Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 34 (1977)
Heft: 4

Artikel: Biochemical study of malnutrition : situation before treatment
Autor: Antener, I. / Verwilghen, A.M. / Geert, C. van
DOI: https://doi.org/10.5169/seals-312273

Acta Tropica *34*, 331–354 (1977)

Research Laboratory Nestlé Vevey, Switzerland (Director: Professor J. Mauron),
Hospital of Yasa-Bonga, Zaïre (Director: Dr. A. M. Verwilghen)

Biochemical study of malnutrition – situation before treatment

I. Antener[1], A. M. Verwilghen, C. Van Geert, J. Mauron

Summary

A biochemical study of malnutrition has been carried out at the rural hospital of Yasa-Bonga in Kwilu (Zaïre), a region where a large number are affected and where the form of malnutrition is fairly serious. The aim of this research work was to determine the regional pathology, so as to establish an optimum treatment, both from the dietetic and clinical point of view. Since we had set up a small laboratory, we were able to check patients regularly, thus helping to reduce recurrence and, most important of all, making prevention easier. Our tests enable us to choose the most suitable diet, to learn the seriousness of the illness and to follow up its evolution. The study has shown that the extent to which the bowel (secondary malabsorption) and the liver (steatosis, fibrosis) are altered determines the recovery of patients in this area. The following tests give us full information on the condition of the bowel and the disorders of intestinal resorption. In total faeces we can follow starch, fat, mono- and disaccharides and measure the isoenzymes of alkaline phosphatase; in faecal ultrafiltrates: measure electrolytes, calcium, magnesium and zinc; in the urine: test xylose and measure vitamins B_1 and B_2; blood serum: measure transferrin. Measuring serum prealbumin, albumin, cholinesterase, γ-glutamyltranspeptidase and γ-glutamyltranspeptidase isoenzymes gives us information on the condition of the liver. Establishing the hydroxyproline and creatinine/height rates is very useful; the former gives us information on growth at the time of the test and the latter on the muscular mass. In order to reduce the number of relapses, patients ought to have a check-up every six months. Our work showed, for the first time, the importance of measuring γ-GT and its isoenzymes when assessing the seriousness of hepatic disorders in malnutrition.

Key words: Metabolism of nitrogen, malabsorption, steatosis of the liver, electrolyte, trace-elements, vitamins, enzymes, γ-GT-isoenzymes.

[1] As a mark of acknowledgment and recognition I dedicate this study to the memory of Professor I. Abelin, M.D. Ph.D., whose instruction has guided me throughout my research.

Correspondence: Dr. I. Antener, Research Laboratory Nestlé, CH-1800 Vevey (Switzerland)

Introduction and aim of our research

We studied malnutrition (local name: *mbwaki*) at the Yasa-Bonga Hospital in Kwilu/Mokamo in Zaïre. The area has a large number of patients and a very severe form of malnutrition (Verwilghen, 1957). Mauron will publish data on the geographic location, the ethnic outline of the area and results of nutritional surveys carried out in villages near the hospital, in another article [40].

Apart from seriously affected children, we were amazed at the large number of young women who, apart from the other clinical symptoms, suffer from general discolouration. The main aim of our research at this hospital was to effect a detailed comprehensive study of the illness, so as to establish the optimum dietetic and clinical treatment. We were able to follow up the patients through our diagnostic tests and through a small number of clinical measuring operations which could be performed on the spot in a laboratory specially installed for this purpose. By continued use of this laboratory, we hope to avoid relapses and, most important of all, give preventive treatment.

Methods

Collecting samples

We examined the serum, urine and faeces over 24 h, immediately freezing them at –20° C. Details on collecting faeces and preparing their ultrafiltrates are described by Antener (1969) and Antener et al. (1972).

Biochemical methods

1. Serum. We used Boehringer's combination tests for measuring the following substances: total proteins (biuret reaction), cholesterol (enzymatic), glucose (hexokinase), total lipids, triglycerides, and free fatty acids and for the following enzymes: GOT, GPT, γ-GT, LDH, LAP, CHE, GLDH, PA. We measured other components as follows: Electrolytes:

- K, Na, using an Instrumentation Laboratory, Flame Photometer 343.
- Mg, Ca, by atomic absorption, Bausch and Laub equipment.

Cl^- with a Corning Eel 920 chloride meter, Cu by Perkin-Elmer atomic absorption; immunoglobulins IgA, IgG, IgM (Tripartigen Hoechst Behring); Ceruloplasmin by immuno-diffusion (immunoplates Human Ceruloplasmin test Heyland).

Prealbumin by radial diffusion (Behring plates and standard); γ-GT isoenzymes were separated on Cellogel by the method of Patel et al. (1973); Alkaline phosphatase isoenzymes were separated by Quick disc Electrophoresis (acrylamide gel) "Canalco", Rockville, Maryland 20852. Protein electrophoresis was carried out with a Microzon Cell Model R-101 and amino acids were separated by the Biochrom aminoanalyzer.

2. Urine. Total nitrogen (Technicon analyzer) and vitamins: microbiological test; electrolytes as for serum except for magnesium which was measured by atomic absorption. Creatinine: Lloyd's method; urea: Boehringer combination test; Hydroxyproline: method developed in our laboratories. Copper, zinc, manganese: atomic absorption (Perkin-Elmer 503 and AGA 70 Perkin-Elmer graphite oven).

3. Faecal Ultrafiltrate. Electrolytes and trace-elements: same methods as for urine. Sugars and short-chain fatty acids were measured by thin-layer chromatography and gas chromatography respectively (both methods were submitted for publication). Lactic acid and glucose: Boehringer's combination tests.

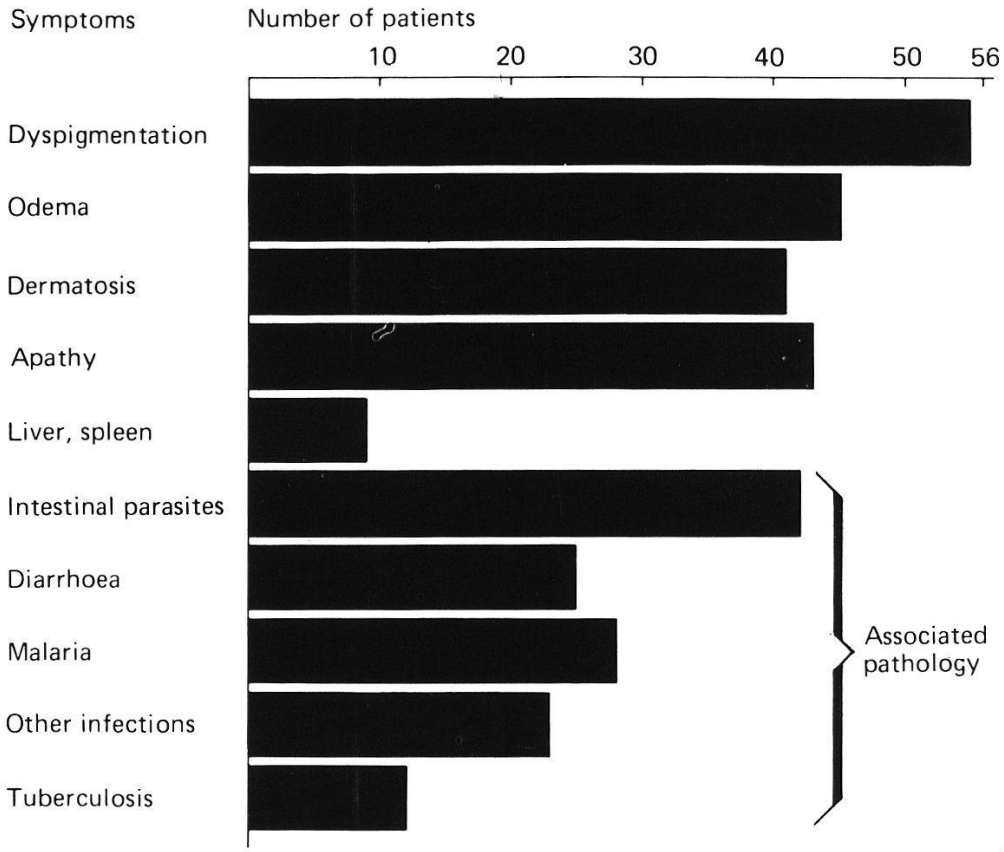


Fig. 1. Clinical symptoms.

4. Total faeces. Starch was detected with a iodine solution, fat with red oil 0 (Chroma). Alkaline phosphatase: electrophoresis on acrylamide gel or agarose: Readyfilm R by Müllener Gerätebau AG, 8957 Spreitenbach (Switzerland); Albumin also with electrophoresis on acrylamide.

Patients studied

Numbers and ages

Between 1971 and 1975 (four different periods) at the Hospital of Yasa, we studied 56 patients, comprising 33 children (aged 1 to 9), one adolescent, twenty women (aged 19 to 25) and two men aged between 40 and 50.

69.7% of the children involved were aged between three and six; this represents wider age limits than those usually described for kwashiorkor (Pereira et al., 1974).

The age of our patients is similar to that studied in Kivu (Zaïre) by De Maeyer (1954), De Maeyer et al. (1958), Dubois et al. (1968).

Clinical symptoms

The main clinical symptoms found in our 56 patients are shown in Fig. 1. 80.3% of our patients exhibit more or less marked oedemata which are very characteristic (Waterlow et al., 1960). The very characteristic discolouration among children – also very striking among the young women – affects 98.2% of patients. Only one child, aged 13 months, was not discoloured. The discolouration first affects the hair, eyebrows and eyelashes and then, in the most serious cases, spreads across the whole body starting with the extremities of the lateral members. This symptom is very characteristic of the form of malnutrition which we are studying.

Van Daele, Trolli, Doucet, and Pieraerts studied this discolouration from 1938 to 1946 for the region where we are carrying out our study, Kwango. There are a considerable number of dermato-

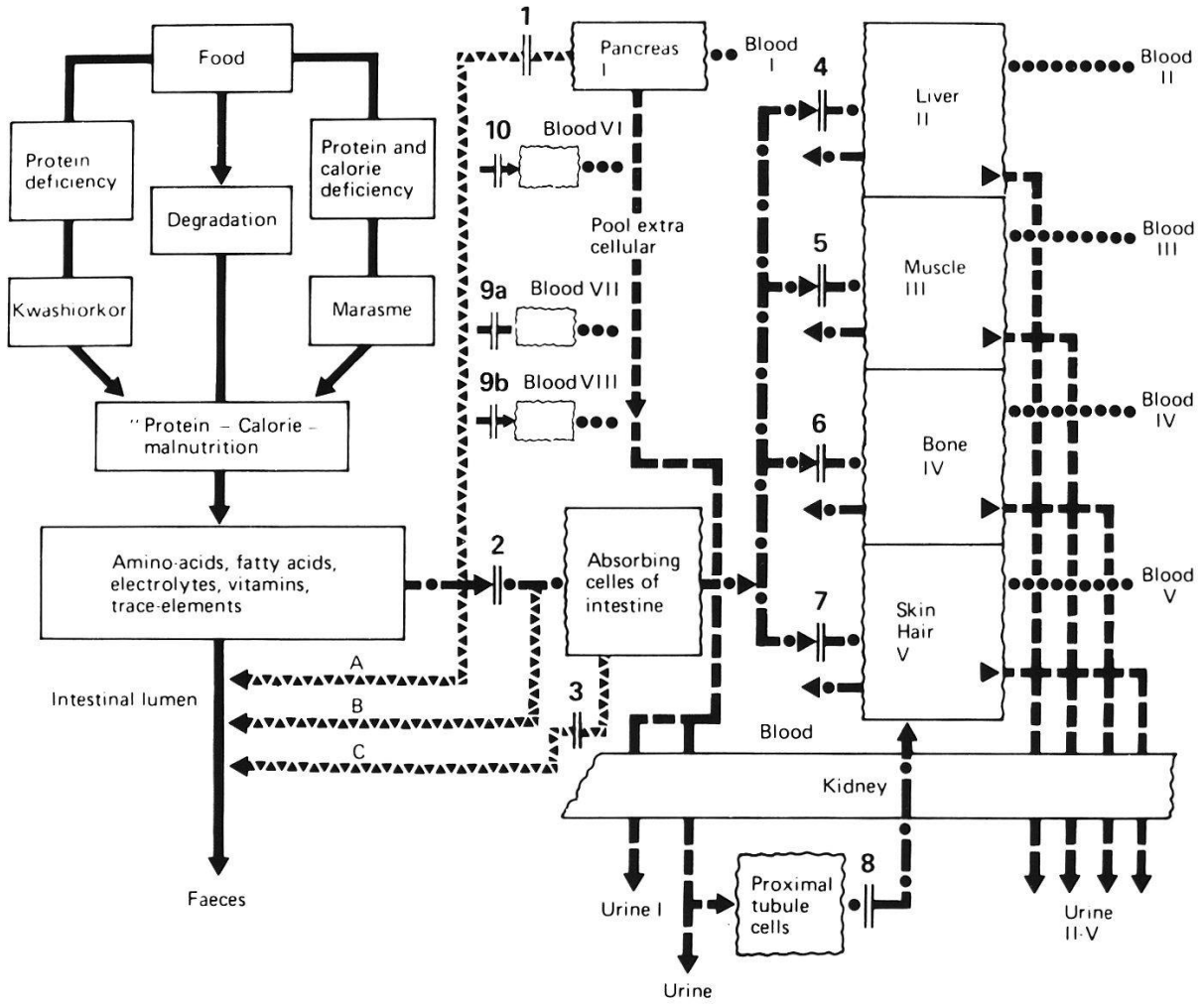


Fig. 2. Physiology of the nutrition. Schema of the analysis in biological fluids.

Metabolic disorders

1 Pancreatic atrophy (maldigestion)
2 Villous atrophy, flat mucosa, enzyme and transport deficiency (secondary malabsorption)
3 Bacterial and viral infections, fermentation, irritability, increase of mobility, diarrhoea
4 Steatose and cellular alterations (precirrhosis, cirrhosis). Reduction of protein synthesis, hypoalbuminaemia, oedema
5 Muscle mass reduction (wasting)
6 Growth retardation (stunting)
7 Dyspigmentation, dermatosis, infection
8 Trouble of tubular reabsorption
9 Endocrine troubles:
a) hypophysis (growth hormone)
b) Isle of Langerhans (Insulin production)
10 Thymus (T-Lymphocytes)

Faeces

A Starch, fat, proteins
B Amino acids, sugars, lactic acid, cholesterol, calcium, magnesium, trace-elements (if possible intestinal biopsy)
C Amino acids, fat, protein, lactic acids, organic acids, sugars, potassium, sodium, iron, trace-elements

Urine

I Amylase
II Total proteins, urea, amino acids, vitamins, urocanic acid, formiminoglutamic acid, vitamin B_6
III Creatinine, potassium, sodium, magnesium, phosphorus
IV Tyrosine, phenylalanine, dopa, copper, manganese, zinc, vitamins, metabolites of tyrosine and phenylalanine

Blood

I Lipase, amylase
II Proteins, enzymes (CHE, γ-GT, GOT, GPT, LDH, GLDH, alkaline phosphatase) IgA, IgG, IgM, copper, amino acids (if possible liver biopsy)
III Potassium, sodium, magnesium, phosphorus, creatinine (if possible muscle biopsy)
IV Calcium, phosphorus, alkaline phosphatase
V Ceruloplasmin, copper, manganese phenylalanine, tyrosine, vitamins
VI Study of isolated lymphocytes
VII Determination of growth hormone
VIII Determination of insulin

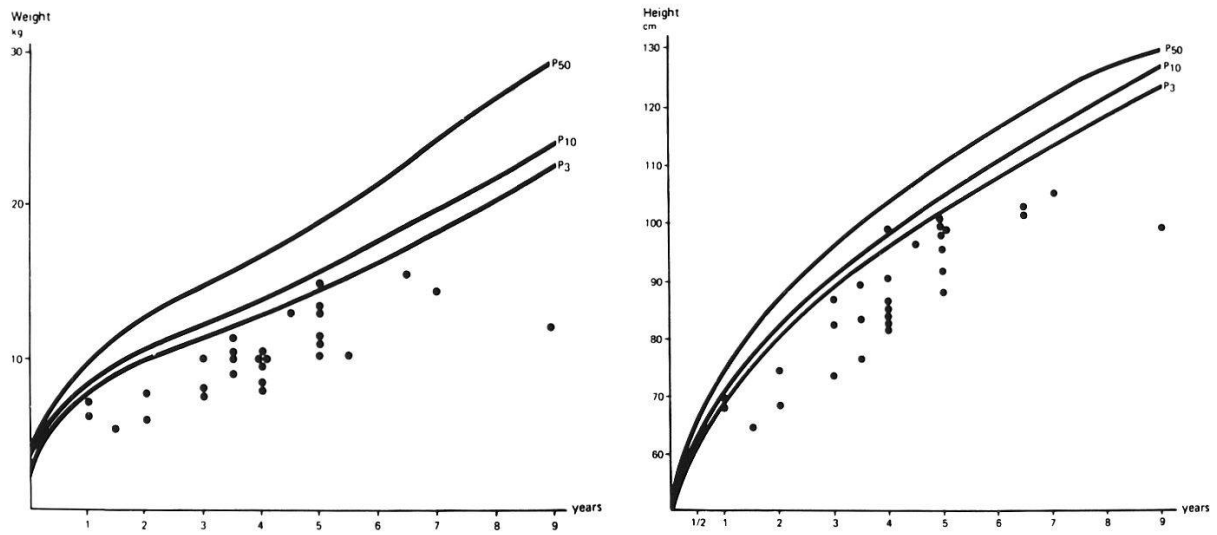


Fig. 3. Anthropometric measurement: weight/age; height/age.

sis cases among our patients: 73.2% of cases are affected, but mainly at the extremities. In our group there were only two patients who had generalized serious dermatosis; these two patients were no longer curable. This is in accordance with Kahn (1959) who states that children affected by acute dermatosis have poor prospects.

Apart from a protein deficiency, a vitamin and trace-element deficiency may be at the root of dermatosis. We found that for some of our patients urinary riboflavin levels were practically nil and that they often suffered from stomatitis. These findings are in line with Mauron's findings on the vitamin B_2 deficiency in this area. Infections also play a major part which we established with the help of a biopsy of the skin, carried out post mortem. In 1970, Vasantha et al. established biochemical proof of the occurrence of skin lesions in Kwashiorkor on the basis of their skin tests.

76.8% of our patients suffered from apathy; the children had lost their smile, which is a very typical symptom (Waterlow, 1960). In 8 patients, i.e. 14.2%, we found the liver to be palpable. Apart from these clinical signs which are typical of malnutrition, we find various kinds of intestinal parasitosis, different kinds of diarrhoea, tuberculosis, measles and infections of the respiratory organs (cf. Fig. 1). In some children we also found "duck's waddle", as described by Dupin (1969), although rachitis is rare in black Africa (Kendall, 1972).

Working plan of biochemical study

The food metabolism of healthy human beings is shown in Fig. 2. A well-balanced and sufficient nutritional supply is required for the metabolism to be normal. Nutritional imbalance is apparent in very characteristic symptoms which occur mainly in 2 diseases: Kwashiorkor which is a protein deficiency, and marasmus which is a combined calorie and protein deficiency. The various forms of these two diseases are classified as "protein-calorie malnutrition", also known as "protein-energy malnutrition". The clinical signs are specific alterations of different organs resulting in a pathological composition of intra and extra-cellular fluids. Through blood, urine and faecal tests and faecal ultrafiltrate tests we showed the above-mentioned pathology. This is indicated in the diagram in the following manner: The main organs affected by malnutrition are designated by Arabic numerals, and the double line in front of the organ indicates the metabolic disorder involved. Numbers 1 to 10 are explained in the text below the diagram under the heading "metabolic disorders". Faecal tests which correspond to disorders Nos. 1 to 3 are to be found below the diagram under A, B and C. The affected organ, and the tests which show the respective urinary and serum pathology are indicated by Roman numerals, both in the diagram and in the column below it (I to VIII blood; I to V urine).

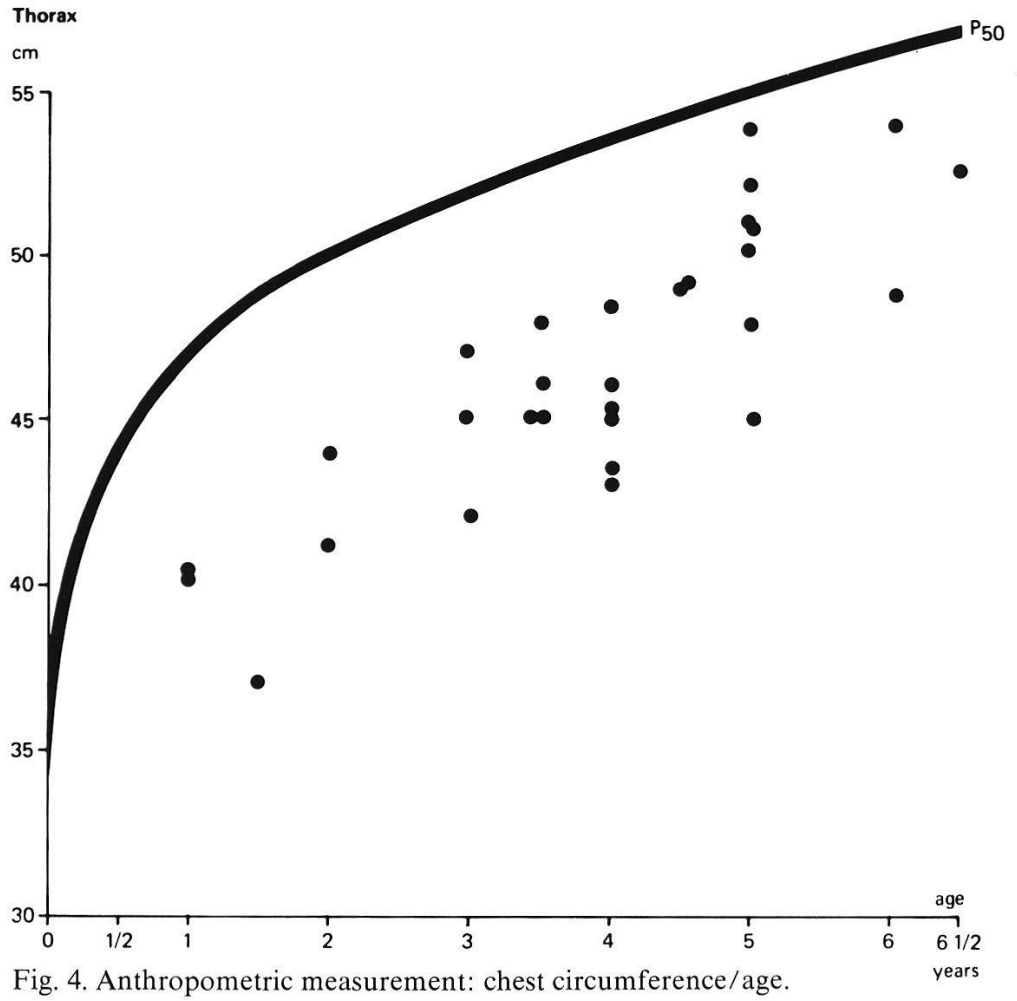


Fig. 4. Anthropometric measurement: chest circumference/age.

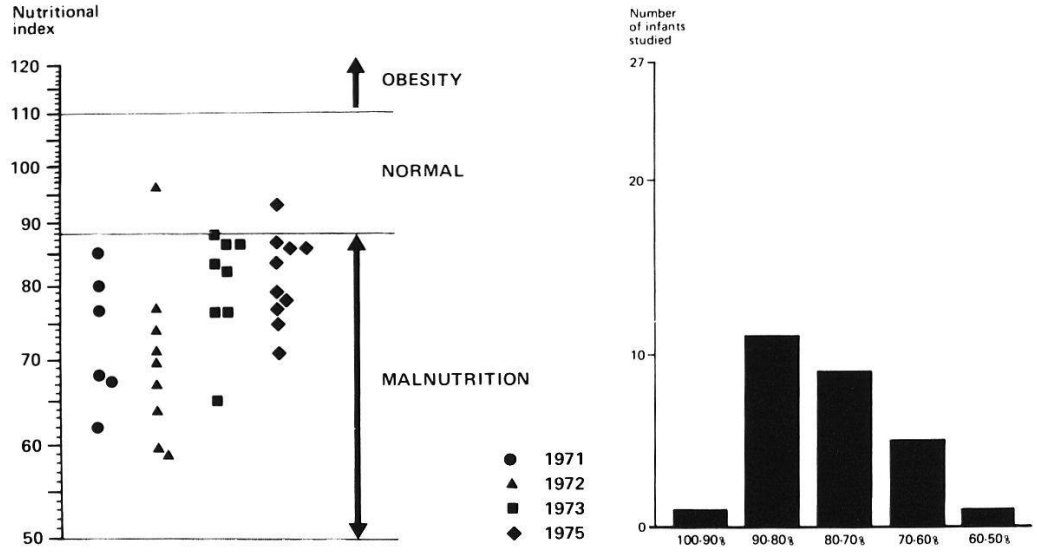


Fig. 5. Anthropometric measurement: on the left side: Nutritional Index (Dugdale). On the right side: Mid-upper-arm circumference (standard Jelliffe).

Results

A. Anthropometric measurements

1. Classification according to age, weight, height and thorax

Figs. 3 and 4 show the classification of our patients according to weight/age, height/age and thorax/age. We used the Boston standard (Nelson, 1964).

Among our patients, the weight/age and thorax/age relations are even more abnormal than the height/age relation.

2. Classification according to measurements, independent from age

Fig. 5 shows the arm measurement and the nutritional index which is based on weight and height. Most of our children have an arm measurement of between 60 and 90% of standard. Except for two, all children fall within the malnutrition part of the nutritional diagram. The latter clearly shows the pathology of patients who were examined in different years.

B. Liver

In a post-mortem biopsy of the liver of one of our patients we found marked steatosis.

14.2% of patients studied had a palpable liver. If we had been able to carry out biopsies of the liver, we should no doubt have found other cases of steatosis.

C. Serum

1. Protein, albumin and globulin, ceruloplasmin, prealbumin

Table 1 shows average values and the standard deviation in our patients – separately for children and adults –, and also average values and standard deviation in our rural controls.

A lowered albumin value is even more characteristic than a lowered total protein figure. The α_2 and β-globulin rate is only slightly lowered in our children, as compared to controls. Among adults we find a marked increase in γ-globulins. The ceruloplasmin (an α_2-globulin) and prealbumin rates are reduced in adults and children.

2. Calcium, magnesium and phosphorus

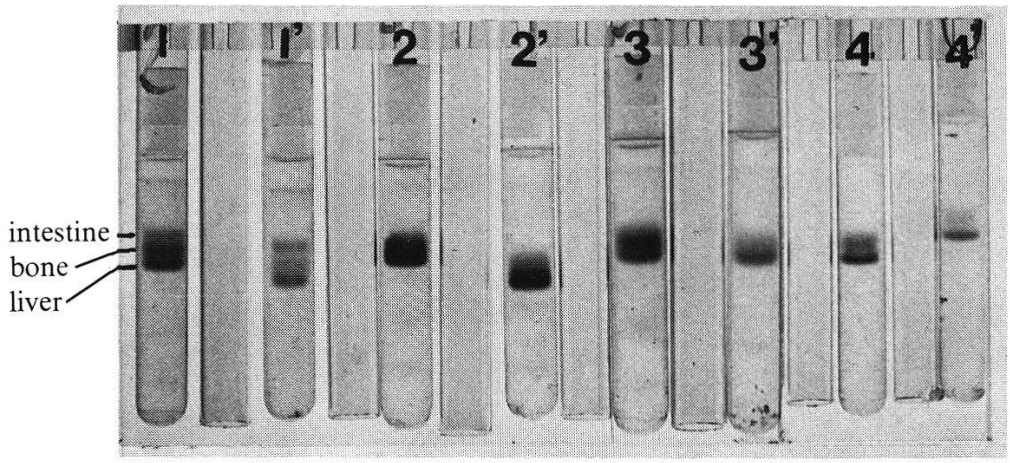
We find marked hypocalcaemia in the majority of our patients. In our children we find a more or less lowered magnesium level, whereas it is considered normal in our adults.

The phosphorus rate is definitely lowered in our children, but to a lesser degree in the adults: cf. Table 1.

Table 1. Serum content and SD of proteins, amino acids, electrolytes and enzymes of patients and controls

	Units	Infants		Adults	
		Patients	Controls of Yasa-Bonga	Patients	Controls of Yasa-Bonga
		Mean ± SD	Mean ± SD (Interval)	Mean ± SD	Mean ± SD (Interval)
Total proteins	g/l	54.6 ± 8.7	71.1 ± 6.9	61.7 ± 10.3	76.1 ± 6.6
Albumin	g%	1.89 ± 0.53	3.31 ± 0.35	1.93 ± 0.50	4.36 ± 0.38
α_1-globulin	g%	0.27 ± 0.09	0.32 ± 0.07	0.31 ± 0.06	0.27 ± 0.08
α_2-globulin	g%	0.56 ± 0.13	0.71 ± 0.18	0.57 ± 0.13	0.48 ± 0.10
β-globulin	g%	0.59 ± 0.15	0.75 ± 0.15	0.66 ± 0.18	0.76 ± 0.13
γ-globulin	g%	2.08 ± 0.51	2.04 ± 0.61	2.69 ± 0.67	1.80 ± 0.49
Ceruloplasmin	mg%	31.1 ± 8.7	56.1 ± 15.3	28.9 ± 7.4	42.4 ± 12.9
Prealbumin	mg%	10.2 ± 4.15	14.3 ± 4.26[1]	11.5 ± 5.7	20.9 ± 7.6
Immunoglobulins IgA	UI/ml	105.9 ± 33.3	(30 − 110)[2]	165.6 ± 88.6	(40 − 190)[2]
Immunoglobulins IgG	UI/ml	291.8 ± 85.2	(60 − 125)[2]	361.1 ± 71.9	(70 − 160)[2]
Immunoglobulins IgM	UI/ml	328.4 ± 120.7	(60 − 200)[2]	645.6 ± 579.6	(110 − 370)[2]
N.E. AA/E.AA		4.35 ± 2.55	(1 − 2)[3]	3.27 ± 1.13	(1 − 2)[3]
Valine/Glycine		0.53 ± 0.38	0.96 ± 0.14[4]	0.52 ± 0.28	0.96 ± 0.14[4]
Phenylalanine/Tyrosine		3.38 ± 4.13	0.87 ± 0.13[4]	2.98 ± 1.30	0.87 ± 0.13[4]
Haemoglobin	g%	6.53 ± 2.24	(11.0 − 12.0)	−	−
Glucose	mg%	63.5 ± 13.6	85.0 ± 15.0	46.7 ± 4.9	85.0 ± 15.0
Total lipids	g/l	4.93 ± 1.83	6.89 ± 2.68	4.94 ± 1.37	5.84 ± 1.23
Cholesterol	mg%	89.9 ± 28.6	132.6 ± 24.8	99.6 ± 38.3	165.0 ± 38.6
Potassium	mEq/l	4.5 ± 0.49	4.52 ± 0.59	4.96 ± 0.51	4.63 ± 0.29
Sodium	mEq/l	136.2 ± 5.80	138.0 ± 1.94	139.6 ± 3.7	140.6 ± 2.3
Chlorine	mEq/l	103.6 ± 7.20	103.2 ± 2.97	104.9 ± 4.5	102.9 ± 3.3
Calcium	mg%	8.3 ± 0.80	9.1 ± 0.45	8.5 ± 0.8	9.8 ± 0.45
Magnesium	mg%	1.83 ± 0.25	2.22 ± 0.11	1.92 ± 0.24	1.99 ± 0.34
Phosphorus	mg%	3.86 ± 0.75	5.20 ± 0.67	3.79 ± 0.8	3.98 ± 0.32
Enzymes γ-GT	U/l	77.2 ± 45.8	8.82 ± 4.12	55.4 ± 35.3	8.82 ± 4.12
Enzymes GOT	U/l	20.8 ± 8.4	< 12[5]	17.7 ± 6.0	< 12[5]
Enzymes GPT	U/l	14.7 ± 10.9	< 12[5]	13.4 ± 4.9	< 12[5]
Enzymes GLDH	U/l	4.9 ± 4.1	< 4[5]	3.5 ± 2.2	< 4[5]
Enzymes LDH	U/l	216.7 ± 50.9	(120 − 195)[5]	362.7 ± 274.9	(120 − 240)[5]
Enzymes LAP	U/l	16.2 ± 3.4	(8 − 22)[5]	19.6 ± 6.0	(8 − 22)[5]
Enzymes CHE	U/ml	1.54 ± 0.66	(3 − 8)	1.96 ± 1.05	(3 − 8)
Enzymes AP	U/l	142.1 ± 95.7	(151 − 471)[5]	134.7 ± 81.6	(60 − 170)[5]

[1] Smith F. R. (1975) [2] O'Brien D. et al. (1968) [3] Whitehead R. G. (1964) [4] Armstrong M. D. et al. (1973)
[5] Normal values of Boehringer Mannheim GmbH



1'-2'-3'-4': heat inactivation at 55° C

Fig. 6. Separation on polyacrylamide of isoenzymes of the alkaline phosphatase in the serum.

3. Potassium, sodium, chlorine

Potassium, sodium and chlorine levels are normal, apart from a few exceptions (Table 1).

4. Total lipids and cholesterol

The total lipid and cholesterol levels are very definitely lowered. Levels in adults vary more than those in children.

5. Amino acids

The pathology shows in an increased ratio between non-essential/essential amino acids and between phenylalanine tyrosine, as well as a lower value for the valine/glycine ratio.

6. Haemoglobin and glucose

The haemoglobin level is definitely lowered in our children. In our patients, the glycemia level is not lowered regularly.

7. Enzymes

We measured the following enzymes: glutamate-oxaloacetate-transaminase (GOT), glutamate-pyruvate-transaminase (GPT), lactate-dehydrogenase (LDH), glutamate-dehydrogenase (GLDH), γ-glutamyl-transpeptinase (γ-GT), leucine-aminopeptidase (LAP), alkaline phosphatase (PA) and cholinesterase (CHE No. 3. 1. 18).

Table 1 shows the enzymatic measurement results. It shows that CHE and γ-GT levels are uniformly pathological. Other enzyme levels (GOT, GPT, LDH, GLDH) are only pathological in some patients. GOT levels are raised more than GPT levels. Increased LDH levels are more characteristic in adults than in children. On the other hand, GLDH levels are higher in our children

Table 2. Serum γ-glutamyltranspeptidase-isoenzymes-content of patients and controls (electrophoresis scanner of CAMAG)

No photo	Serum	γ-GT U/l	α_1-γGT		α_2-γGT		β_1-γGT		0-γGT	
			%	U/l	%	U/l	%	U/l	%	U/l
1	Control 1	4	9	0.3	91	3.7	–	–	–	–
2	Mb.	57	–	–	100	57	–	–	–	–
3	Mp.	86	72	61.9	28	24.1	–	–	–	–
4	Moy.	86	62	53.3	36	31	–	–	2	1.7
5	Kim.	47	52	24.5	38	18.8	–	–	10	4.7
6	Mas.	129	72	92.9	27	34.8	–	–	1	1.3
7	Luy.	182	22	40	38	69.2	10	18.2	30	54.6
8	Control 2	9	8	0.7	92	8.3	–	–	–	–

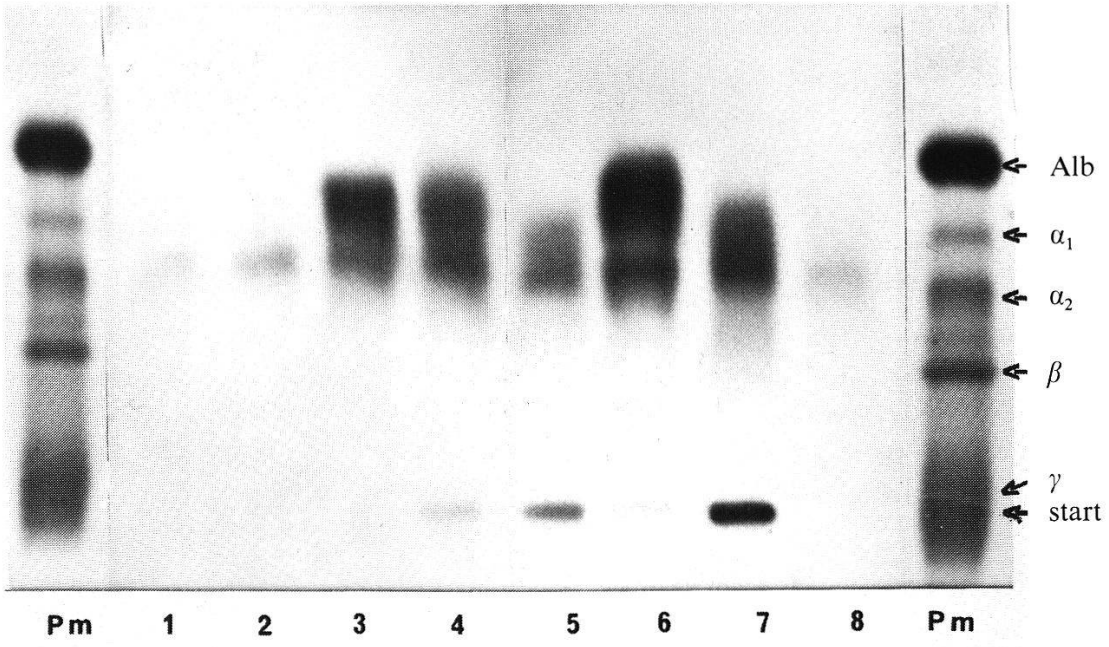


Fig. 7. Separation of serum γ-glutamyltranspeptidase isoenzymes using Cellogel Electrophoresis. Controls 1 and 8: α_2-γ-GT, α_1-GT (very low); patient 2: α_2-γ-GT; patient 3: α_1-γ-GT, α_2-γ-GT; patient 4: α_1-γ-GT, α_2-γ-GT, 0 (start)-γ-GT; patients 5 and 6: α_1-γ-GT, α_2-γ-GT, 0-γ-GT; patient 7: α_1-γ-GT, α_2-γ-GT, β_1-γ-GT, 0-γ-GT, see Table 2.

than in the adults. The LAP level is slightly higher in some children and adults. The PA level is higher among the adults.

Separating serum isoenzymes from alkaline phosphatase enables us to see two bands which correspond to the liver and the bones. We found the intestinal band only once, in a little girl aged two (Fig. 6). Heat inhibits activity (15′ at 56°) by about 80% for the bone enzyme and by about 20% for the liver enzyme. We can therefore distinguish the intensity of the liver strip from that of the bone strip. Fig. 6 shows a patient in whom the liver strip predominates, and this is

especially visible after inhibition (2.2′), whereas, in one of the others, the bone strip is more intense (3.3′). In other patients (1.1′, 4.4′) bone and liver strips are of about equal intensity.

8. γ-glutamine transpeptidase isoenzymes

In 6 patients with a high γ-GT activity level, we found the following 4 bands relative to the protein marker: a_1-γ-GT, α_2-γ-GT, β_1-γ-GT, 0γ-GT (start); cf. Fig. 7 and Table 2. The γ-GT_1 spot is located nearer the albumin and it remains to be seen whether it has special peculiarities. It also remains to be shown whether the trails which we found in the β_1 area of the protein marker (Pm) are of special significance or whether they simply represent a diffusion phenomenon. The two controls, with their total activity of 4 and 9 U/l, have very low α_1-γ-GT levels: 8% and 9% respectively; the main fraction is represented by α_2-γ-GT: 91% and 92%. On the other hand, all patients except one (Mb.) have a raised α_1-γ-GT level (22% to 72%) and a marked reduction of the α_2-γ-GT band. In addition, 4 patients have a band at the start (0-γ-GT) and one patient has trails in the β_1 area. The increases in the α_1-γ-GT band, in the band at the origin and in the β_1 area of the protein marker are pathological. The separation method used must be stated, because migration on various supports may differ.

D. Urine

Table 3 shows urinary excretion of the substances which we measured.

1. Nitrogen, urea, creatinine, hydroxyproline

Nitrogen, urea and creatinine excretion levels are very low; the index for creatinine/height and hydroxoproline are definitely reduced.

2. Electrolytes: calcium, magnesium, potassium, sodium, chlorine, phosphorus

The calciuria level is definitely lowered in our children, being almost nil in some of them; it is also low in the adults. We find the same reduced levels for magnesium in both children and adults. In addition, the raised phosphaturia levels are striking in some of the children, but less so in the adults. Lowered potassium and sodium excretion levels are also found in some of the children and adults. Chlorine follows the sodium variations.

3. Trace-elements and vitamins

Some of our patients, both children and adults, have a low copper, zinc and manganese excretion level. Moreover, in some of our patients, we found a low vitamin B_1, B_2, B_6 and, in particular, PP excretion level (Table 3).

Table 3. Urinary excretion of electrolytes, trace-elements, total nitrogen, urea, creatinine, hydroxyproline, vitamins and xylose, the latter after loading

Substances	Units	Patients Infants		Controls Infants of Yasa-Bonga Interval (Mean − SD)	Patients Adults		Controls Adults of Yasa-Bonga Interval (Mean − SD)
		Mean ± SD	Interval		Mean ± SD	Interval	
Potassium	mEq/24 h	10.5 ± 8.8	0.2 − 29	19 − 53	16.40 ± 12.4	1 − 31	29 − 60
Sodium	mEq/24 h	11.9 ± 9.8	0.1 − 32	12 − 27	21.9 ± 19.9	0.25 − 70	33 − 158
Chlorine	mEq/24 h	14.6 ± 9.2	1.1 − 37	22 − 42	25.0 ± 17.8	4.0 − 53	33 − 159
Calcium	mg/24 h	0.6 ± 0.9[1]	0.02 − 4.95[1]	1.1 − 7.4[1]	10.8 ± 7.5	1.5 − 20.3	3 − 25
Magnesium	mg/24 h	1.2 ± 0.9[1]	0.05 − 3.6[1]	0.9 − 5.2[1]	36.0 ± 25.2	7.9 ± 84.9	30 − 90
Phosphorus	mg/24 h	31.2 ± 29.1	0.7 − 102	98 − 247	102.3 ± 82.3	2.5 − 188	127 − 381
Copper	µg/24 h	15.0 ± 9.2	5 − 46	13 − 23	37.6 ± 19.9	15 − 73	40 − 60
Zinc	µg/24 h	113.5 ± 74.2	12 − 29	121 − 254	341.6 ± 260.6	119 − 1067	256 − 1500
Manganese	µg/24 h	3.3 ± 2.4	1 − 11	3 − 10	4.2 ± 3.1	1 − 10	3 − 23
Nitrogen	g/24 h	0.4 ± 0.2	0.08 − 0.96	1.3 − 2.2	1.2 ± 0.6	0.6 − 2.2	1 − 4
Urea	g/24 h	0.5 ± 0.3	0.05 − 1.0	1.4 − 4.1	1.8 ± 1.3	0.5 − 4.4	2 − 3
Creatinine	mg/24 h	99.8 ± 53.6	16.4 − 201.3	186 − 311	285.7 ± 104.2	129 − 481	300 − 953
Hydroxyproline	mg/24 h	6.9 ± 3.1	1.9 − 13.7[2]	38.9 ± 8.7 (1–4*); 62 ± 14.2 (4–8*)	14.5 ± 7.8	2.4 − 30.6	5.7 − 44.9[3]; (20.8 ± 7.72)
Index hydroxyproline	–	0.7 ± 0.29	0.15 − 1.4	3.0 ± 0.8[4]	–	–	–
Index creatinine height	–	0.45 ± 0.19	0.12 − 0.77	0.9 − 1.2[5]	–	–	–
Xylose loading	% elimin.	19.9 ± 10.2	5.8 − 36.7	20 − 36			
Vitamin B_1	µg/24 h	8.8 ± 6.2	1 − 15	(276 ± 59)[6]	25.6 ± 23.9	4.2 − 50	–
Vitamin B_2	µg/24 h	205 ± 238	25 − 625	(228 ± 48)[6]	386 ± 324	53 − 925	–
Vitamin PP	µg/24 h	111 ± 81.5	5 − 251	(412 ± 72)[6]	182 ± 175	60 − 491	280 − 491
Vitamin B_6	µg/24 h	21.1 ± 15.7	2.5 − 35.5	(89.9 ± 12.9)[6]	49 ± 54.7	6.7 − 178	–
Folic acid	µg/24 h	1.6 ± 1.2	0.5 − 3.5	–	1.4 ± 1.3	0.4 − 4.3	–

[1] mg/24 h/kg [2] Nusgens B. et al. (1973) [3] Haury H. (1972) [4] Viteri F. E. et al. (1970) [5] Whitehead R. G. (1965)
[6] Nordio S. et al. (1968) * Years

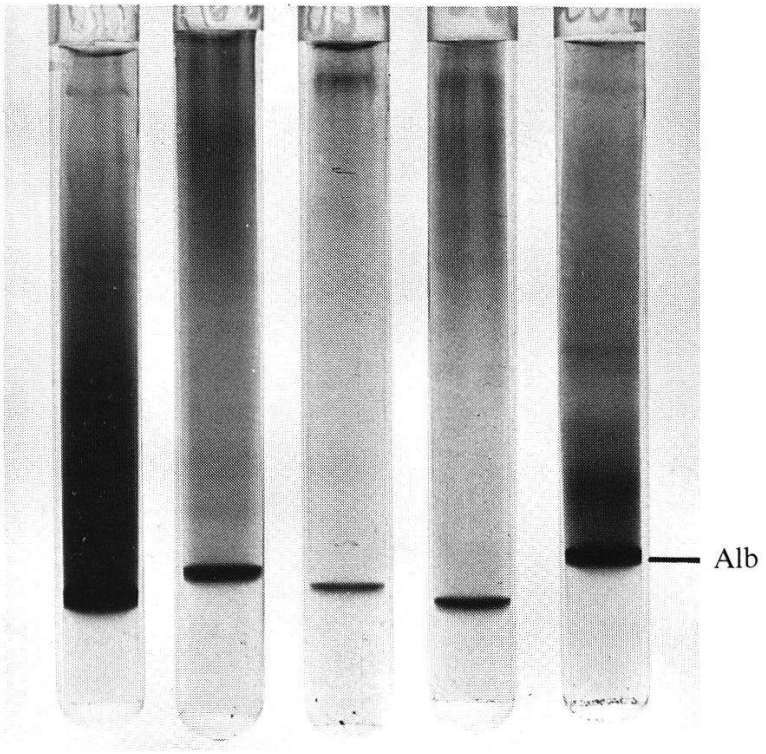


Fig. 8. Albumin in the faeces (Separation on polyacrylamide).

E. Intestines and faeces

1. Biopsy

We showed the very serious atrophy of the intestinal mucosae in one of our patients by carrying out a post-mortem biopsy in the best possible conditions. We add that intestinal biopsies cannot be carried out at a rural hospital. This is why we carried out a very detailed study of the faeces.

2. Faecal quantity and frequency

We were mainly struck by the large number of daily excretions of some of our patients, mainly children. Nearly all had periods of diarrhoea and some had very voluminous and pale faeces.

3. Screening tests

a) *Starch* (total faeces). The test which was carried out on 32 patients shows the following distribution: 46.9% highly positive, 21.9% positive, 31.2% negative. Microscopic preparation shows grains of starch which are still whole.

b) *Fat* (total faeces). Steatorrhea plays a minor part when the patient enters hospital, because fat supply is low. During treatment, when fat supply increases, conditions change.

c) *Albumin.* We have shown albumin in faeces by the method of electrophoresis on acrylamide gel (Fig. 8).

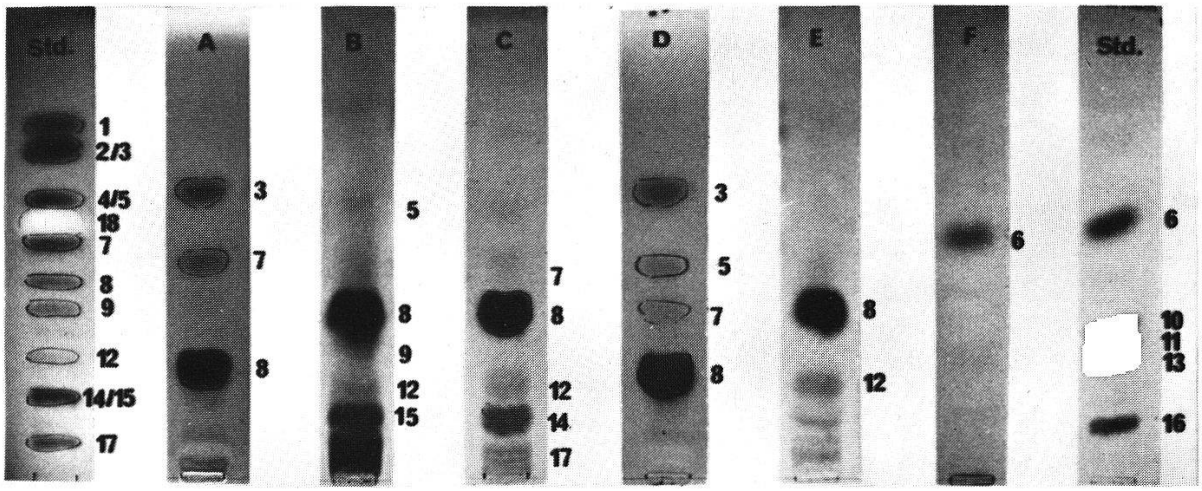


Fig. 9. Thin-layer chromatography of sugars in the stool-ultrafiltrate: A–E: glucose (8); B: lactose (15), galactose (9); C: isomaltose (14); A–D: xylose (5), ribose (3), arabinose (7); B, C, E: maltose (12); F: fructose (6).

Table 4. Evaluation of sugars from the thin-layer plates

Results	Maltose (n=22)		Isomaltose (n=22)		Lactose (n=22)		Glucose (n=32)	
	n	%	n	%	n	%	n	%
Highly positive	7	32	5	23	2	9	24	75
Positive	2	9	2	9	0	0	3	9
Negative	13	59	15	68	20	91	5	16
Total	22	100	22	100	22	100	32	100

d) *Sugar chromatography in faecal ultrafiltrate.* Thin-layer chromatography enabled us to detect the following sugars in faecal ultrafiltrates: maltose, isomaltose and lactose as disaccharides; glucose, galactose and fructose as monosaccharides; ribose, arbinose and xylose as pentoses (Fig. 9). Since using our technique, isomaltose and lactose migrate together, the test must be repeated after hydrolysis, so as to identify galactose.

After development the TLC-plates show the following sugar distribution (Table 4): We find glucose in 84%, maltose in 41%, and isomaltose in 32% of our patients. The lactose % is low (9%), because only two of our patients were still being breast-fed, whereas the others did not drink any milk. Among controls, the disaccharide test of the faeces is negative, whereas there are traces of glucose. We were not able to show saccharose in either our patients or our controls and we did not test pentose excretion, because it was of no physiological significance.

e) *Alkaline phosphatase isoenzymes.* Fig. 10 shows the separation of alkaline

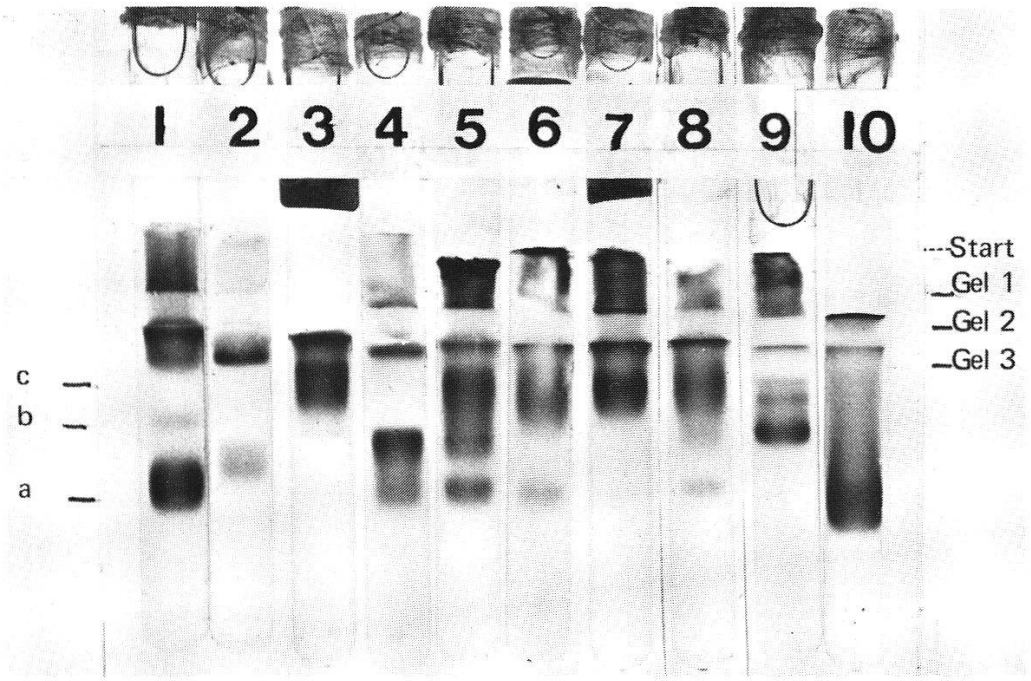


Fig. 10. Separation of alkaline phosphatase in the stool using polyacrylamide gel electrophoresis. a = band of the intestine; b = band of the liver (probably); c = band 4a named by Benic-Fiser.

Table 5. Composition of the stool-ultrafiltrate: electrolytes, trace-elements, nitrogen, glucose, lactic acid and short-chain fatty acids

Substances	Units	Patients Infants		Patients Adults	
		Mean ± SD	Interval	Mean ± SD	Interval
Potassium	mEq/l	16.5 ± 6.5	8 – 26	15.3 ± 5.8	8 – 24
Sodium	mEq/l	3.9 ± 4.0	1 – 16	4.8 ± 2.6	1 – 8
K/Na		8.6 ± 7.7	0.5 – 26	4.9 ± 3.8	1.7 – 12
Calcium	mg/100 ml	23.7 ± 20.3	5 – 81	30.3 ± 24.1	11 – 89
Magnesium	mg/100 ml	12.1 ± 7.9	3 – 27.8	14.1 ± 8.8	4 – 36.8
Phosphorus	mg/100 ml	17.5 ± 17.0	1 – 59	16.4 ± 10.5	3.4 – 36.1
Copper	µg/100 ml	36.3 ± 21.9	5 – 100	31 ± 9.9	15 – 43
Zinc	µg/100 ml	590.7 ± 261.6	250 – 1260	476.3 ± 162.6	300 – 700
Manganese	µg/100 ml	263.7 ± 192	35 – 760	320 ± 118.7	120 – 460
Nitrogen	mg/100 ml	14.6 ± 8.5	4.3 – 34.2	15.0 ± 5.5	7.1 – 26.6
Glucose	mg/100 mg N_2	783.4 ± 1475	3.5 – 5395	340.9 ± 432.7	9 – 956
Lactic acid	mg/100 mg N_2	348.3 ± 748.0	4.7 – 691	71.3 ± 102.0	6.8 – 347.9
Acetic acid	mg/100 mg N_2	309.1 ± 225.3	39.1 – 897.2	332.1 ± 206.1	18.6 – 583.8
Propionic acid	mg/100 mg N_2	185.1 ± 145.7	17.4 – 566.9	172.6 ± 128.7	5.3 – 346.6
Isobutyric acid	mg/100 mg N_2	4.6 ± 3.1	1.2 – 12.1	3.4 ± 3.0	1.2 – 9.0
Butyric acid	mg/100 mg N_2	94.9 ± 130.8	1.0 – 406.6	113.4 ± 99.4	0.5 – 315.3
Isovoleric acid	mg/100 mg N_2	5.8 ± 6.5	0 – 25.3	3.6 ± 5.1	0 – 15.1
Valeric acid	mg/100 mg N_2	4.6 ± 6.0	0 – 17.9	7.2 ± 8.3	0 – 22.0
Caproic acid	mg/100 mg N_2	0.7 ± 1.8	0 – 68	–	–

phosphatase iso-enzymes. We observed several bands, but could only identify the source of three. Band a) which migrates fastest comes from the intestine. Control No. 10 shows a very marked band. It is more or less intense in patients Nos. 1, 3, 4, 5, 6 and 8 and is completely missing in Nos. 2, 7 and 9. Band b) probably comes from the liver. It cannot be seen in the control, but it is to be found in patients Nos. 1, 3, 4, 5 and 8. Benic and Fiser identified band 4a (5 nucleotidase) in some of our patients. We think that band c) on our acrylamide gel corresponds to band 4a of these authors. Band c) is found in patients Nos. 3, 5, 7 and 8.

4. Quantitative tests

a) *Measuring electrolytes, trace-elements and nitrogen in faecal ultrafiltrates.* Table 5 shows average levels, standard deviation and differences between values. Although patients eat the same food, electrolyte levels vary a great deal. It is interesting to see that the standard deviation is of the same magnitude for children and adults, as far as potassium, calcium, magnesium and the K/Na relation is concerned. On the other hand, standard deviation is higher in children than in adults for phosphorus, sodium and nitrogen. The adults have a higher excretion rate of the trace-elements Zn, Cu, and Mn than the children.

b) *Measuring glucose, lactic acid and short-chain fatty acids in faecal ultrafiltrates.* Results are presented in Table 5. Some of the children and adults have a high glucose and lactic acid excretion rate. The average short-chain fatty acid rate is only slightly higher in adults than in children. The highest value is that for acetic acid, followed by propionic and butyric acid. The results for isobutyric, isovaleric, valeric and caproic acid are lower.

c) *Xylose test.* The xylose test carried out on 11 children after one week's treatment showed that 7 children have a raised urinary xylose level after 5 h (Table 3).

Discussion of results

A. Anthropometric measurements

The weight/age and thorax/age relation is more disturbed in the children than is the height/age relation, which leads us to the conclusion that our patients suffer from marasmic Kwashiorkor (Vis, 1968). This is confirmed by arm measurements according to Burgess et al. (1969) if we use Jelliffe's standard (1966) and the nutritional index of Dugdale (1971).

B. Liver

In those children who suffered from the chronic form of malnutrition where the condition of the liver plays a considerable part, anamnesis showed

their bad nutritional state. Biopsy showed considerable steatosis of the liver. We showed the presence of steatosis in our patients by measuring their γ-GT rate which amounted to 77.22 ± 45.83 U/L (children) and 55.42 ± 35.29 U/L (adults). Other authors find similar levels in hepatic statosis (Bel et al., 1973, chronic steatosis 75 ± 67 U/L; Mayr, 1973; Hegner et al., 1975; Henning et al., 1974). The lowered CHE figures reflect the seriousness of the illness (Waterlow et al., 1969; Barclay et al., 1973).

Transaminase sensitivity is much lower, especially that of GPT. On the other hand, the LDH enzyme is raised to a greater or lesser degree in 66.2% of our patients suggesting that it is released after damage of the hepatic cells. The GLDH enzyme which is found in the mitochondria is raised in 28% of our patients. This fact confirms the alterations found in the mitochondria with the help of an electronic microscope (Bhamarapravati, 1975). The bile vessels do not seem to be affected in our children, because the LAP rate (Weber, 1969) is normal for all but three of them, but it is raised in 37% of adults, which shows that here the bile vessels are impaired. On the other hand, the PA rate is raised in 26% of children and in 65% of adults. Since the level for this enzyme is raised more than that for LAP, we may conclude that other factors play a part, such as growth in children and active osteopathies or an oestrogen treatment in adults. In some patients suffering from malnutrition, the distribution of γ-GT enzymes is very characteristic. Values found in the Hepato-Quick test were normal. For alkaline phosphate isoenzymes: in the faeces, the liver band along with band 4c according to Benic-Fiser are diagnostically helpful, as are the blood alkaline phosphatase bands from the liver.

C. Serum and urine

1. Nitrogen substances

Results found for albumin are similar to those found by De Maeyer (1954), Vis et al. (1965), Dubois et al. (1968) in an area fairly close to ours. In some of our patients we identified the prealbumin fraction which is very sensitive to treatment (Smith et al., 1975). β and α_2 globulins are only slightly lowered compared to controls (Whitehead et al., 1973; Kader et al., 1972). γ-globulins are only significantly raised in our adults if we compare them with controls (Ezeilo et al., 1971). The creatinine/height index (Viteri et al., 1970) is very useful for classifying our patients. Arroyave (1969) also observed this. Southgate (1971) discussed its limitations. The hydroxyproline index (Whitehead, 1965; McLaren et al., 1970; Cabacungang et al., 1973) was a very sensitive test for our group. It is lowered in all patients and indicates that, at the time of our examination, growth was retarded.

2. Electrolytes

We managed to show a magnesium deficiency indirectly, because the serum and urinary magnesium rates were lowered and because there was faecal

loss. Our urinary levels are definitely lower than those given by Paunier et al. (1970) and Ghazali et al. (1974) for normal children. Our findings are in line with Montgomery et al. (1961), Linder et al. (1963), Rosen et al. (1970), Harris et al. (1971), who found a lowered magnesium rate. The serum potassium rate is normal, apart from three exceptions where it is definitely lowered. Rates are similar to those found by Vis and Dubois in Zaïre. Urinary potassium is lowered in 42% of our patients. Ingenbleek and Satge (1972) also found a lowered excretion rate before treatment.

A lowered excretion rate is merely a qualitative indication of deficiency. Muscular biopsies yield more reliable figures, since muscles are the largest potassium reservoir (Vis et al., 1965). The use of the ^{42}K isotope (Smith et al., 1960) and counting potassium^{40} with a whole-body counter (Mann et al., 1975) constitute very precise methods of showing this specific deficiency. The phosphataemia rate is lowered in our children and some of them have a low phosphorus excretion rate, because both inorganic and organic muscle phosphorus is reduced (Metcoff, 1967). Urinary phosphate varies with food phosphorus supplied. It can be almost nil in cases where the protein supply is reduced and the carbohydrate supply increased (Willenbockel, 1969). Dricot et al. (1951) also found a reduced blood phosphorus rate in children in the Kwango area.

3. Lipids

The impaired fat metabolism shows in a reduced total lipid and cholesterol rate (Taylor, 1971; Truswell, 1975; Flores et al., 1970). For the latter, apart from an impairment of the liver and of the intestines, a low nutritional supply may play a part (Kudchodkar et al., 1973).

4. IgA, IgG, IgM immunoglobulins

We mainly noticed an increase in IgG and IgM, whereas the increase is less marked for IgA. We found similar figures to those found and communicated by Neumann et al. (1975). Our patients suffer from a form of Kwashiorkor aggravated by infections. This mainly results in an increased IgG rate. In the serious cases, IgA, IgG and IgM are all raised. We were struck by very high IgM levels in some women. We assume that a specific infection is at the root of this increase. The diseases in the area where we carried out our study which can raise these rates are malaria, tuberculosis and some forms of intestinal parasitosis (McFarlane, 1973).

5. Trace-elements

We have very few data on the urinary excretion of trace-elements because the latter is very low. But modern methods available enable us to establish small quantities of trace-elements accurately.

In some of our patients we found definitely reduced rates of urinary cop-

per, zinc and manganese excretion. The urinary copper excretion probably represents copper-linked albumin (Cartwright et al., 1964), a very interesting fact within the physiopathology of the illness.

Although urinary zinc excretion does not exclusively depend on supply, we wondered whether these low rates did not indicate that the body is on the borderline of having its zinc requirements covered. We measured urinary manganese, because this trace-element plays a part in the synthesis of melanin (Cotzias et al., 1964).

6. Vitamins

Although the specificity of urinary measurements is limited, we measured a few vitamins, mainly before treatment, so as to have a detection method which would enable us to assess optimum vitamin additions during treatment. For vitamin B_1, the urinary excretion rate does not indicate the actual state of deficiency but reflects the quantity supplied at the moment of the test. Out of the four patients whom we examined, two had lowered rates (4, 2 and 6 γ/24 h). In 1962, ICNND considers figures of 10 γ/24 h deficient and those between 10 and 24 γ lowered.

Thanangkul (1975) found pathological levels (between 0 and 5 γ). Lowered vitamin B_2 urinary excretion rates must be connected with Mauron's nutritional survey. We found a few lowered vitamin B_6 and PP results, but folic acid rates seemed normal to us. We may therefore conclude that our patients were on the borderline of vitamin deficiencies.

F. Faeces

Faecal examination showed:

1. Manioc starch maldigestion caused frequent, voluminous and discoloured faeces. This intolerance to starch is caused by an atrophy of the pancreas and of the intestinal mucosae (Auricchio et al., 1968).
2. We did not observe steatorrhea before treatment and faecal albumin loss was very low compared to supply.
3. Secondary intestinal disaccharide and monosaccharide malabsorption is the result of villus anomalies, villus atrophy and atrophy of the wall of the small intestine, as we found by doing a biopsy (Anderson, 1975; Axton, 1972). We showed these disorders by identifying maltose, isomaltose, lactose and measuring faecal glucose and carrying out a xylose test (Jones et al., 1963).
4. We found an imbalance between potassium and sodium, as a result of diarrhoea on the one hand and of a low supply rate, especially for Na, on the other hand (Sickinger, 1973).
5. Faecal electrolyte excretion rates vary a great deal. The low rates found, especially in our children, prove that supply is low: if it remains low over a long period, this leads to a calcium, magnesium, phosphorus and, mainly,

potassium deficiency. Raised electrolyte excretion rates are the result of a quick passage through the intestine and of intestinal resorption disorders. This pathology mainly affects calcium, magnesium and zinc and the disorder mainly affects children.

6. The quantity and composition of short-chain fatty acids in the faeces depends on the intestinal flora, the quantity of non-reabsorbed sugar and on parasitoses. Research along these lines must be pursued systematically, so as to help us to differentiate this very complex pathology.
7. Measuring alkaline phosphatase isoenzymes in total faeces must be continued, so as to facilitate differentiation between the pathology of the liver and of the bowel (intestine and liver band).

Final conclusions

The comprehensive biochemical study carried out at the rural hospital of Yasa-Bonga in the Kwilu area (Zaïre) shows that malnutrition is present in a fairly serious form in our patients. It affects not only children (70% are aged 3–6 years), but also young mothers who live in poverty.

Its pathology becomes localized in the bowel, the pancreas, the liver, the muscles and the skin; this results in secondary intestinal malabsorption, maldigestion and specific cellular impairments in the liver, the muscles, the bones and the skin. Various infections must also be borne in mind, and this makes the pathology even more complex. Protein deficiency in our patients is apparent in a considerable hypoproteinaemia, hypoalbuminaemia, hypoprealbuminaemia, hypoceruloplasminaemia and hypoaminoacidaemia.

As a result of this protein deficiency, total urinary nitrogen and urea excretion is also lowered. The enzymatic changes in the amino-acid metabolism show in an imbalance between the various amino acids in the blood, i.e. between essential and non-essential amino acids, between phenylalanine and tyrosine, and between valine and glycine. The hydroxyproline index is lowered in all our patients. The creatinine/height index is in line with the reduced weight/thorax relation and arm measurement.

The very low haemoglobin levels are due to the general enteropathy (malabsorption, parasitoses) and to the protein and vitamin (B_6) deficiency.

The raised serum IgA, IgG and IgM rates show infection and enteropathy and enable us to conclude that the B-lymphocyte system does function, but that the function of the T-lymphocyte system is considerably lowered.

Serum electrolyte rates (potassium, sodium, chlorine) are normal. But in some of our patients we found a magnesium, phosphorus and potassium deficiency. The calcium problem is especially striking. The local calcium supply is low and, in addition, we found that some of our patients suffered from high faecal calcium, magnesium and zinc losses (reabsorption disorders, excessively rapid passage through the intestine). The lowered cholesterolaemia rate is the

result of an impairment in the liver and intestine, but the low nutritional supply must also be borne in mind. The same reasoning holds good for total lipids. The atrophy of the pancreas and the alterations of the intestinal mucosae and of intestinal transport cause starch maldigestion, and disaccharide and monosaccharide malabsorption in our patients.

Lactic acid and short-chain fatty acid measurements show the extent of fermentation of non-reabsorbed sugar and amino acids. The xylose load test is very suitable for showing these impairments. Before treatment, when fat supplies are low, we find no steatorrhea. Total faecal alkaline phosphate isoenzymes enable us to show the intestinal band. In these serious cases, secondary intestinal malabsorption may extend to all nutrients and we therefore need to know that these impairments exist, as well as their extent, in order to prescribe the best possible treatment.

The condition of the liver is as important as that of the intestine, especially for assessing the evolution of the illness. Moreover, we found a very characteristic enzymatic activity in the blood. Measuring γ-glutamyl-transpeptidase – an enzyme which is found in the membrane – proved to be a very sensitive way of showing steatosis of the liver. The separation of γ-glutamyltranspeptidase isoenzymes showed a very characteristic dispersion of bands and it is very important for assessing the stage of the illness, from the point of view of the patient's recovery.

The cholinesterase levels (a membrane enzyme synthesized in the liver) give us information on the seriousness of the illness. Our first attempts at separating the alkaline phosphatase isoenzymes in the serum and in the total faeces encouraged us greatly to go on with this research. It is necessary to look out for possible vitamin deficiencies and to make sure that trace-elements supplied are sufficient.

Acknowledgements. We thank Miss A. Lubrano, Miss F. Givel, Mrs. T. Homewood, Mr. J. Schifferle and Mrs. A. Berger of the laboratory of Clinical Chemistry, Nestlé Vevey, as well as Miss A. Nabholz and Mr. R. Besson of the Control Laboratory, Nestlé Vevey, for their valuable technical assistance. Many thanks also to Citizen Mangunza, nurse at the hospital of Yasa-Bonga and his colleagues for their effective help during our stage.

1 Anderson C. M.: Approach to the child with malabsorption. Conférence tenue à la réunion de la Soc. suisse de pédiatrie, Sion (Suisse) 1975. Helv. paediat. Acta Suppl. 35, p. 3–6 (1975).
2 Antener I.: Recent methods for determining amino acids in paediatrics and interpreting the results. Ann. Nestlé, No 20, 1–29 (1969).
3 Antener I., Nordio S., Kaeser M., Gatti R.: Clinical study of the intestinal absorption of amino acids. Determination of amino acids in the stools. Ann. Nestlé, No 28, 17–50 (1972).
4 Armstrong M. D., Stave U.: A study of plasma free amino acid levels. II. Normal values for children and adults. Metabolism *22*, 561–569 (1973).
5 Arroyave G.: Proposed methodology for the biochemical evaluation of protein-calorie malnutrition in children. In: Protein calorie-malnutrition (ed. by A. Von Muralt), p. 48–56. Springer-Verlag, Berlin 1969.

6 Auricchio S., Ciccimarra F., Della Pietra D., Moauro L.: Pathophysiologie der Stärkeverdauung beim Säugling. Ernährungsforschung *13*, 89–100. Akademie-Verlag, Berlin 1968.
7 Axton J. H. M.: Intestinal intolerance to sugars in children. A review. Centr. Afr. J. Med. *18*, 118–121 (1972).
8 Barclay G. P. T., Path M. R.: Pseudocholinesterase activity as a guide to prognosis in malnutrition. Amer. J. clin. Path. *59*, 712–716 (1973).
9 Bel A., Trouyez R., Dechelette P., Lenglet I. P.: Valeurs cliniques de la γ-glutamyltranspeptidase sérique à propos de 920 dosages. In: Colloque international γ-GT organisé par Boehringer Paris, p. 37–47 (1973).
10 Benic V., Fiser-Herman M.: Über alkalische Phosphatasen in menschlichen Faeces. Z. klin. Chem. klin. Biochem. *8*, 458–464 (1970) and *13*, 437–444 (1975).
11 Bhamarapravati N.: The liver in protein-calorie malnutrition. In: Protein-calorie malnutrition (ed. by Robert E. Olson), p. 119–141. Academic press, New York 1975.
12 Burgess H. J. L., Burgess A. P.: The arm circumference as a public health index of protein-calorie malnutrition of early childhood. J. trop. Pediat. *15*, 189–192 (1969).
13 Cabacungan N. B., Miles C. W., Abernathy R. P., Ritchey S. J.: Hydroxyproline excretion and nutritional status of children. Amer. J. clin. Nutr. *26*, 173–176 (1973).
14 Cartwright G. E., Wintrobe M. M.: Copper metabolism in normal subjects. Amer. J. clin. Nutr. *14*, 224–232 (1964).
15 Cotzias G. C., Papavasiliou P. S., Miller S. T.: Manganese in melanin. Nature (Lond.) *201*, 1228–1229 (1964).
16 De Maeyer E. M.: Traitement diététique du kwashiorkor. Ann. Soc. belge Méd. trop. *34*, 139–154 (1954).
17 De Maeyer E. M., Vanderborght H.: A study of different sources in the feeding of african children. J. Nutr. *65*, 335–352 (1958).
18 Doucet G.: Le «Mbuaki» ou maladie de carence observé au Kwango. Rec. Trav. Sci. méd. Congo belge *5*, 261–273 (1946).
19 Dricot C., Beheyt P., Charles O.: Contribution à l'étude de kwashiorkor (buaki du Kwango). Ann. Soc. belge Méd. trop. *31*, 581–630 (1951).
20 Dubois J., Cremer M., Vis H. L.: Etudes des troubles électrolytiques accompagnant le kwashiorkor marastique. Rev. franç. Etud. clin. biol. *13*, 976–983 (1968).
21 Dugdale A. E.: An age-independent anthropometric index of nutritional status. Amer. J. clin. Nutr. *24*, 174–176 (1971).
22 Dupin H.: Les enquêtes nutritionnelles. Editions CNRS, Paris 1969.
23 Ezeilo G. C., Ekeke G.: Serum protein pattern in african neonates. Arch. Dis. Childh. *46*, 310–313 (1971).
24 Flores H., Pak N., Maccioni A., Monckeberg F.: Lipid transport in kwashiorkor. Brit. J. Nutr. *24*, 1005–1011 (1970).
25 Ghazali S., Barratt T. M.: Urinary excretion of calcium and magnesium in children. Arch. Dis. Childh. *49*, 97–101 (1974).
26 Harris I., Wilkinson A. W.: Magnesium depletion in children. Lancet *1971*/II, 735–736.
27 Haury H.: Zur routinemässigen Bestimmung von Hydroxyproline. Z. klin. Chem. klin. Biochem. *10*, 25–28 (1972).
28 Hegner H., Dölle W., Engelhardt A.: Die Bedeutung der γ-Glutamyl-transpeptidase (γ-GT) in der klinischen Diagnostik. Medizinische Universitätsklinik Marburg/L. Aktuelle Diagnostik Boehringer Mannheim, 1–23 (1975).
29 Henning H., v. Braun H. H., Lüders C. J., Probst P.: Gammaglutamyl-transpeptidase in der Diagnostik chronischer Leberkrankheiten. Diagnostik *7*, 732–736 (1974).
30 Ingenbleek Y., Satge P.: Importance théorique et pratique des troubles du métabolisme du potassium et du magnésium dans le kwashiorkor. Bull. Soc. méd. Afr. noire Langue franç. *13*, 895–904 (1968).
31 ICNND Report: Nutrition survey in Thailand (1962).

32 Jelliffe D. B.: The assessment of the nutritional status of the community. World Health Organization, Genève 1966.
33 Jones W. O., di Sant'Agnese P. A.: Laboratory aids in the diagnosis of malabsorption in pediatrics. J. Pediat. *62*, 50–56 (1963).
34 Kader A. M., Rahman A. M.: Studies on the biochemical characteristics of malnutrition in Pakistani infants and children. Brit. J. Nutr. *28*, 191–200 (1972).
35 Kahn E.: Prognostic criteria of severe protein malnutrition. Amer. J. clin. Nutr. *7*, 161–165 (1959).
36 Kendall A. C.: Rickets in the Tropics and Sub-Tropics. Centr. Afr. J. Med. *18*, 47–49 (1972).
37 Kudchodkar B. J., Sodhi H. S., Horlick L.: Absorption of dietary cholesterol in man. Metabolism *22*, 155–163 (1973).
38 Linder G. C., Hansen J. D. L., Karabus C. D.: The metabolism of magnesium and other inorganic cations and of nitrogen in acute kwashiorkor. Pediatrics *31*, 552–568 (1963).
39 Mann M. D., Bowie M. D., Hansen J. D. L.: Total body potassium and serum electrolytes concentrations in protein energy malnutrition. Sth. Afr. med. J. *49*, 76–78 (1975).
40 Mauron J.: Conférence à Kinshasa le 11 novembre 1975 au UNAZA.
41 Mayr K.: Die γ-Glutamyltranspeptidase in der klinischen Diagnose. Wien. klin. Wschr. *85*, 83–87 (1973).
42 McFarlane H.: Immunoglobulins in populations of subtropical countries. In: Advanc. in clin. Chem. *16*, 154–238 (ed. by O. Bodansky and A. L. Later). Academic Press, New York 1973.
43 McLaren D. S., Loshkajian H., Kanawati A. A.: Urinary creatinine and hydroxyproline in relation to childhood malnutrition. Brit. J. Nutr. *24*, 641–651 (1970).
44 Metcoff J.: Biochemical effects of protein-calorie malnutrition in man. Ann. Rev. Med. *18*, 377–422 (1967).
45 Montgomery R. D.: Magnesium balance studies in marasmic kwashiorkor. J. Pediat. *59*, 119–123 (1961).
46 Nelson W. E.: Growth and development in the infant and the child. In: Textbook of pediatrics. W. B. Saunders Company, Philadelphia/London 1964.
47 Neumann C. G., Lawlor G. J., Stiehm E. R., Swendseid M. E., Newton C., Herbert J., Amman A. J., Jacob M.: Immunologic responses in malnourished children. Amer. J. clin. Nutr. *28*, 89–104 (1975).
48 Nordio S., Antener I., Gatti R., Dentan E.: The molecular conception of rickets pathogenesis. Med. exp. (Basel) *18*, 211 (1968).
49 Nusgens B., Lapiere M.: The relationship between proline and hydroxyproline urinary excretion in human as an Index of collagen catabolism. Clin. chim. Acta *48*, 203–211 (1973).
50 O'Brien D., Ibott F. A., Rodgerson D. O.: Laboratory manual of pediatric microbiochemical techniques, p. 283. Hoerber Medical Division, Harper & Row Publishers, New York/Evanston/London 1968.
51 Patel S., O'Gorman P.: Demonstration of serum Gamma-Glutamyltranspeptidase. Isoenzymes using cellogel electrophoresis. Clin. chim. Acta *49*, 11–17 (1973).
52 Paunier L., Borgeaud M., Wyss M.: Urinary excretion of magnesium and calcium in normal children. Helv. paediat. Acta *25*, 577–584 (1970).
53 Pereira S. M., Begum A.: The manifestations and management of severe protein-calorie malnutrition. Wrld Rev. Nutr. Diet. *19*, 1–50 (1974).
54 Pieraerts G.: Etude sur le syndrome dépigmentation-œdeme au Kasai. II. A. Nature du syndrome. Bull. Soc. Path. exot. *39*, 226–235 (1946).
55 Rosen E. U., Campbell P. G., Moosa G. M.: Hypomagnesemia and magnesium therapy in protein-calorie malnutrition. J. Pediat. *77*, 709–714 (1970).
56 Sickinger K.: Quantitative Stuhlanalyse. Med. Klin. *68*, 1243–1247 (1973).
57 Smith R., Waterlow J. C.: Total exchangeable potassium in infantile malnutrition. Lancet *1960/I*, 147–149.
58 Smith F. R., DeWitt Goodman S., Zaklama M. S., Gabr M. K., El Maraghy S., Patwardhan V.

N.: Serum vitamin A, retinol-binding protein, and prealbumin concentrations in protein-calorie malnutrition. Amer. J. clin. Nutr. *26*, 973–981 (1973) and *28*, 732–738 (1975).
59 Southgate D. A. T.: Creatinine-height index in malnourished children. Nutr. Rev. *29*, 134–137 (1971).
60 Taylor G. O.: Serum triglycerides and fatty acids in kwashiorkor. Amer. J. clin. Nutr. *24*, 1212–1215 (1971).
61 Thanangkul O.: Water soluble vitamins in protein-calorie malnutrition. In: Protein-calorie malnutrition (ed. by R. E. Olson), p. 149–161. Academic Press, New York/San Francisco/London 1975.
62 Trolli C.: Résumé des observations réunis au Kwango au sujet de deux affections d'origine indéterminée. Brussels 1938.
63 Truswell A. S.: Carbohydrate and lipid metabolism in protein calorie malnutrition. In: Protein-calorie malnutrition (ed. by R. E. Olson), p. 119–141. Academic Press, New York/San Francisco/London 1975.
64 Van Daele G.: Sur une affection de carence et de déséquilibre diététique observé au Congo (Buaki des indigènes). Ann. Soc. belge Méd. trop. *18*, 653–669 (1938).
65 Vasantha L., Skrikantia S. G.: Biochemical changes in the skin in kwashiorkor. Amer. J. clin. Nutr. *23*, 78–82 (1970).
66 Verwilghen A. M.: Contribution à l'étude de l'ulcère gastro-duodénal au Kwango. Ann. Soc. belge Méd. trop. *37*, 757–772 (1957).
67 Vis H., Dubois R., Vanderborght H., de Maeyer E.: Etudes des troubles électrolytiques accompagnant le kwashiorkor marastique. Rev. franç. Etud. clin. biol. *10*, 729–741 (1965).
68 Vis H. L.: General and specific metabolic patterns of marasmic kwashiorkor in the Kiwu area. In: Calorie deficiencies and protein deficiencies (ed. by R. A. McLane and E. M. Widdonsen), p. 119–134. J. & A. Churchill Ltd., London 1968.
69 Viteri F. E., Alvarado J.: The creatinine height index: Its use in the estimation of the degree of protein depletion and repletion in protein calorie malnourished children. Pediatrics *46*, 696–706 (1970).
70 Waterlow J. C., Cravioto J., Stephen Joan M. L.: Protein malnutrition in Man. In: Advances in protein chemistry (ed. by C. B. Anfinsen and M. L. Anson), 15. 131–238. Academic Press, New York/London 1960.
71 Waterlow J. C., Stephen J. M. L.: Enzymes and the assessment of protein nutrition. Symp. Proc. *28*, 234–242 (1969).
72 Weber H.: Die Bedeutung der Leucin-Aminopeptidase. Dtsch. med. Wschr. *94*, 181–184 (1969).
73 Whitehead R. G., Dean R. F. A.: Serum amino acids in kwashiorkor. Amer. J. clin. Nutr. *14*, 313–319 (1964).
74 Whitehead R. G.: Hydroxyproline creatinine ratio as an index of nutritional status and rate of growth. Lancet *1965*/II, 567–570.
75 Whitehead R. G., Coward W. A., P. G.: Serum-albumin concentration and the onset of kwashiorkor. Lancet *1973*/I, 63–66.
76 Willenbockel U.: Zur Physiologie und Pathologie des Phosphatstoffwechsels. Beihefte zum Archiv für Kinderheilkunde (ed. by O. Vivell and W. Burmeiser) *60*, 1–110 (1969).