134**, 59.
Faloon, W. W., Eckhardt, R. D., Cooper, A. M. & Davidson, C. S. (1949). *J. clin. Invest.* **28**, 595.
Flynn, F. Y. & de Mayo, P. (1951). *Lancet*, **261**, 235.
Gomori, G. (1949). *J. Lab. clin. Med.* **34**, 275.
Hodges, R. G., Sperry, W. M. & Anderson, D. H. (1943). *Amer. J. Dis. Child.* **65**, 858.
Holmes, E. G., Stanier, M. W., Semambo, Y. B. & Jones, E. R. (1952). *Trans. R. Soc. trop. Med. Hyg.* **45**, 371.
Janssen, E. & le Roux, J. S. (1950). *S. Afr. J. clin. Sci.* **1**, 100.
King, E. J. (1951). *Micro-analysis in Medical Biochemistry*, 2nd ed. London: J. and A. Churchill.
Kunkel, H. G. (1947). *Proc. Soc. exp. Biol., N.Y.*, **66**, 217.
Kunkel, H. G. & Tiselius, A. (1951–2). *J. gen. Physiol.* **35**, 89.
Leslie, S. H. & Ralli, E. P. (1947). *Endocrinology*, **41**, 1.
McCance, R. A. (1951). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 275.
Maclagan, N. F. (1948). *Brit. med. J.* ii, 892.
Majoor, C. L. H. (1947). *J. biol. Chem.* **169**, 583.
Man, E. B. & Gildea, E. F. (1936). *J. clin. Invest.* **15**, 203.
Man, E. B., Kartin, B. L., Durlacher, S. H. & Peters, J. P. (1945). *J. clin. Invest.* **24**, 623.
Michel, H. O. (1949). *J. Lab. clin. Med.* **34**, 1564.
Nataf, B., Mickelsen, O., Keys, A. & Petersen, W. E. (1948). *J. Nutr.* **36**, 495.
Nothman, M. M. (1951). *Ann. intern. Med.* **34**, 1358.
Nothman, M. M., Pratt, T. D. & Benotti, J. (1948). *J. Lab. clin. Med.* **33**, 833.
Ralli, E. P., Robson, J. S., Clarke, D. & Hoagland, C. L. (1948). *J. clin. Invest.* **24**, 316.
Schales, O. & Schales, S. S. (1941). *J. biol. Chem.* **140**, 879.
Schoenheimer, R. & Sperry, W. M. (1934). *J. biol. Chem.* **106**, 745.
Seligman, A. M. & Nachlas, M. M. (1950). *J. clin. Invest.* **29**, 31.
Shay, H., Kolm, R. & Fels, S. S. (1945). *Gastroenterology*, **4**, 257.
Sherlock, S. & Walshe, V. M. (1951). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 275.
Shorr, E., Zweifach, B. W. & Baez, S. (1948). *Fed. Proc.* **7**, 115.
Sobel, A. E. & Meyer, A. M. (1945). *J. biol. Chem.* **157**, 255.
Sperry, W. M. (1935). *J. biol. Chem.* **111**, 467.
Srere, P. A., Chaikoff, I. L., Treitman, S. S. & Burstein, L. S. (1950). *J. biol. Chem.* **182**, 629.
Stransky, E. & Dauis-Lawas, D. F. (1950). *Ann. paediat.* **174**, 226.
Thompson, M. D. & Trowell, H. C. (1952). *Lancet*, **262**, 1031.

Trowell, H. C., Davies, J. N. P. & Dean, R. F. A. (1952). *Brit. med. J.* ii, 798.
Van der Sar, A. (1951). *Docum. neerl. indones. Morb. trop.* 3, 25.
Véghelyi, P. V. (1949). *Pediatrics*, 3, 749.
Viollier, G. (1951). *Liver Disease.* A Ciba Foundation Symposium. London: J. and A. Churchill.
Walt, F., Wills, L. & Nightingale, R. P. (1950). *S. Afr. med. J.* 24, 920.
Waterlow, J. C. (1948). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 263.
Waterlow, J. (1950). *Lancet*, 258, 908.
Waterlow, J. C. (1951). *W. Indies med. J.* 1, 15.

The Relationship between Calcium Retention and Body Stores of Calcium in the Rat: Effect of Age and of Vitamin D

By KATHLEEN M. HENRY AND S. K. KON

National Institute for Research in Dairying, University of Reading

(Received 6 June 1952)

It is known that the rate of calcium absorption decreases with progressive calcification of the skeleton (see e.g. Fairbanks & Mitchell, 1936; Rottensten, 1938; Mitchell, 1939; Macy, 1942; Nicolaysen, 1943; Henry & Kon, 1947; Kane & McCay, 1947); normally an adult stage is reached when the animal no longer deposits Ca in its bones and when excretion balances the intake. However, with advancing age the process goes further and increased katabolism leads to loss of bone salts (McCay, Crowell & Maynard, 1935; Meulengracht, 1938; Todd, 1942; Stare, 1943; Henry & Kon, 1947). Henry & Kon (1947) found that rats, having retained when 1 month old 96% of the Ca from a diet containing 0·13% of it and 0·23% phosphorus, 2 years later not only retained no Ca when given the same diet but, on the contrary, lost it from their bodies. Between the metabolic tests, of which there were, all told, four during the 2 years, the animals received our stock-colony diet (Folley, Ikin, Kon & Watson, 1938), which supplies ample quantities of Ca, and there is no doubt that while on this diet they had full opportunity to replenish their stores. It is known that in young rats the efficiency of absorption of Ca at any one age depends on the degree of saturation of the body, the retention being better when the stores are relatively low (Fairbanks & Mitchell, 1936; Rottensten, 1938; Nicolaysen, 1943). Outhouse, Kinsman, Sheldon, Twomey, Smith & Mitchell (1939) found similar relationships in children.

As just pointed out, the old rats of Henry & Kon (1947) were in all probability well calcified before the metabolic tests with the low-Ca diet on which they went into negative Ca balance. It seemed of interest, therefore, to determine the behaviour in relation to age of rats with body stores saturated to different degrees.

EXPERIMENTAL

General plan of the experiments

In long-term experiments litter-mate rats were given diets containing different levels of Ca and P. The diet lowest in Ca supplied it in amounts definitely suboptimal for young, rapidly growing animals. This diet was used for the metabolic study, at

10-2