Congenital β-Lipoprotein Deficiency*

F. S. P. VAN BUCHEM, M.D., G. POL, PH.D., J. DE GIER, PH.D.,
Groningen *Wageningen* *Utrecht*
C. J. F. BÖTTCHER, PH.D. *and* C. PRIES, PH.D.
Leiden

BASSEN and Kornzweig [*1*] in 1950 called attention to a syndrome characterized by neuromuscular disturbances simulating Friedreich's ataxia, atypical retinitis pigmentosa and the presence of thorny-appearing erythrocytes. This syndrome was observed in an eighteen year old girl and her nine year old brother. Singer et al. [*2*] described a similar patient (thirteen years of age) and called the malformed erythrocytes acanthrocytes (*acantha* is the Greek name for thorn), a name which was later changed to acanthocytes for etymological reasons. In the patient described by Singer, Jampel and Falls [*3*] later found a serum cholesterol content of 37 mg. per cent. Salt et al. [*4*] were the first to observe in such a case a marked decline in the plasma β-lipoprotein content. There are at least thirteen additional reports of such cases [*5–17*]. In all these reports the presence of steatorrhoea during the first years of life is mentioned, in particular bulky stools with a high fat content observed microscopically and/or chemically. As far as could be discerned from the examinations carried out, the patients suffered from a disturbance in absorption of I^{131}-triolein, carotene and vitamin A [*6,8–10,15*]. Glucose absorption was normal and xylose absorption usually was normal. The duodenal juice contained normal quantities of bile acids and normal lipolytic and tryptic activity [*4,15*], but the cholesterol content was reduced [*6*]. The jejunal biopsy specimen [*4,7,10,11,15,18*] consistently revealed well developed villi with normal thickness of mucosa and lamina propria, but the epithelial cells showed vacuolization and contained much fat [*11,18*]. Electromicroscopically the mitochondria and endoplasmic reticulum of the jejunal epithelium were normal. No lactase activity was found in the mucosa and lactose absorption was disturbed [*18*]. The findings in the jejunum, therefore, differ substantially from those in non-tropical sprue. In the course of years the stools may become more normal, but in the cases cited the absorption coefficient remained low. A gluten-free diet has no influence on the steatorrhoea.

Sobrevilla et al. [*17*] described a thirty-six year old woman who had no diarrhoea but whose fat absorption was not determined. Autopsy did not reveal any abnormalities of the small intestine.

Neuromuscular Disturbances. Neuromuscular disturbances were absent only in the four year old child described by Salt et al. [*4*], but they developed between the second and seventeenth years of life; in most patients they are manifest before the age of ten. In almost all patients described there was ataxia of the trunk and extremities, the tendon reflexes were absent, and muscular weakness and disturbed sensibility were often present. Electric stimulation did not yield any indications of denervation of the muscles. Muscle biopsy disclosed few abnormalities. Schwartz et al. [*15*] performed two nerve biopsies; on one occasion no abnormalities were found, and on the other, demyelinization was revealed, most fibres being devoid of myelin. Nevertheless, Schwartz et al. did not consider a general involvement of myelin to be present.

The neuromuscular disturbances are progressive and disability may develop within a few years. Up to the present the results of only

* From the Department of Internal Medicine, State University of Groningen, Groningen, The Netherlands Institute of Nutrition, Wageningen, the Laboratory of Organic Chemistry, Department of Biochemistry, State University of Utrecht, Utrecht and the Gaubius Institute, State University of Leiden, Leiden, The Netherlands. This study was made possible by funds supplied by The Netherlands Organization for Nutrition and Food Research T.N.O. and U. S. Public Health Service Grant H 5471. Manuscript received September 16, 1965.

Reprint requests should be addressed to Dr. van Buchem, Duinroosplantsoen 15, Haarlem, The Netherlands.

one autopsy have been recorded [*17*], in which were found demyelinating lesions of the anterior columns, spinocerebellar tracks and cerebellum with loss of anterior horn and cerebellar nuclei. Sections of peripheral nerve showed focal areas of demyelinization.

Retinal Abnormalities. Retinal abnormalities have been observed in many patients, especially in those of more advanced age, but sometimes also in younger patients, for example, in the patient described by Schwartz et al. [*15*] who was nine years old. Frequently, the first finding is reduced vision; Lamy and co-workers [*11*] found initial electroretinographic deviations (scotopic impairment). The retinal affections have been described as (atypical) retinitis pigmentosa, retinitis punctata albescens and myopic choroiditis. The patient described by Bassen became blind at the age of thirty-four.

Fat Metabolism. In all patients examined β-lipoprotein has been absent in the plasma or present in only trace amounts; both the low-density and the very low-density lipoproteins were deficient. This deficiency associated with a decrease in the plasma total lipids, free cholesterol and cholesterol esters (total cholesterol, 25 to 86 mg. per cent), triglycerides, phospholipids and free fatty acids, especially linoleic acid and arachidonic acid [*18*]. In respect to the phospholipids, this is true for lecithin and sphingomyelin, as well as cephalin, but proportionately the reduction in lecithin is greater than that in sphingomyelin, i.e., there is a lower lecithin-sphingomyelin ratio. In contrast to the plasma, the cholesterol and phospholipid contents in the erythrocytes change but little, although the phospholipids show a decrease in lecithins and an increase in sphingomyelin [*9,13,19*], with the exception of the case reported by Schwartz. This shift in the phospholipid distribution was present not only in the younger erythrocytes and reticulocytes, but also in the older erythrocytes [*19*]. The linoleic acid content was decreased in plasma, acanthocytes and normally shaped erythrocytes [*19*].

Isselbacher et al. [*18*] found a decrease in linoleic acid also in the fat of the jejunal mucosa, the liver and the subcutaneous adipose tissue.

In three patients [*4,9,12*] the α-lipoprotein content of the serum also was reduced.

Acanthocytosis. In all typical cases acanthocytosis has been found in the peripheral blood, but not in the bone marrow. This abnormal appearance of the red blood cells remains unchanged on incubation in normal serum at 37°C. and after repeated washings with isotonic saline solution [*2*]. The shape of normal erythrocytes does not change on incubation in the serum of these patients. The osmotic fragility is somewhat decreased or normal, but the mechanical resistance is reduced and the lysolecithin fragility markedly increased [*2*]. The red blood cells proved to be more rapidly permeable for haemoglobin during incubation at 37°C. and 4°C. for 48 hours than erythrocytes of normal subjects, and this was particularly true for the older erythrocytes. This autohaemolysis could be inhibited by the addition of normal serum and with separated low-density and high-density lipoproteins [*20,21*]. The life span of the erythrocytes sometimes was found to be normal [*15*], sometimes shortened [*5,8*]. Two patients showed increased haemolysis (hyperbilirubinaemia, reticulocytosis) [*5,11*]. The reaction to the direct Coombs' test, determined in six patients, was found to be negative [*1,2,5,8,11,12*]. Mild anaemia was often observed. The serum vitamin B_{12} content was normal [*4,16,18*] and results of the Schilling test revealed no abnormalities [*16,18*]. The acanthocytosis does not depend on the lowered lecithin-sphingomyelin ratio of the erythrocytes, as this was not found in a patient with acanthocytosis [*15*], nor on the low linoleic acid content, as this is also found without acanthocytosis, e.g. in the malabsorption syndrome [*19*] and in haemolytic anaemias [*22,23*]. The acanthocytes assumed a normal shape after intravenous injection of cottonseed oil emulsion for thirty-nine days [*24*] and after the injection of Tween® 80 [*25*].

Acanthocytosis is said not to be pathognomonic for β-lipoprotein deficiency, as Isselbacher et al. [*18*] found this condition also in alcoholism with liver cirrhosis and anaemia, and in anaemia with pyruvate kinase deficiency of the red blood cells.

The leucocytes and thrombocytes have been found to be normal except in the patient described by Stöcker [*16*] who had thrombocytopenia but no haemorrhagic diathesis.

This survey of the literature implies a syndrome with rather sharply circumscribed clinical and biochemical characteristics. The present report is intended to call attention to cases in which this syndrome is only partially manifest, a point of importance both from the clinical point of view and for the understanding of the pathogenesis.

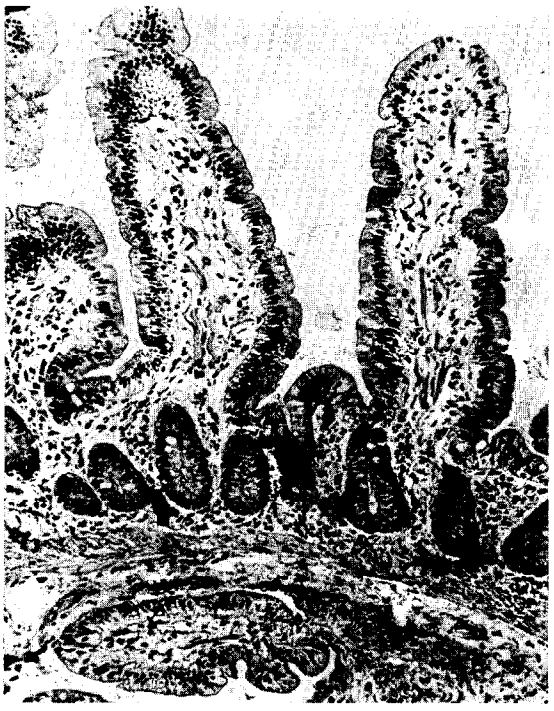

FIG. 1. Jejunal biopsy specimen. There is no fat accumulation in the epithelium. Original magnification × 200.

CASE REPORT

In a forty-six year old man (patient A) who was entirely symptom-free, a remarkably low serum cholesterol level (70 to 80 mg. per cent) was found several times during a mass screening program under the supervision of one of us (van Buchem [*26,27*]). Nine years earlier the patient had been treated for lymph node swellings (neck and axillas). The reaction to the Mantoux test was negative and lymph node biopsy revealed abnormalities that were not characteristic but which might fit into the picture of Besnier-Boeck's disease. After this episode the patient had never been ill, although occasionally his skin had appeared somewhat yellow. A few small hard lymph nodes were still palpable in the axillas; they were not painful to pressure and did not adhere to the adjacent parts and the skin. On general examination the only further finding was an enlarged spleen, which reached one fingerbreadth under the costal arch. The patient's height was 176 cm. and his weight 75.5 kg. The stools were always normal and contained only a few fatty acid needles and a trace of fat; the fat absorption coefficient was 95 to 95.5 per cent. Results of the xylose test were normal (excretion in 5 hours was 4.8 gm. of xylose); the blood sugar curve did not demonstrate any abnormalities after loading with 50 gm. of glucose (fasting, 102 mg. per cent; after 30 minutes, 155 mg. per cent; after 60 minutes, 163 mg. per cent; after 90 minutes, 112 mg. per cent; after 120 minutes, 105 mg. per cent; after 150 minutes, 85 mg. per cent). There was no neuropathy (for example, the tendon reflexes were normal; Romberg's test and the finger-nose and heel-knee test were within normal limits; there was no dysdiadochokinesis or nystagmus; speech and gait were normal). Vision was normal after correction (myopic astigmatism) and vision in twilight was normal. Very fine shining dots were seen in both fundi and also in the walls of the veins and arterioles (observed by Dr. A. C. Copper). Four years previously, when the patient was forty-two years old, there were no abnormalities in the fundus oculi. Jejunal biopsy revealed no changes (Fig. 1); the content of disaccharidases in the jejunal wall was normal. Liver biopsy revealed a mild degree of steatosis but no indications of Besnier-Boeck's disease. (Fig. 2.)

Paper electrophoresis showed a marked reduction of the β-lipoproteins. (Fig. 3.) A normal α_1-lipoprotein content was found by immunoelectrophoresis. The α_2- and β-lipoproteins cannot be separated by these methods, as both possess the same antigen determinants. In the β-lipoprotein fraction there was a marked reduction of α_2- and β-lipoproteins, both with anti-α_2 and anti-β-lipoprotein (determined by L. Ruinen). On immunoelectrophoresis and immunodiffusion, serum from normal men of the same age as the patient reacted at a dilution of 1:32 whereas the patient's serum reacted only at a dilution of 1:2 (determined by Dr. F. Peetoom). The peripheral blood showed no acanthocytosis, haemoglobin was 13.3 to 14 gm., erythrocytes 3,940,000 per cu. mm., leucocytes 5,000 per cu. mm. with normal distribution, thrombocytes 276,000 per cu. mm., reticulocytes 4.4 to 11.8 per cent, haematocrit 40 per cent, mean corpuscular volume 104 cu. μ, mean corpuscular haemoglobin 36 μμg., mean corpuscular haemoglobin concentration 34 per cent, diameter of erythrocytes 7.3 μ, serum bilirubin (direct) 0.5 units and (indirect) 2.3 units, alkaline phosphatase 10.7 King-Armstrong units, thymol turbidity 0.4 units, erythrocyte sedimentation after 1 hour 2 mm., serum total proteins 8.05 per cent, albumin 69.5 per cent, α_1-globulin 3.1 per cent, α_2-globulin 6.2 per cent, β-globulin 7.4 per cent, γ-globulin 13.8 per cent, serum glutamic oxaloacetic transaminase 32 units, serum glutamic pyruvic transaminase 18 units, lactic dehydrogenase 240 units, fraction I 63 per cent, fraction II 29.6 per cent, fraction III 7.4 per cent. Serum iron was 100 μg. per 100 ml., copper 100 μg. per 100 ml., calcium 10.5 mg. per 100 ml., protein-bound iodine 6.6 μg. per 100 ml., vitamin A 230 I.U. per 100 ml. and carotene 20 μg. per 100 ml. The blood group was A, rhesus positive; the reactions to the tests for syphilis were negative.

Beginning haemolysis occurred at 0.46 per cent, complete haemolysis at 0.38 per cent; haptoglobin content was decreased; results of the direct Coombs'

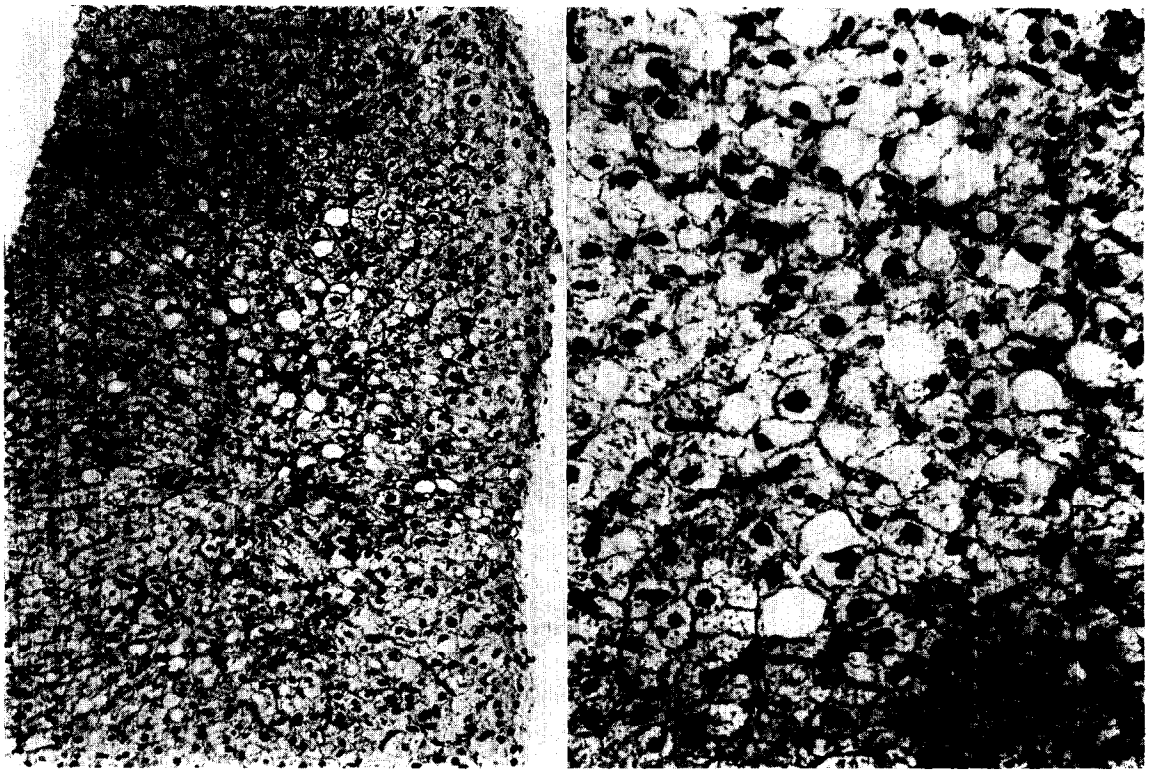

FIG. 2. Liver biopsy specimen showing mild steatosis. *Left*, original magnification × 200. *Right*, original magnification × 500.

test were positive with antigammaglobulin serum, and negative with anticomplement serum; reactions to the tests for warm and cold haemolysis were negative, weak incomplete autoantibodies against erythrocytes were demonstrable (bromalin method). The glucose-6-phosphate dehydrogenase content of the erythrocytes was normal. The erythrocyte apparent half survival time (Cr^{51}) was ten days (normal, about thirty days). The spleen-liver index for Cr^{51} rose in ten days from 2.32 to 4.19; the spleen-heart index from 1.30 to 3.78 and the liver-heart index from 0.56 to 0.88. The Coombs' consumption test with leucocytes, thrombocytes and nuclei gave negative results.

Examination of the urine gave the following results: protein negative, glucose negative, urobilin positive, sediment normal, 17-ketosteroids 18.3 mg. and 17-hydroxysteroids 7 mg. per 24 hours. During two successive days of ACTH infusion (75 units) urinary excretion was, on the first day, 15.1 mg. 17-ketosteroids and 10.7 mg. 17-hydroxysteroids, on the second day, 24.9 mg. 17-ketosteroids and 16.8 mg. 17-hydroxysteroids. The urine contained increased amounts of 4 amino-5 imidazol carboxamide(aica) (determined by Dr. S. Wadman).

Iliac crest biopsy yielded spongy bone with many erythrocytes and slightly fatty bone marrow, rich in cells, with active production of all elements; a cell-

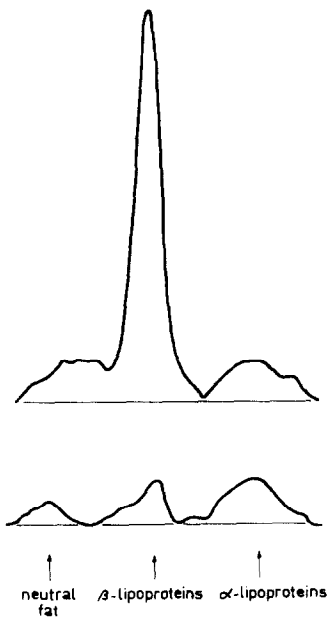

FIG. 3. Serum lipoproteins. *Top*, paper electrophoresis of pooled serum of seven normal subjects. *Bottom*, paper electrophoresis of serum of patient A showing marked decrease in β-lipoprotein.

TABLE I
RESULTS OF TESTS IN PATIENT A AND HIS FAMILY

Data	Salt et al. [4] Father	Salt et al. [4] Mother	Kuo and Basset [14] Brother	Kuo and Basset [14] Sister	Kuo and Basset [14] Sister	Patient A	Subject B	Subject C	Subject D	Son of Patient A (19 yr.)	Daughter of Patient A (13 yr.)
Acanthocytosis	−	−	+	+	+	−	−	−	−	−	−
Neuromuscular signs	−	−	+	−	−	−	−	−	−	−	−
Retinopathy	−	−	−	−	−	+	−	−	−	−	−
Steatorrhea	−	−	−	−	−	−	−	−	−	−	−
α-Lipoprotein	Normal	Normal	↓	↓	↓	Normal	Normal	Normal	Normal	Normal	Normal
β-Lipoprotein (%)	±50	±50	↓	↓	↓	±10	±24	↓	...	100	100
β-Lipoprotein-α-lipoprotein ratio	0.7	1.0	...	...	...	0.6	1	1.6	...	...	...
Plasma cholesterol (mg./100 ml.)	107	127	...	...	...	76	109	126	251	171	201
Phospholipids (mg./100 ml. plasma)	139	142	...	...	...	150	131	165	320	190	235
Triglycerides (mg./100 ml. plasma)	...	...	...	...	...	29	32	44	258	77	93
Free fatty acids (mg./100 ml. plasma)	...	...	...	...	...	9	13	11	12	...	...
Total lipids (mg./100 ml. plasma)	460	460	...	...	...	302	340	410	967	583	688

NOTE: + = present, − = absent, ↓ = decreased.

rich bone marrow with enhanced erythropoiesis. Roentgenograms of thoracolumbal vertebrae, skull and pelvis showed a normal bone structure.

Results of the Pirquet test and the Mantoux test with bovine and human purified protein derivative were negative.

A roentgenogram of the thorax revealed extensive fibrosis, in both upper fields with prominent hilar markings.

The results of the analysis of the lipids in plasma and red blood cells are given in Tables I to X.

METHODS

The chemical analyses were carried out in various institutions. Fat extraction of plasma was performed by the method of Folch et al. [*28*], or by a modification of this method [*29*]; the erythrocytes were extracted by the method of Reed et al. [*30*]. Cholesterol was determined by the Liebermann-Burchard reaction in various modifications: in Wageningen according to Anderson and Keys [*31*], in Utrecht according to Abell et al. [*32*] and in Leiden according to Sperry and Webb [*33*]. The triglyceride content was determined according to Carlson [*34*] in Wageningen and according to van Handel and Zilversmit [*35*] in Leiden. In Wageningen the method of Dole [*36*] was used for the fatty acid determination, in Leiden the method of Pries and Böttcher was employed [*37*]. The content of phospholipids was determined gravimetrically after isolation by means of silica gel column chromatography. If isolation was not necessary, the phosphorus content of lipoid was determined according to Böttcher et al. [*38*] and then the phospholipid content was calculated from this, assuming that the average phosphorus content of the fraction was 4.0 per cent. Plasma phospholipids were estimated by a combination of silica gel column chromatography and stepwise hydrolysis according to Pries et al. [*39*]. The phospholipids of the red blood cells were separated by means of thin layer chromatography, and then phosphorus determinations in the individual patches gave the distribution according to type.

The nonsaponified fat extracts were fractionated by

TABLE II
COMPOSITION OF PLASMA LIPIDS
(PER CENT OF TOTAL LIPIDS)

Data	Pool Group*	Patient A	Subject B	Subject C	Subject D
Phospholipids	36.8	49.9	38.5	40.3	33.1
Cholesterol (free)	8.2	7.2	8.7	8.5	7.3
Cholesterol esters	37.0	30.4	39.7	37.8	31.7
Triglycerides	15.6	9.7	9.3	10.7	26.7
Free fatty acids	2.4	2.8	3.8	2.7	1.2

TABLE III
COMPOSITION OF PLASMA PHOSPHOLIPID
(PER CENT OF TOTAL PHOSPHOLIPIDS)

Data	Pool Group	Patient A	Subject B	Subject C	Subject D
Phosphatidic acid	0.7	...	0.4	1.3	...
Cephalin*	1.5	3.3	3.9	1.6	6.3
Lysocephalin*	1.8	...	8.7	2.3	...
Plasmalogens	2.3	2.5	1.1	2.8	2.3
Lecithin	68.3	57.4	53.1	51.5	62.1
Lysolecithin	3.1	2.8	5.2	3.1	6.4
Cerebrosides	2.9	2.6	3.6	2.7	2.5
Sphingomyelin	17.1	25.8	21.3	27.7	20.4
Unknown phospholipids	1.9	5.6	2.6	7.0	...

*The sum of phosphatidylethanolamines, phosphatidylserines and inositides.

TABLE IV
PRINCIPAL FATTY ACIDS OF CHOLESTEROL ESTERS, GLYCERIDES AND PHOSPHOLIPIDS IN SERUM

Chain Length and Double Bonds	Cholesterol Esters mg./100 ml.	Cholesterol Esters % Total Fatty Acids	Triglycerides mg./100 ml.	Triglycerides % Total Fatty Acids	Phospholipids mg./100 ml.	Phospholipids % Total Fatty Acids
			Patient A			
C-14	0.56	1.2	1.39	3.4	0.44	0.5
C-16	9.59	20.4	11.48	28.0	38.28	43.5
C-16:1	2.26	4.8	1.80	4.4	2.29	2.6
C-18	0.85	1.8	3.32	8.1	12.67	14.4
C-18:1	13.63	29.0	17.10	41.7	14.08	16.0
C-18:2	19.04	40.5	3.49	8.5	12.85	14.6
C-20:4	0.71	1.5	...	...	3.61	4.1
>22	...	...	...	...	1.06	1.2
			Pool Group			
C-14	1.11	1.0	2.31	2.2	1.04	0.6
C-16	12.43	11.2	28.56	27.2	72.73	41.8
C-16:1	4.77	4.3	7.14	6.8	4.18	2.4
C-18	1.00	0.9	5.04	4.8	30.45	17.5
C-18:1	23.09	20.8	40.32	38.4	24.53	14.1
C-18:2	60.05	54.1	11.76	11.2	20.71	11.9
C-20:4	4.55	4.1	5.25	5.0	6.44	3.7
>22	0.78	0.7	0.63	0.6	8.00	4.6

column chromatography according to the method of Barron and Hanahan [*40*]. Cholesterol analyses were made on the sterol ester fraction and on the triglyceride-free cholesterol fraction. Both fractions were saponified, methylated and analysed by gas chromatography for fatty acids according to the method of Böttcher et al. [*41*]; however, a succinate column was used instead of an adipate column. The phospholipid fractions were re-esterified with methanol hydrochloride after which the fatty acids were also determined.

The plasma of patient A was found to have a

TABLE V
ERYTHROCYTE LIPIDS (PER CENT OF TOTAL LIPIDS)

Data	Patient A	Subject B	Normal Subjects
Cholesterol	23.0	24.1	22.7 (22–24.5) [*10,30,42*]
Lipoid phosphorus	2.1	2.3	2.2 (2.1–2.4) [*10,30,42*]
Triglycerides	3.2	3.1	2.5 (2.5–3.1) [*42*]
Total fatty acids	42.1	44.0	45.1
Cholesterol lipoid phosphorus	10.9	10.4	10.2 (9.8–10.8) [*10,30,42*]

NOTE: Numbers in parentheses represent ranges.

TABLE VI
PLASMA FATTY ACIDS (PER CENT OF TOTAL FATTY ACIDS)

Chain Length and Double Bonds	Cholesterol Esters Pool Group	Cholesterol Esters Subject B	Cholesterol Esters Subject C	Cholesterol Esters Subject D	Triglycerides Pool Group	Triglycerides Subject B	Triglycerides Subject C	Triglycerides Subject D	Free Fatty Acids Pool Group	Free Fatty Acids Subject B	Free Fatty Acids Subject C	Free Fatty Acids Subject D
C-12:0	0.1	0.1	0.1	0.1	0.5	0.1	0.1	0.4	0.3	0.3	0.5	0.3
C-14:0	1.1	0.6	0.6	1.0	3.1	1.6	1.4	3.7	2.7	2.6	2.5	2.7
C-16:0	12.0	11.5	12.0	11.9	25.3	21.7	23.9	35.7	23.7	23.5	21.8	24.6
C-16:1	4.6	2.8	3.5	6.2	4.8	4.0	4.5	6.5	4.3	4.1	4.6	4.7
C-18:0	1.5	2.9	1.6	1.3	4.1	6.9	5.9	6.8	11.2	10.0	10.3	10.4
C-18:1	21.1	20.9	22.4	24.4	37.8	44.8	44.7	35.0	39.3	43.7	42.0	43.4
C-18:2	49.8	50.8	49.0	42.5	17.3	11.4	12.4	7.3	9.9	7.9	9.5	6.0
C-18:3	0.3	...	0.3	2.5	0.5	0.7	...	...	0.3	0.4	...	...
C-20:3	0.9	0.9	0.6	0.6	0.6	0.7	0.3	0.1	0.4	...	0.4	0.6
C-20:4	5.4	6.0	6.4	6.4	1.0	1.2	1.3	0.9	0.6	0.3	0.8	0.6

TABLE VII
SERUM LIPID (MG. PER 100 ML. SERUM)

Data	Pool Group	Patient A: Admission	Patient A: Before Diet*	Patient A: 2 wk. After Diet*	Patient A: 4 wk. After Diet*
Total lipids	672.0	327.0	336.0	372.0	385.0
Total cholesterol	192.0	81.0	80.0	82.0	84.0
Esterified cholesterol	146.0	51.0	51	54.0	59.0
Lipoid phosphorus	...	5.4	4.7	5.2	5.4
Phospholipids	245.0	127.0	...	...	...
Triglycerides	116.0	43.0	37.9	36.5	34.4
Fatty Acids (% of total fatty acids)					
Sterol Ester					
C-16:0	11.2	20.4	12.4	12.4	12.2
C-16:1	4.3	4.8	3.2	3.1	2.5
C-18:0	0.9	0.9	1.3	1.2	1.9
C-18:1	20.8	29.0	26.5	15.7	14.8
C-18:2	54.1	40.5	43.4	57.3	58.1
C-20:4	4.1	1.5	8.5	6.9	6.7
Triglycerides					
C-16:0	27.2	28.0	20.1	20.4	19.5
C-16:1	6.8	4.4	3.8	4.3	4.2
C-18:0	4.8	8.1	6.7	19.4	14.3
C-18:1	38.4	41.7	51.5	31.0	31.8
C-18:2	11.2	8.5	12.5	16.5	23.1
C-20:4	5.0	...	1.1	1.1	1.0
Phospholipids					
C-16:0	41.8	43.5	32.1	26.3	29.2
C-16:1	2.4	2.6	2.0	1.8	1.5
C-18:0	17.5	14.4	12.8	15.3	15.3
C-18:1	14.1	16.0	16.5	12.4	11.0
C-18:2	11.9	14.5	17.6	19.9	21.6
C-20:4	3.7	4.1	9.5	10.0	7.0

* Diet contained only fat with 50 per cent linoleic acid.

markedly reduced content of total lipids, cholesterol, cholesterol esters, phospholipids and especially triglycerides. (Tables I and II.) The lecithin-sphingomyelin ratio was decreased. (Table III.) The plasmologen percentage of the phospholipids of patient A was the same as that found for the control group (pool). (Table III.) The linoleic acid content (C-18:2) and the arachidonic acid content (C-20:4) of the cholesterol esters, the triglycerides and the phospholipids was markedly reduced, but proportionately the greatest reduction was in the cholesterol esters and triglycerides. (Table IV.) The erythrocytes (Table V) of patient A had a normal cholesterol and phospholipid content, as compared with the values found by Hill et al. [*12*], Reed et al. [*30*] and Ways et al. [*10*], but also a decreased lecithin-sphingomyelin ratio (Table X) and linoleic and arachidonic acid content. (Table IX.)

Patient A had, therefore, in addition to the biochemical phenomena of β-lipoprotein deficiency, a

TABLE VIII
LIPID COMPOSITION OF ERYTHROCYTES (MG. PER 100 ML. PACKED CELLS)

Data	Patient A: Before Diet*	Patient A: 2 wk. After Diet*	Patient A: 4 wk. After Diet*	Normal Subjects
Cholesterol	125	119	129	123
Phospholipids	322	291	311	311
Cholesterol-phospholipid ratio	0.39	0.41	0.38	0.40

* Diet contained only fat with fatty acids comprising 35 per cent of linoleic acid.

compensated increased haemolysis with shortened life span of the erythrocytes, as found in only a few patients with the Bassen-Kornzweig syndrome [*5,11*]. Remarkably enough, in our patient the reaction to the direct Coombs' test was positive with anti γ-globulin serum, a reaction always found to be negative in other similar patients.

We have had the opportunity to examine the two children (a nineteen year old boy and a thirteen year old girl) and three brothers of patient A; examination was not possible for his two sisters. The children and one brother (subject D) had no β-lipoprotein deficiency and did not present any abnormalities. The haptoglobin content of the son was decreased; he had no increased haemolysis. The brothers (subjects B and C), thirty-eight and forty-nine years old, respectively, also had a decreased β-lipoprotein content, but to a lesser degree than patient A. (Fig. 4.) With immunoelectrophoresis and immunodiffusion, the serum of subject B reacted with a 1:8 dilution, whereas the serum of three normal men of the same

TABLE IX
ERYTHROCYTE LIPIDS (MG. PER 100 ML. BLOOD)

Data	Patient A: Admission	Patient A: Before Diet*	Patient A: 2 wk. After Diet*	Patient A: 4 wk. After Diet*	Pool Group
Total lipids	237.0	215.5	231.0	198.5	215.0
Total cholesterol	54.6	50.1	55.7	49.6	48.8
Lipoid phosphorus	5.0	4.5	5.1	4.4	4.8
Cholesterol (% of total lipids)	23.0	23.2	24.1	25.0	22.7
Lipoid phosphorus (% of total lipids)	2.1	2.1	2.2	2.2	2.2
Fatty Acids (Phospholipids)					
C-16:0	20.8	22.9	17.9	20.9	16.7
C-16:1	1.1	1.6	0.9	0.9	1.6
C-18:0	18.4	20.4	16.7	13.9	14.9
C-18:1	16.1	19.1	14.2	12.6	17.7
C-18:2	8.7	7.6	10.0	9.5	12.1
C-20:4	12.9	10.8	13.0	14.7	15.0

* Diet contained only fat with 50 per cent linoleic acid.

TABLE X
ERYTHROCYTE DISTRIBUTION OF PHOSPHOLIPIDS (PER CENT)

Data	Patient A			Normal Subjects
	Before Diet*	2 wk. After Diet*	4 wk. After Diet*	
Lysolecithin	3.5	6.0	5.8	5.6
Sphingomyelin and phosphatidylserin	41.5	37.9	41.5	35.9
Lecithin	25.4	25.7	25.9	29.7
Phosphatidylethanolamine	29.4	30.4	25.2	28.3

* Diet contained only fat with fatty acids comprising 50 per cent of linoleic acid.

age group reacted at a dilution of 1:32. Subjects B and C had no complaints and no diarrhoea. Examination did not reveal any abnormalities, in particular, there were no neurologic disturbances and no involvement of the fundus oculi. The fat absorption coefficient of subject B was 95.7 per cent. There was no acanthocytosis. In subjects B and C the plasma cholesterol, phospholipids and especially triglycerides were reduced. (Tables I and II.) Corresponding with the reduction in the lipids, the linoleic acid content also was decreased, but the distribution of the various fatty acids was about the same as that in the control group (pool). (Table VI.)

COMMENTS

Patient A showed a marked β-lipoprotein deficiency, with the same change in the lipid pattern of the plasma and erythrocytes as occurs in the classic Bassen-Kornzweig syndrome. However, the characteristic clinical manifestations of steatorrhoea and neuropathy, as described in this syndrome, were absent. The fundus oculi did show changes, in particular, scattered, very fine, shiny dots on both sides. In the patient's fortieth year of age, the fundus was still unchanged. There was no acanthocytosis, although the red blood cells did have the lowered lecithin-sphingomyelin ratio and the reduced linoleic acid content of the lipids, and the plasma showed the characteristic changes in the lipids. This seems to be of importance in view of the suggestions of Ways and Simon [*21*] that "abnormal plasma environment in acanthocytosis does influence cell shape and autohemolytic behavior." It is possible that in our patient the alteration of the lipid pattern was not sufficiently marked to cause acanthocytosis. This might also explain the absence of neuropathy. However, the normal fat absorption coefficient, which was found many times, is of importance in understanding the part played by the steatorrhoea in the pathogenesis of the disease. Isselbacher et al. [*18*] proved that in the classic cases with steatorrhoea, fatty acids and diglycerides are taken up by the intestinal mucosa and converted into triglycerides. These investigators believe that there is either a disturbance in the formation of chylomicrons in the intestinal mucosa or a disturbed release of chylomicrons into the lymph vessels. Due to this blockade in the removal of triglycerides, the fat accumulates in the intestinal mucosa, becoming manifest in the accumulation of fat in the epithelial cells. This might lead to steatorrhoea. This conception is consistent with the fact that in our patient, who had no steatorrhoea, no fat accumulation in the jejunal mucosa was observed. There was apparently still sufficient β-lipoprotein for removal of the fat. This also explains why patient A's brothers, who had mild β-lipoprotein deficiency, did not suffer from steatorrhoea. Patient A did not show the decreased lactase activity of the jejunum observed by Isselbacher et al. [*18*].

The fatty liver, found by Isselbacher et al. [*18*], would also be the result of the disturbed removal of the triglycerides. Liver fat consists mainly of triglycerides and cholesterol esters. Our patient showed a mild steatosis, which might be explained by the insufficient synthesis of pre-β-lipoprotein.

The possibility has been raised that in this disease there is a primary deficiency in essential fatty acids. There is a marked reduction of linoleic acid and arachidonic acid in plasma and red blood cells, but such a decrease has also been observed in the malabsorption syndrome with-

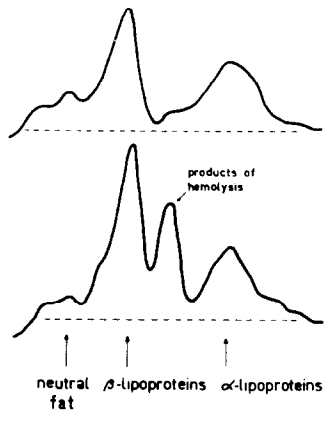

FIG. 4. Serum lipoproteins. *Top*, paper electrophoresis of serum of subject B showing moderate decrease in β-lipoprotein. *Bottom*, paper electrophoresis of the serum of subject C showing slight decrease in β-lipoprotein.

out acanthocytosis and without neurologic disturbances. Moreover, there is no increase of eicosatrienoic acid in erythrocytes and other tissues, which is characteristic for essential fatty acid deficiency [*21*]. We were also unable to find an increase of C-20:3 in plasma and erythrocytes.

The evidence that the primary disturbance is a deficiency of β-lipoprotein is more impressive [*7,18,24*]. That this is really a deficiency in β-lipoprotein, and not an inability of this protein to bind fat, is shown by immunoelectrophoresis.

With the help of puromycin, which disturbs lipoprotein synthesis, Seymour et al. [*43*] and Isselbacher et al. [*18*] were able to elicit the same biochemical and morphologic picture.

The deficiency of the lipids in the plasma is understandable, as normally β-lipoprotein contains about 75 per cent of the total circulating lipids [*44*].

Still to be explained, however, is the striking reduction in the linoleic acid content of the plasma, the red blood cells and the adipose tissue. It has been suggested that this is correlated with the fall in the lecithin content. In our patient it was shown, however, that the linoleic acid content of the serum phospholipids was proportionately less reduced than the linoleic acid content of the cholesterol esters and triglycerides. (Table IV.) In the red blood cells the decline in the lecithin content was relatively less than that of the linoleic acid content. (Tables IX and V.)

Isselbacher et al. [*18*] believe that the low linoleic acid content is caused by disturbed absorption. We have studied this in our patient, who also showed this marked decrease in linoleic acid content, and there was no disturbance of the absorption of fat, xylose and glucose. Therefore we instituted a four weeks' diet containing a fat with fatty acids 50 per cent linoleic acid. The serum and erythrocyte lipids were determined before, and two and four weeks after, beginning the diet. The serum contents of total lipids, cholesterol and phospholipids showed only slight differences; the triglyceride content became somewhat lower. (Table VII.) Analysis of the fatty acid composition of the lipids demonstrated a marked influence of the diet rich in linoleic acid. The linoleic acid content of the phospholipids, the cholesterol esters and especially the triglycerides increased strikingly, whereas the oleic acid content in all three fractions decreased. (Table VII.) The lipid pattern of the erythrocytes changed but little in respect to the cholesterol and phospholipid contents. (Tables VIII.) However, the fatty acid pattern changed markedly under the influence of the diet: the stearic acid and oleic acid contents decreased significantly and the linoleic acid and arachidonic acid contents increased. (Table IX.)

Farquehar et al. [*45*] found an increase in linoleic acid but not in arachidonic acid in normal persons after such a regimen. The distribution of the phospholipids in the red blood cells did not change (Table X) under the influence of the diet rich in linoleic acid. The cephalin (phosphatidylethanolamine) content also did not show significant differences.

These results indicate that the intestinal mucosa of our patient certainly was able to absorb linoleic acid, and not to a slight degree, in view of the sharp increase in the linoleic acid content of the erythrocytes, the serum triglycerides and cholesterol esters, certainly when sufficient linoleic acid was present in the diet. Why the linoleic acid content is reduced in these patients, as well as why there is a shift in the lecithin-sphingomyelin ratio in plasma and erythrocytes, remains unknown. Linoleic acid deficiency of itself does not cause a decrease in the lecithin-sphingomyelin ratio of plasma and red blood cells.

Therapy. Therapy directed at the cause is, of course, not possible. To cure the possible steatorrhoea, a low fat diet has proved to be the best method; it is followed by a considerable improvement in the general condition [*11*]. In their plan of treatment, Isselbacher et al. [*18*] started with the premise that fatty acids with a chain length less than C-12 are normally not esterified in the intestinal mucosa, and reach the liver via the portal vein as nonesterified fatty acids bound to albumin. They gave their patient a diet with 80 per cent octanoate (C-8) and 2 per cent decanoate (C-10). With 30 to 45 gm. of these medium-chain triglycerides, the patient soon gained weight and the loss of fat in the stools decreased from 20 to 8 per cent. After eight months the general condition had improved and muscular strength increased, but the neurologic disturbances remained unchanged.

Heredity. Bassen and Kornzweig [*1*] suggested the hereditary nature of this syndrome. Their two patients were siblings from a consanguineous marriage (first cousins). Parental consanguinity was also present in the patients

described by Lamy et al. [*11*] (half brother and half sister), Druez [*5*] (the grandparents were first cousins), Singer et al. [*2*] (the parents were second cousins) and Schwartz [*15*] (the parents were fourth cousins). A sibling from the family of the patient described by Di George et al. [*24*] was said to have died at the age of four from the same disease. In a maternal uncle of the patient described by Mier et al. [*8*] a gradually progressive neuropathy with ataxia and visual disturbances developed at the age of nineteen. In a paternal cousin a tumour of the head, disturbances of gait and gradual deterioration of vision developed at the age of six.

Our observation of β-lipoprotein deficiency in three brothers corresponds with the observations of Kuo and Basset [*14*] who found it in one male and two female members of a family (Table 1), and those of Salt and co-workers [*4*] who found β-lipoprotein deficiency in the two parents of a young patient who had the classic syndrome. (Table 1.) The parents of our patients were not related. Several times, the parents of other patients have been examined, but no β-lipoprotein deficiency was found on these occasions. These data make it probable that an autosomal recessive gene with variable penetrance is involved in this hereditary disease.

Since it has become clear (and is confirmed by our observations) that many degrees of this β-lipoprotein deficiency exist, even in subjects who are entirely symptom-free, it is probable that this deficiency does not occur as rarely as has been supposed. Only if traces of β-lipoprotein are present, or if it is entirely absent, do steatorrhoea, neuromuscular disturbances, acanthocytosis and retinal changes develop. A slighter degree of β-lipoprotein deficiency may, however, also be associated with the characteristic lipid pattern of the plasma and red blood cells, and the greater the deficiency, the more characteristic the pattern.

A particularly low serum cholesterol content need not be the result of a β-lipoprotein deficiency, but can also be caused by a α_1-lipoprotein deficiency. In this case the serum content of total and free cholesterol and of phospholipids also is decreased, but the triglyceride content is, instead, on the high side [*46*]. The patients described by Frederickson et al. [*46*] had very large tonsils but no neurologic disturbances. Tonsils and lymph nodes contained many foam cells; the fat of these cells consisted mainly of cholesterol esters, chiefly cholesterol oleate.

SUMMARY

There are several degrees of β-lipoprotein deficiency. If there is no β-lipoprotein present, or if there are only traces of it, the Bassen-Kornzweig syndrome develops. A constant feature of this syndrome is disturbed fat absorption with accumulation of fat in the epithelium of intestinal mucosa and acanthocytosis; ultimately neuropathies, and usually disturbances of the ocular fundus, develop. If there is still about 10 to 20 per cent β-lipoprotein present, steatorrhoea, fat accumulation in the epithelium of the intestinal mucosa, neurologic disturbances and acanthocytosis are absent. The changes in the lipids and fatty acid patterns in plasma and red blood cells are the same as those present in the absence of β-lipoproteins. We found that after consumption of food containing much linoleic acid, the linoleic acid content of the erythrocytes, and of the serum cholesterol esters, phospholipids and triglycerides, increased considerably. β-Lipoprotein deficiency is a hereditary disease; probably an autosomal recessive gene is involved.

Acknowledgment: Our thanks are due to Prof. E. Mandema for the clinical observation of our patient and to Dr. F. Peetoom for his help with the immunoelectrophoresis of the serum.

REFERENCES

1. Bassen, F. A. and Kornzweig, A. L. Malformation of the erythrocytes in a case of atypical retinitis pigmentosa. *Blood*, 5: 381, 1950.
2. Singer, K., Fisher, B. and Perlstein, M. Acanthrocytosis, a genetic erythrocytic malformation. *Blood*, 7: 577, 1952.
3. Jampel, R. S. and Falls, H. F. Atypical retinitis pigmentosa, acanthrocytosis and heredodegenerative neuromuscular disease. *Arch. Ophth.*, 59: 818, 1958.
4. Salt, H. B., Wolff, O. H., Lloyd, J. K., Forsbrooke, A. S., Cameron, A. H. and Hubble, D. V. On having no beta-lipoprotein. A syndrome comprising a-beta-lipoproteinaemia, acanthocytosis and steatorrhoea. *Lancet*, 2: 325, 1960.
5. Druez, G. Un nouveau cas d'acanthocytose. *Rev. hemat.*, 14: 3, 1959.
6. Friedman, J. S., Cohn, H., Zymaris, M. and Golder, M. G. Hypocholesteremia in idiopathic steatorrhoea. *Arch. Int. Med.*, 105: 112, 1960.
7. Marcy, C. C., Di George, A. M. and Auerbach, V. H. Studies concerning the defect in a patient with acanthocytosis. *Clin. Res.*, 8: 371, 1960.
8. Mier, M., Schwartz, S. O. and Boshes, B. B. Acanthocytosis, pigmentary degeneration of the retina and ataxic neuropathy. A genetically determined syndrome with associated metabolic disorder. *Blood*, 16: 1586, 1960.
9. Wolff, J. A. and Bauman, W. O. Studies concern-

ing acanthocytosis. A new syndrome with absent beta-lipoprotein. *Am. J. Dis. Child.*, 102: 478, 1961.

10. WAYS, P., REED, C. F. and HANAHAN, D. J. Abnormalities of erythrocytes and plasma lipids in acanthocytosis. *J. Clin. Invest.*, 40: 1088, 1961.
11. LAMY, M., FRÉZAL, J., POLONOVSKI, J. and REY, J. L'absence congénitale de betalipoprotéines. *Presse méd.*, 69: 1511, 1961.
12. LAMY, M., FRÉZAL, J., POLONOVSKI, J., DRUEZ, G. and REY, J. Congenital absence of bêta-lipoproteins. *Pediatrics*, 31: 277, 1963.
13. PHILLIPS, G. B. Quantitative chromographic analysis of plasma and red blood cell lipids in patients with acanthocytosis. *J. Lab. & Clin. Med.*, 59, 357, 1962.
14. KUO, P. T. and BASSET, D. R. Blood and tissue lipids in a family with hypo-beta-lipoproteinemia. *Circulation*, 26: 660, 1962.
15. SCHWARTZ, J. F., ROWLAND, L. P., EDER, H., MARKS, P. A., OSSERMAN, E. F., HIRSCHBERG, E. and ANDERSON, H. Bassen-Kornzweig syndrome: deficiency of serum betalipoprotein. *Arch. Neurol. & Psychiat.*, 8: 438, 1963.
16. STÖCKER, J. P. Acanthocytose. *Nederl. tijdschr. Geneesk.*, 108: 1830, 1964.
17. SOBREVILLA, L. A., GOODMAN, M. L. and KANE, C. A. Demyelinating central nervous disease, macular atrophy and acanthocytosis. *Am. J. Med.*, 37: 821 1964.
18. ISSELBACHER, K. J., SCHEIG, R., PLOTKIN, G. R. and CAULFIELD, J. B. Congenital beta-lipoprotein deficiency, an hereditary disorder involving a defect in the absorption and transport of lipids. *Medicine*, 43: 347, 1964.
19. WAYS, P., REED, C. F. and HANAHAN, D. J. Red cell and plasma lipids in acanthocytosis. *J. Clin. Invest.*, 42: 1248, 1963.
20. SIMON, E. R. and WAYS, P. Incubation hemolysis and red cell metabolism in acanthocytosis. *J. Clin. Invest.*, 43: 1311, 1964.
21. WAYS, P. and SIMON, E. R. The role of serum in acanthocyte auto hemolysis and membrane lipid composition. *J. Clin. Invest.*, 43: 1322, 1964.
22. DE GIER, J., VAN DEENEN, L. J. M., VERLOOP, M. C. and VAN GASTEL, C. Phospholipid and fatty acid characteristics of erythrocytes in some cases of anemia. *Brit. J. Haematol.*, 10: 246, 1964.
23. VAN DEENEN, L. J. M. and DE GIER, J. Chemical composition and metabolism of lipids in red cells of various animal species. In: The Red Blood Cell, chap. 7, pp. 298–300. New York, 1964. Academic Press, Inc.
24. DI GEORGE, A. M., MABRY, C. C. and AUERBACH, V. H. Specific disorder of lipid transport (acanthrocytosis). Treatment with intravenous lipids. *Am. J. Dis. Child.*, 102: 580, 1961.
25. SWITZER, S. and EDER, A. H. Interconversion of acanthocytes and normal erythrocytes with detergents. *J. Clin. Invest.*, 41: 1404, 1962.
26. VAN BUCHEM, F. S. P. Atherosclerosis and nutrition. *Nutritio et dieta*, 4: 122, 1962.
27. VAN BUCHEM, F. S. P. and DRION, E. F. Cardiovascular disease and serum cholesterol. *Angiology*, 15: 424, 1964.
28. FOLCH, J., ASCOLI, I., LEES, M., MEATH, J. A. and LE BARON, F. N. Preparation o' lipid extracts from brain tissue. *J. Biol. Chem.*, 191: 833, 1951.
29. PRIES, C., MEYER, J. W. A., DE PAGTER, H. A. TH, BÖTTCHER, C. J. F. and VAN BUCHEM, F. S. P. Composition of blood plasma-lipids from atherosclerotic cardiovascular diseased donors and of normal donors, in press.
30. REED, C. F., SWISHER, S. N., MARINETTI, G. V. and EDEN, E. G. Studies of the lipids of the erythrocyte. I. Quantitative analysis of the lipids of normal human red blood cells. *J. Lab. & Clin. Med.*, 56: 281, 1960.
31. ANDERSON, J. T. and KEYS, A. Cholesterol in serum and lipoprotein fractions, its measurement and stability. *Clin. Chem.*, 2: 145, 1960.
32. ABELL, L. L., LEVY, B. B., BRODIE, B. B. and KENDALL, F. E. A simplified method for the estimation of total cholesterol in serum. *J. Biol. Chem.*, 195: 357, 1952.
33. SPERRY, W. M. and WEBB, M. A revision of the Schoenheimer-Sperry method for cholesterol. *J. Biol. Chem.*, 187: 97, 1950.
34. CARLSON, L. A. Determination of serum triglycerides. *J. Atherosclerosis Res.*, 3: 334, 1963.
35. VAN HANDEL, E. and ZILVERSMIT, D. B. Micro method for the determination of serum triglycerides. *J. Lab. & Clin. Med.*, 50: 152, 1957.
36. DOLE, V. P. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. *J. Clin. Invest.*, 35: 150, 1956.
37. PRIES, C. and BÖTTCHER, C. J. F. Ultra microtitration of fatty acids in non-aqueous single phase systems. *Anal. chim. acta*, 31: 293, 1964.
38. BÖTTCHER, J. C. F., VAN GENT, C. M. and PRIES, C. A rapid and sensitive submicro phosphorus determination. *Anal. chim. acta*, 24: 203, 1961.
39. PRIES, C., AUMONT, A. and BÖTTCHER, C. J. F. Analysis of phospholipids by combined silica gel column chromatography and stepwise hydrolysis. *Biochim. et biophys. acta*, in press.
40. BARRON, E. J. and HANAHAN, D. J. Observations on the silicic acid chromatography of the neutral lipids in rat liver, beef liver and yeast. *J. Biol. Chem.*, 231: 493, 1958.
41. BÖTTCHER, C. J. F., WOODFORD, E. P., BOELSMA-VAN HOUTE, E. and VAN GENT, C. M. Methods for the analysis of lipids extracted from human arteries and other tissues. *Rec. trav. chim. Pays-Bas*, 78: 794, 1959.
42. HILL, J. G., KUKSIS, A. and BEVERIDGE, J. M. R. The constancy of red blood cell lipids in man during extreme variations of dietary fat intake. *J. Am. Oil Chemists' Soc.*, 41: 393, 1964.
43. SEYMOUR, M. G., DRAMMERY, G. D., BUTZ, D. M. and ISSELBACHER, K. J. Inhibition of protein synthesis. A mechanism for the production of impaired fat absorption. *J. Clin. Invest.*, 43: 1281, 1964.
44. EDER, A. H. The lipoproteins of human serum. *Am. J. Med.*, 23: 269, 1957.
45. FARQUEHAR, J. W. and AHRENS, E. H., JR. Effect of dietary fats on human erythrocyte fatty acid patterns. *J. Clin. Invest.*, 42: 675, 1963.
46. FREDERICKSON, D. S., ALTROCCHI, P. H., AVIOLI, L. V., DE WITT, S. and GOODMAN, H. C. Tangier disease. *Ann. Int. Med.*, 55: 1016, 1961.